

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



# Liver Progenitor Cells, Cancer Stem Cells and Hepatocellular Carcinoma

Janina E.E. Tirnitz-Parker<sup>1,2</sup>, George C.T. Yeoh<sup>2</sup> and John K. Olynyk<sup>1,2</sup>

<sup>1</sup>Curtin University,

<sup>2</sup>Western Australian Institute for Medical Research, University of Western Australia  
Australia

## 1. Introduction

There is great interest in the biology of liver progenitor cells (LPCs) because of their stem cell-like ability to regenerate the liver when the hepatocyte pool is exhausted. Barely detectable in healthy tissue, they emerge upon chronic insult in periportal regions, proliferate and migrate to injury sites in the parenchyma and eventually differentiate into hepatocytes and cholangiocytes to restore liver mass, morphology and function. The increasing worldwide shortage of livers for orthotopic transplantation means LPCs have assumed more prominence as candidates for cell therapy as an alternative therapeutic approach for the treatment of various liver diseases. However, an LPC response is usually seen in pre-cancerous liver pathologies and their high proliferation potential makes them possible transformation targets; associations that overshadow their restorative capability. This mandates that we continue to investigate the factors that govern their activation, proliferation and especially their differentiation into mature, functional cells to effectively direct transplanted cells towards regeneration and not tumorigenicity.

## 2. Normal liver tissue turnover

Tissue regeneration and maintenance in healthy intestine and skin is achieved within days and weeks respectively. In contrast healthy liver has a very slow cell turnover rate and the vast majority of hepatocytes is considered to be in the quiescent, non-proliferative G<sub>0</sub> phase of the cell cycle. It has been estimated that at any one time only 1 in 20,000 to 40,000 hepatocytes is undergoing mitotic cell division with an average life span of 200 to 300 days (Bucher & Malt, 1971).

The mechanisms by which hepatic cells are replaced in healthy liver are controversial. An early model, the “streaming liver” hypothesis is based on the metabolic zonation and differential gene expression patterns of periportal compared to pericentral hepatocytes. Periportal cells were proposed to proliferate and migrate (“stream”) towards the central area with maturation during the journey and terminal differentiation achieved when the cells reached the central zone (Zajicek *et al.*, 1985; Arber *et al.*, 1988; Sigal *et al.*, 1992). However there is no convincing evidence for a periportal to pericentral differentiation gradient and while hepatocytes in opposing lobular areas are responsible for different

metabolic functions, cells in either location are considered to be fully differentiated. By reversing the blood flow in the liver, Thurman and Kauffman demonstrated that this lobular zonation is not dependent on hepatocyte lineage progression but rather due to metabolite-induced gene regulation (Thurman & Kauffman, 1985). Retroviral marking studies provided additional evidence against the “streaming liver” model since transplanted cells, traceable by  $\beta$ -galactosidase expression, remained in the original location for 15 months (Bralet *et al.*, 1994). Furthermore, experiments performed with mosaic livers of chimeric rats (Ng & Iannaccone, 1992) as well as approaches using transgenic hAAT/ $\beta$ -gal mice (Kennedy *et al.*, 1995) demonstrated that hepatocytes proliferate clonally during normal tissue renewal throughout the whole liver lobule. Collectively, these findings led to the conclusion that normal liver cell plates lack the existence of a main proliferative compartment and instead randomly distributed hepatocytes mediate normal liver turnover by slow clonal expansion without involvement of a liver stem cell (Ponder, 1996).

### 3. Liver regeneration

The liver has an enormous capacity to regenerate by (1) replication of remaining, healthy hepatocytes, (2) activation, expansion and differentiation of a stem cell compartment, or (3) by a combination of these processes. Which pathway is employed depends on the nature of the injury, its severity and duration. This is discussed in greater detail in the sections to follow.

#### 3.1 Hepatocyte-mediated regeneration

The hepatic regenerative capacity is most clearly seen after surgical removal of liver mass. This model, referred to as partial hepatectomy (PHx), was introduced by Higgins and Anderson (Higgins & Anderson, 1931) and it is unquestionably the best studied liver regeneration model due to its simplicity of design and reproducibility. In the rat two-thirds PHx is performed, whereas in the mouse usually only the left lobe is removed due to technical difficulties in the performance of two-thirds PHx surgery in mice, with resultant high mortality (Fausto *et al.*, 2006). The removed lobes do not re-grow. Instead there is compensatory, hyperplastic growth of all residual cellular populations until the size of the organ achieves proportionality to the body size, as determined by metabolic demands of the organism (Kawasaki *et al.*, 1992; Starzl *et al.*, 1993). The different liver cell types do not divide simultaneously but show different kinetics in DNA synthesis. Periportal hepatocytes, with a presumably shorter  $G_1$  phase than pericentral cells (Rabes, 1976), are the first to undergo a wave of mitosis but DNA synthesis progresses to eventually involve the whole lobule with the exception of a few glutamine synthetase-positive, pericentral cells (Gebhardt, 1988). The proliferating hepatocytes are thought to provide mitogenic stimuli for the other hepatic cell populations. Biliary ductular cells, Kupffer and hepatic stellate cells (HSCs) and finally sinusoidal endothelial cells enter DNA synthesis about 24 hours later (Michalopoulos & DeFrances, 1997) with synchronised proliferation of each cell type for at least the first wave of replication. The greatest increase in liver mass can be seen by 72 hours with complete mass restoration after about one week (Grisham, 1962).

Although it was known from early experiments that repeated PHx does not exhaust hepatocyte growth (Simpson & Finck, 1963), the enormous proliferative capacity of adult

hepatocytes has previously been underestimated. Rhim *et al.* showed that newborn uPA overexpressing mice with continuous hepatocytic necrosis could be rescued by transplantation of a small number of hepatocytes that required between 10 to 15 rounds of replication to generate sufficient liver mass (Rhim *et al.*, 1994; Rhim *et al.*, 1995). In addition, serial transplantation experiments performed in tyrosinemic mice caused by a deficiency for fumarylacetoacetate hydrolase (FAH) revealed that hepatocytes are capable of undergoing more than 70 cell doublings without loss of functionality (Overturf *et al.*, 1997). Conversely there is also recent evidence that hepatocytes might reach a state of “replicative senescence” under certain chronic conditions such as advanced cirrhosis, perhaps due to telomere shortening (Paradis *et al.*, 2001; Wiemann *et al.*, 2002).

### 3.2 Liver progenitor cell-mediated regeneration

Repeated replication of healthy hepatocytes is the most efficient way to restore liver mass and function during normal tissue renewal and repair. If this process is inhibited or blocked during chronic chemical or carcinogenic hepatocyte insult, the liver relies on stem cell-like LPCs for its restoration. These cells are also referred to as “oval cells” in rodents (Fausto & Campbell, 2003) and the “Ductular Reaction” in humans due to their rather ductular phenotype in most human chronic liver diseases (Roskams & Desmet, 1998; Theise *et al.*, 1999).

#### 3.2.1 History, origin and features of liver progenitor cells

The appearance of oval-like cells in the livers of rats treated with the azo dye “Butter Yellow” was originally reported in 1937 (Kinosita, 1937). Two decades later, Farber introduced the term “oval cell” for this population after observing small ovoid cells with a scant basophilic cytoplasm and a high nuclear to cytoplasmic ratio following treatment of rats with carcinogenic agents (Farber, 1956a, 1956b). Shortly after, Wilson and Leduc documented the proliferation of ductular cells that gave rise to hepatocytes and possibly new interlobular bile ducts in mice fed a methionine-rich, bentonite-supplemented diet and they were the first to suggest the existence of a bipotential liver progenitor or stem cell (Wilson & Leduc, 1958). Many experimental models involving toxins and carcinogens, alone or in combination with other surgical or dietary regimes, have since been developed and these facilitated the study of these progenitor cells, which are now widely accepted to represent adult LPCs; the progeny of hepatic stem-like cells.

The precise origin of LPCs remains uncertain, even though many researchers have addressed this question. The lack of definite evidence regarding the cellular source of LPCs may reflect differences in the models used to induce them and has also been hampered by a lack of specific LPC markers. Lenzi *et al.* suggested bile ducts as the structure of origin and argued that LPCs express biliary markers such as cytokeratin (CK) 7 and CK19 and lack expression of the mesenchymal cell markers vimentin and desmin. Additionally, the degree of LPC proliferation during early ethionine-induced carcinogenesis was found to be proportional to the increase in biliary tree volume and the authors claimed that LPCs are simply part of spatially expanded cholangioles (Lenzi *et al.*, 1992).

Other investigators have proposed an extrahepatic origin for LPCs. After it became apparent that some LPCs share c-kit, CD34 and Thy-1 expression with haematopoietic stem cells

(Fujio *et al.*, 1994; Omori *et al.*, 1997; Petersen *et al.*, 1998a), Petersen *et al.* were the first to suggest that LPCs could be derived from epithelial precursors in the bone marrow (Petersen *et al.*, 1999). Bone marrow-derived cells that potentially contribute to liver regeneration would enter via the portal vasculature and locate adjacent to the ducts in the periportal region, which is why Sell extended the preceding proposition by suggesting the periductular LPC as the candidate cell for an extrahepatic, bone marrow-derived stem cell in the liver (Sell, 2001). To test the hypothesis that cells from the bone marrow contribute to the formation of LPCs and hepatocytes, several investigators performed cell transplantation studies. They generally followed the fate of male bone marrow cells or purified haematopoietic stem cells transplanted into lethally irradiated female recipients that were in most cases subjected to liver injury. It was demonstrated that very minor fractions of LPCs or hepatocytes were donor-derived in both healthy and diseased livers (Petersen *et al.*, 1999; Theise *et al.*, 2000a, 2000b; Krause *et al.*, 2001; Wang *et al.*, 2002). The responsible population in the bone marrow capable of repopulating the liver was thought to be of  $c\text{-kit}^{\text{high}}\text{Thy}^{\text{low}}\text{Lin}^{\text{neg}}\text{Sca-1}^{\text{pos}}$  phenotype (Lagasse *et al.*, 2000). Soon after, the bone marrow was found to contain another stem cell subpopulation, the multipotent adult progenitor cell (MAPC), which can be induced to express hepatocyte phenotype and functions *in vitro* (Schwartz *et al.*, 2002) and is capable of differentiating into hepatocyte-like cells when transplanted into the liver (Jiang *et al.*, 2002). When donor-derived hepatocytes were examined genotypically, it was noted that they contained both donor and host genetic markers, indicating cell fusion as the likely mechanism by which hepatocytes are generated from bone marrow and not by transdifferentiation of haematopoietic stem cells (Vassilopoulos *et al.*, 2003; Wang *et al.*, 2003b). On the other hand, haematopoietic stem cells co-cultured with injured liver tissue separated by a trans-well membrane were shown to convert to a hepatocyte phenotype without fusion due to humoral factors released from the liver tissue. When engrafted into injured liver the haematopoietic stem cells differentiated into functional hepatocytes and their plasticity was proposed to facilitate the conversion, rather than the rare cell fusion event that was only seen at later stages of the experiment (Jang *et al.*, 2004). Recently it was demonstrated by transplantation of lacZ-transgenic bone marrow into virally or steatotically challenged mice that the contribution of extrahepatic cells to LPC-generated hepatocytes is minimal (Tonkin *et al.*, 2008). Collectively, these experiments show that some bone marrow cells are capable of producing hepatocytes (with or without fusion, depending on the model and cell population used) to restore injured liver. However, it occurs at a low frequency and efficiency unless a strong selective pressure is applied (Thorgeirsson & Grisham, 2006). It is likely that the more significant role of bone marrow cells is to generate non-parenchymal cells during liver regeneration (Forbes *et al.*, 2004). The usual regeneration processes after acute and chronic liver injuries appear to rely predominantly on intrahepatic cells.

The most widely accepted view is that LPCs originate from liver-resident precursor or stem cells, which lie dormant and present in such low numbers as to be undetectable in normal liver. However, they can be activated to proliferate under certain pathological conditions (Fig. 1). Evidence from experiments showing that LPCs always emerge from periportal liver zones and the fact that selective periportal damage inhibits the LPC response (Petersen *et al.*, 1998b) have led to the conclusion that the precursor cell likely resides somewhere in the vicinity of the portal triad. Grisham and Porta found ductular proliferation in carcinogen-treated rats that they attributed to activated stem-like cells from the Canals of Hering, the anatomical boundary between terminal bile ducts and the most distal hepatocytes of the

hepatic plate (Grisham and Porta, 1964). Microscopic studies of early histological changes in rats following 2-acetylaminofluorene (2-AAF)/partial hepatectomy (PHx) treatment also show elongated ductular branches that are formed by proliferating LPCs, which originate from a stem cell compartment located in these canaliculo-ductular junctions. The newly formed biliary structures represent cellular extensions of the Canals of Hering and remain connected to the terminal biliary ductules by a continuous basement membrane (Paku *et al.*, 2001). Reid and colleagues suggested epithelial cell adhesion molecule (EpCAM) as a suitable marker for isolation and study of these Canals of Hering-derived LPCs (Schmelzer *et al.*, 2007). Lineage tracing of Sry (sex determining region Y)-box 9 (Sox9)-expressing cells supports the hypothesis that LPCs derive from the epithelial lining of bile ducts (Furuyama *et al.*, 2011). Theise *et al.* conducted studies comparing normal with acetaminophen-induced necrotic liver and identified the human equivalent to the rodent Canals of Hering, a niche which is similarly thought to harbour stem-like cells that give rise to LPCs or the Ductular Reaction (Theise *et al.*, 1999).

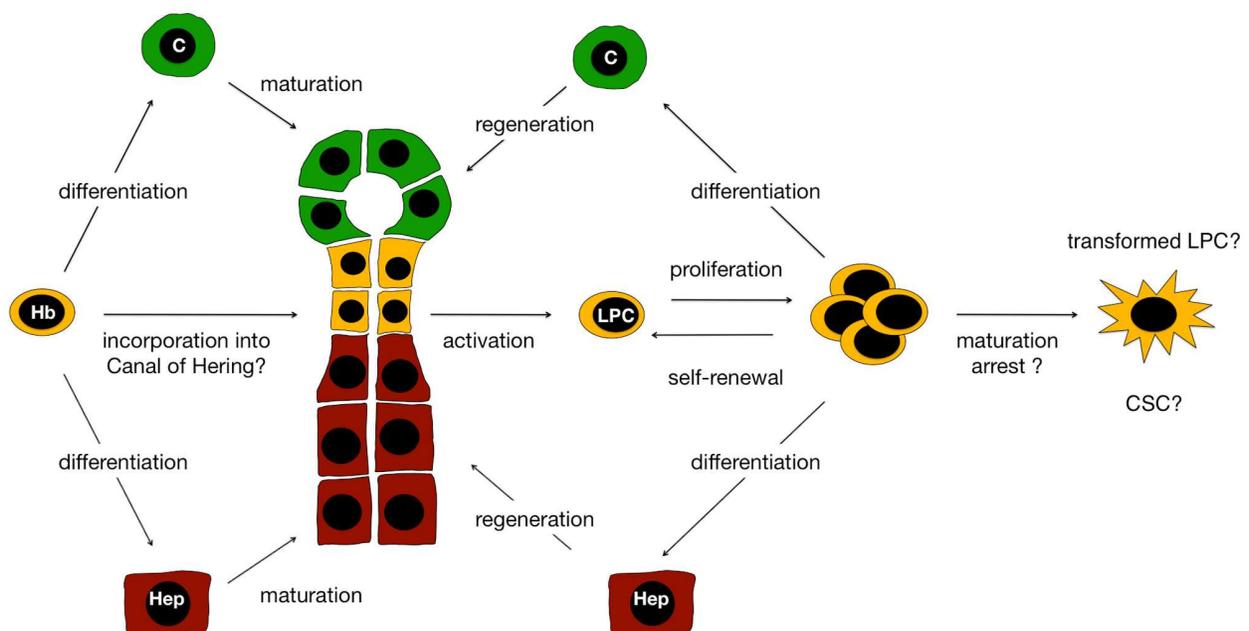


Fig. 1. LPC ontogeny. During liver development hepatoblasts (Hb) differentiate into cholangiocytes (C) and hepatocytes (Hep) and might be incorporated into the Canals of Hering to serve as a stem cell compartment during chronic liver injury. Activated liver progenitor cells (LPC) proliferate after appropriate stimuli, are capable of self-renewal and later commit towards either the cholangiocytic or hepatocytic lineage to regenerate the liver. If kept in a proliferative state, LPCs are likely candidates for transformation and might represent cancer stem cells (CSCs).

LPCs are a heterogeneous cell population and immature as well as intermediate phenotypes are observed before cells that express a differentiated phenotype are identified. Importantly, from activation to differentiation or transformation, they continuously change their morphology, phenotype and accordingly marker expression. LPCs express different combinations of phenotypic markers from both the hepatocytic and biliary lineage (Fig. 2) and also share epitopes with haematopoietic cells and cancer stem cells (CSCs; see table 1).

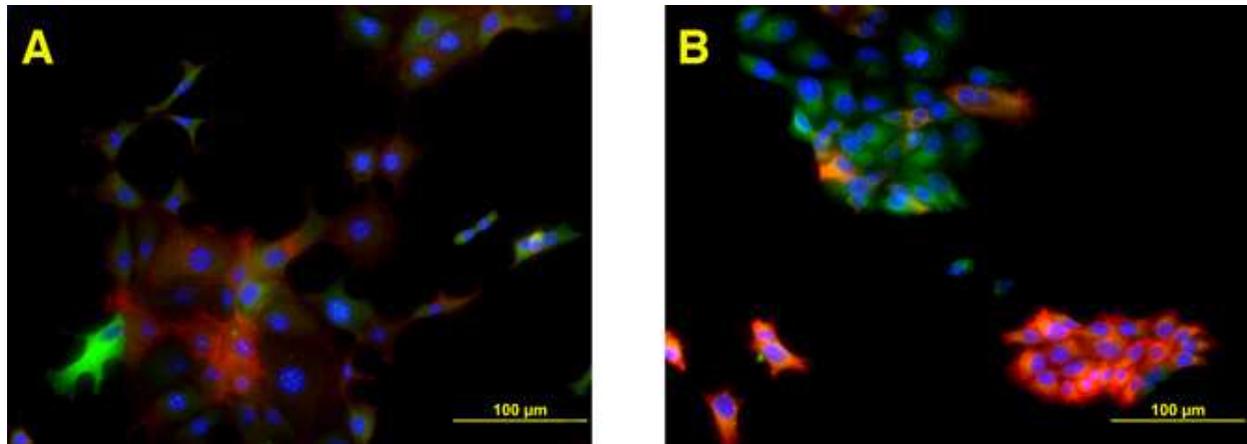


Fig. 2. Bipotentiality of LPCs. Immunofluorescent characterisation of the clonally established LPC line BMOL (Tirnitz-Parker *et al.*, 2007) demonstrates the cells' bipotentiality. Immature BMOL cells co-express the hepatocytic markers muscle 2-pyruvate kinase (A, green) and transferrin (B, green) with the biliary markers A6 (A, red) and CK19 (B, red).

Marker	Hepatocytes	Cholangiocytes	LPCs	CSCs	References
A6	-	+	+	?	Engelhardt <i>et al.</i> , 1993; Tirnitz-Parker <i>et al.</i> , 2007
AFP	fetal	-	+	(+)	Sell, 1978; Everts <i>et al.</i> , 1987; Smith <i>et al.</i> , 1996; Ishii <i>et al.</i> , 2010
Alb	+	-	+	(+)	Sell, 1978; Tian <i>et al.</i> , 1997; Yamashita <i>et al.</i> , 2010
CD24	?	(+)	+	+	Lee <i>et al.</i> , 2011; Qiu <i>et al.</i> , 2011
CD34	-	+	+	(+)	Omori <i>et al.</i> , 1997; Petersen <i>et al.</i> , 2003
CD133	?	?	+	+	Suetsugu <i>et al.</i> , 2006; Ma <i>et al.</i> , 2007; You <i>et al.</i> , 2010; Colombo <i>et al.</i> , 2011
CK7	-	+	+	?	Golding <i>et al.</i> , 1995; Clouston <i>et al.</i> , 2005
CK8	+	(+)	+	?	Sarraf <i>et al.</i> , 1994; Golding <i>et al.</i> , 1995; Sasaki <i>et al.</i> , 2008
CK14	-	(+)	(+)	(+)	Bisgaard <i>et al.</i> , 1993; Rogler, 1997; Zhang <i>et al.</i> , 2010
CK18	+	(+)	+	(+)	Golding <i>et al.</i> , 1995; Zhang <i>et al.</i> , 2010
CK19	-	+	+	?	Sarraf <i>et al.</i> , 1994; Golding <i>et al.</i> , 1995; Colombo <i>et al.</i> , 2011
c-kit	-	(+)	+	(+)	Fujio <i>et al.</i> , 1994; Fujio <i>et al.</i> , 1996; Knight <i>et al.</i> , 2008
Cx32	+	-	(+)	(+)	Zhang & Thorgeirsson, 1994; Paku <i>et al.</i> , 2004; Kawasaki <i>et al.</i> , 2011
Cx43	-	+	+	?	Zhang & Thorgeirsson, 1994; Paku <i>et al.</i> , 2004
Dlk	-	-	+	(+)	Jensen <i>et al.</i> , 2004; Yanai <i>et al.</i> , 2010
E-cad	low	high	high	?	Tirnitz-Parker <i>et al.</i> , 2007; Ueberham <i>et al.</i> , 2007; Van Hul <i>et al.</i> , 2009
EpCAM	(+)	fetal	+	+	Schmelzer <i>et al.</i> , 2007; Yamashita <i>et al.</i> , 2009; Okabe <i>et al.</i> , 2009; Yoon <i>et al.</i> , 2011
GGT IV	-	+	+	?	Petersen <i>et al.</i> , 1998a; Holic <i>et al.</i> , 2000
M <sub>2</sub> PK	fetal	+	+	?	Tian <i>et al.</i> , 1997; Lowes <i>et al.</i> , 1999; Tirnitz-Parker <i>et al.</i> , 2007
OV-6	-	+	+	?	Dunsford <i>et al.</i> , 1989; Zhang <i>et al.</i> , 2010; Cao <i>et al.</i> , 2011
$\pi$ -GST	fetal	-	+	?	Tee <i>et al.</i> , 1992; Lowes <i>et al.</i> , 1999; Oliva <i>et al.</i> , 2010
Sca-1	-	-	+	+	Petersen <i>et al.</i> , 2003; Qiu <i>et al.</i> , 2011
Thy-1	-	-	+	+	Petersen <i>et al.</i> , 1998a; Colombo <i>et al.</i> , 2011

Table 1. Marker expression by adult liver cells. A6, murine marker, epitope unknown; AFP,  $\alpha$ -fetoprotein; Alb, albumin; CD, cluster of differentiation; CK, cytokeratin; c-kit, CD117, stem cell factor receptor; Cx, connexin; Dlk, delta-like protein; E-cad, E-cadherin; EpCAM, epithelial cell adhesion molecule; GGT IV,  $\gamma$ -glutamyl transpeptidase IV; M<sub>2</sub>PK, muscle 2-pyruvate kinase; OV-6, rat and human marker, epitope shared by CK14 and 19;  $\pi$ -GST, pi-glutathione-S-transferase; Sca-1, stem cell antigen 1; Thy-1, thymocyte differentiation antigen 1.

They have been shown to differentiate at least bipotentially into hepatocytes and cholangiocytes (Tirnitz-Parker *et al.*, 2007), and in some models display multipotentiality, also producing intestinal and pancreatic lineages (Tatematsu *et al.*, 1985; Yang *et al.*, 2002; Leite *et al.*, 2007). Hence it is not surprising that there is still not a single LPC-specific marker available and a combination of phenotypic markers is required for their identification or isolation.

LPCs infiltrate the parenchyma in close spatial and temporal association with hepatic stellate cells (HSCs). Following activation, HSCs differentiate from quiescent, vitamin A-rich cells into  $\alpha$ -smooth muscle actin-positive myofibroblastic cells, which are capable of matrix degradation to generate space for cell migration as well as fibrogenesis and collagen deposition to provide chronically injured liver with architectural support. The activation, proliferation, migration and differentiation status of LPCs and HSCs, as well as their beneficial as opposed to pathological contributions, are controlled by key cytokines. LPCs and HSCs have been reported to influence each other's behaviour through paracrine signalling. LPCs produce a range of cytokines, including lymphotoxin  $\beta$  (LT $\beta$ ), which signals via the LT $\beta$  receptor on HSCs to activate the NF $\kappa$ B pathway. This results in production of intercellular adhesion molecule 1 (ICAM-1) and regulated upon activation, normal T-cell expressed and secreted (RANTES), which then act as chemotactic agents for LPCs and inflammatory cells involved in the wound healing response to chronic liver injury (Ruddell *et al.*, 2009). Several other factors mediating the LPC response have been identified, including tumour necrosis factor (TNF), TNF-like weak inducer of apoptosis (TWEAK), interferon gamma (IFN $\gamma$ ), and transforming growth factor beta (TGF $\beta$ ) among others (Knight *et al.*, 2000; Akhurst *et al.*, 2005; Knight *et al.*, 2005; Knight & Yeoh, 2005; Knight *et al.*, 2007; Tirnitz-Parker *et al.*, 2010). Abrogation of these key signalling pathways inhibits the LPC response to injury and prevents or diminishes liver fibrosis in animal models (Davies *et al.*, 2006; Lim *et al.*, 2006; Knight *et al.*, 2008). In the setting of impaired wound healing combined with chronic inflammation, the regenerative fibrotic response turns into pathological fibrogenesis, which can progress to cirrhosis and eventually hepatocellular carcinoma (HCC).

### 3.2.2 Rodent liver progenitor cell induction models

The majority of commonly used LPC induction models was originally developed to study the process of hepatocarcinogenesis. They generally combine an injuring mitotic stimulus, usually in the form of functional liver mass loss (chemically or otherwise-induced), with a manipulation that chronically damages hepatocytes or blocks their ability to divide and prevents them from contributing to the liver regeneration process. Described below are four examples of the most commonly used regimens.

#### 3.2.2.1 D-galactosamine

This model is mainly used to induce liver injury in the rat. Administration of D-galactosamine inhibits RNA and protein synthesis in centrilobular hepatocytes by trapping and depleting uridine-nucleotides and UDP-glucose (Decker & Keppler, 1972), leading to acute necrosis. Hepatocyte replication is not fully blocked in this model; the response is only delayed. LPCs are resistant to the chemical as they do not metabolise D-galactosamine and are induced to proliferate within 48 hours after injury. They migrate into the parenchyma, where they generate both ductular cells and small hepatocytes (Lemire *et al.*, 1991; Dabeva & Shafritz, 1993).

### 3.2.2.2 Solt-Farber model and the modified 2-AAF/PHx regime

In this model, which is commonly used in rats and only rarely in mice, injection of the ethylating hepatocarcinogen diethylnitrosamine (DEN) is followed two weeks later by a two-week treatment with 2-AAF and PHx one week into 2-AAF feeding (Solt & Farber, 1976). The most commonly used regimen is a modification to the original Solt-Farber protocol, in which the “initiation” step of DEN injection is omitted and 2-AAF is administered four days before and after PHx, the 2-AAF/PHx regime (Tatematsu *et al.*, 1984). Both models induce proliferation of ductular or periductular LPCs, which accelerates when 2-AAF feeding is terminated, indicating that not only hepatocytes are growth-inhibited by 2-AAF but also LPCs, although to a lesser extent. LPCs differentiate more efficiently into hepatocytes at low doses of 2-AAF, whereas they tend to undergo apoptosis at higher dosages (Alison *et al.*, 1997). As a consequence, the rate at which LPCs differentiate into hepatocytes can easily be controlled through variation of the 2-AAF dose (Paku *et al.*, 2004).

### 3.2.2.3 Choline-deficient, ethionine supplemented diet (CDE diet)

A dietary deficiency of the lipotrope choline is known to induce hepatic steatosis (Lombardi *et al.*, 1966; Lombardi *et al.*, 1968). This pathology reflects an impaired release of triglycerides in the form of very low-density lipoprotein (VLDL) from hepatocytes, leading to intracytoplasmic deposition of fat vacuoles within a few hours of choline withdrawal. Choline-deficiency has also been reported to induce hepatocarcinogenesis (Ghoshal & Farber, 1984; Yokoyama *et al.*, 1985; Locker *et al.*, 1986). Similar effects were shown for another well-known carcinogen, DL-ethionine. Administered alone, ethionine is an antagonist of methionine and as such an inhibitor of *de novo* choline-biosynthesis thus induces fatty liver (Farber, 1967) and also leads to HCC (Farber, 1956a). When tested in combination with choline-deficiency, ethionine enhances the formation of liver tumours (Shinozuka *et al.*, 1978b), yet surprisingly diminishes the formation of fatty liver during choline-deficiency (Sidransky & Verney, 1969).

An interesting observation during early choline-deficient, ethionine-supplemented (CDE) diet-induced hepatocarcinogenesis studies in rats was the massive proliferation of  $\alpha$ -fetoprotein-positive LPCs in the liver (Shinozuka *et al.*, 1978a). Numerous studies using this model to provoke an LPC response in rats were subsequently described. Due to the extensive availability of genetically engineered mouse strains, it became desirable to apply this regimen to mice. The conventional CDE diet used in rats however caused high mortality in mice and was therefore modified to a CD diet with separate administration of 0.165% DL-ethionine in the drinking water. This customised CDE diet (Akhurst *et al.*, 2001) reliably induces the proliferation of LPCs (Fig. 3, Tirnitz-Parker *et al.*, 2007; Tirnitz-Parker *et al.*, 2010) as well as inflammatory cells (Knight *et al.*, 2005) and serves as a murine model of hepatic fibrogenesis (Ruddell *et al.*, 2009; Van Hul *et al.*, 2009) and tumorigenesis following prolonged CDE diet exposure (Knight *et al.*, 2000; Knight *et al.*, 2008).

### 3.2.2.4 3,5-diethoxycarbonyl-1,4-dihydro-collidine diet (DDC diet)

The hepatotoxin 3,5-diethoxycarbonyl-1,4-dihydro-collidine is also an effective inducer of LPCs as it causes extensive and prolonged liver damage while the diet is administered (Jakubowski *et al.*, 2005). However, in contrast to the CDE diet (see above), a fraction of hepatocytes continue to proliferate for the duration of diet administration (Wang *et al.*, 2003a). Thus the model is unusual in that liver regeneration is accomplished by both

hepatocytes and LPCs. It offers an alternate model to investigate mechanisms that regulate LPC proliferation and differentiation. In the context of liver cancer, the DDC model has been used extensively to demonstrate a link between LPCs and HCC. LPCs isolated from p53 null mice subjected to a DDC diet are able to generate both hepatocarcinomas and cholangiocarcinomas following transplantation into immunodeficient mice (Suzuki *et al.*, 2008). By placing a Hepatitis B Virus X transgenic mouse on a DDC diet, Wang and colleagues were able to show that LPCs overexpressing HBx were tumorigenic (Wang *et al.*, 2012). Interestingly, over the same period of seven months, DDC treatment did not induce tumours in wild type mice. In another study, the importance of the Hippo-Salvador pathway, working through inhibition of the yes-associated protein YAP, was shown by subjecting mice with liver-specific ablation of WW45 (drosophila homolog of Salvador and adaptor for the Hippo kinase) to a DDC diet. These mice displayed liver tissue overgrowth, an enhanced LPC response and they developed liver tumours with HCC as well as cholangiocarcinoma characteristics that appeared to be LPC-derived (Lee *et al.*, 2010).

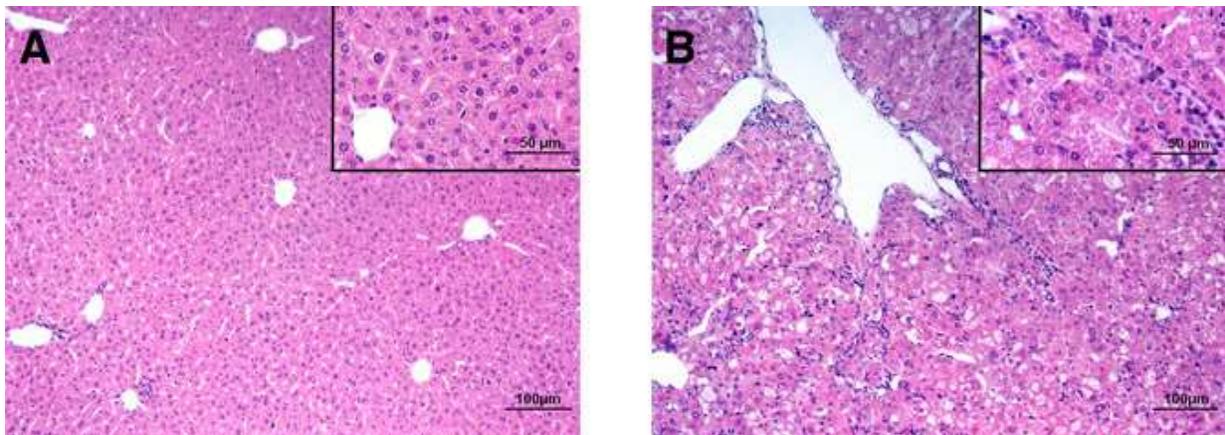


Fig. 3. Histology of normal and chronically injured liver. Adult mice on a control diet display normal liver architecture with orderly cords of hepatocytes and sinusoidal structures in-between the plates (A). On day 21 of the CDE diet, the liver architecture is highly disrupted by steatosis, scattered aggregates of infiltrated inflammatory cells and proliferating LPCs (B).

### 3.2.3 Liver progenitor cells in human pathologies

LPCs have been identified in a variety of human liver pathologies and are activated like their rodent counterparts to regenerate chronically injured liver (Haque *et al.*, 1996; Theise *et al.*, 1999). Like oval cells in rodents, human LPCs are usually associated with prolonged fibrosis, hepatocellular necrosis, cirrhosis and chronic inflammatory liver diseases. Hence, their proliferation is frequently seen in patients with hereditary haemochromatosis, chronic hepatitis B or C infection, alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD) when hepatocytes are inhibited by DNA-damaging oxidative stress (Lowes *et al.*, 1999; Roskams *et al.*, 2003a; Clouston *et al.*, 2005). The degree of stem cell activation and the number of proliferating LPCs in these pathologies was demonstrated to correlate with the progression and severity of the underlying liver disease (Lowes *et al.*, 1999). The activation of human LPCs is characterised by the appearance of reactive ductules, also referred to as Ductular Reaction. Cirrhotic livers have been shown to contain nodules that are usually in close contact with reactive ductules and consist entirely of intermediate hepatocytes, which

strongly suggests they originate from LPCs (Roskams *et al.*, 2003a; Roskams *et al.*, 2003b, Falkowski *et al.*, 2003). LPCs always emerge in pathologies with a predisposition to cancer and their proliferation in an environment rich in inflammatory mediators, growth factors or reactive oxygen species renders them likely targets for transformation. Furthermore, inhibition of the LPC response has been demonstrated to reduce the formation of cancerous lesions, strongly supporting a role for LPCs in hepatocarcinogenesis (Davies *et al.*, 2006; Knight *et al.*, 2005; Knight *et al.*, 2008). Very recently LPCs have not only been discussed as cellular precursors for liver cancer but also as potential liver cancer stem cells, which could be responsible for tumour maintenance and recurrence (Marquardt *et al.*, 2011; Rountree *et al.*, 2012).

#### 4. Cancer stem cells

The similarities between adult stem cells and CSC have led to confusion regarding their identity and it has often not been clear in the literature whether CSCs represent transformed progenitor or stem cells or whether both cell types are distinct cell populations that only share the expression of certain cell markers and display a similar biology. Adult tissue stem cells and CSCs are both defined by (i) highly efficient self-renewing ability through asymmetrical cell division and (ii) differentiation capacity along at least two if not more cell lineages. CSCs manifest the additional property of tumour initiation and/or maintenance. Nowadays the consensus is that the term CSC simply describes a cell's potential for self-renewal and ability to give rise to the hierarchic organisation of the heterogeneous lineages of cancer cells that constitute the tumour and does not consider the cell's origin. CSCs may arise from the differentiation arrest and transformation of a normal adult stem cell through oncogenic and/or epigenetic aberrations or the dedifferentiation of a mature cell that subsequently acquires self-renewing capacity. The CSC concept has been debated for many decades and compelling evidence of their existence has only emerged in the past decade.

##### 4.1 Haematopoietic cancer stem cells

The existence of CSCs was first demonstrated in the haematological malignancy acute myeloid leukaemia (AML). Dick and colleagues isolated human AML cells from peripheral blood and transplanted them into non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice. The vast majority of cells was unable to induce leukaemia, even when transplanted in larger numbers, despite the fact that they displayed a leukaemic blast phenotype such as the CD34<sup>+</sup>/CD38<sup>+</sup> subpopulation. Only 0.01-1% of all AML cells, the CD34<sup>+</sup>CD38<sup>-</sup> fraction, initiated AML and gave rise to a heterogeneous leukaemia tumour cell mass, classifying them as CSCs. The CD34<sup>+</sup>CD38<sup>-</sup> cells could be serially transplanted and reliably developed AML with the same morphology and cell surface marker expression as the original tumour (Bonnet & Dick, 1997). Additional tumour-initiating AML cell populations were later identified and described to be of a CD34<sup>+</sup>CD19<sup>-</sup> or CD34<sup>+</sup>CD10<sup>-</sup> and CD34<sup>+</sup>CD4<sup>-</sup> or CD34<sup>+</sup>CD7<sup>-</sup> phenotype (Cox *et al.*, 2004; Cox *et al.*, 2007).

##### 4.2 Solid tumour cancer stem cells

Using similar approaches involving cell transplantation into immunodeficient mice, CSCs have subsequently been identified in a variety of solid tumours, including breast, brain and liver cancer.

#### 4.2.1 Breast cancer stem cells

Metastatic breast cancer was the first solid tumour in which CSCs were identified and prospectively isolated. The CD44<sup>+</sup>CD24<sup>-/low</sup>Lineage<sup>-</sup> cell population initiated tumours upon transplantation into mice with as few as 100 cells per injection. Importantly, they could be serially passaged and reliably reproduced the heterogeneous phenotype of the original breast cancer. In contrast, unsorted cells from the primary tumour or injection of a large number of alternate phenotypes, such as CD44<sup>+</sup>CD24<sup>+</sup> cells, failed to form tumours (Al-Hajj *et al.*, 2003). Furthermore, it was established that increased expression of the detoxifying enzyme aldehyde dehydrogenase (ALDH) identifies the tumorigenic breast stem cell fraction and high ALDH1 activity correlates with poorer prognosis (Ginestier *et al.*, 2007).

#### 4.2.2 Central nervous system cancer stem cells

The discovery of breast CSC was reported in the same year as the identification of tumour-initiating stem cells in the brain. Singh and colleagues identified and prospectively isolated a CD133<sup>+</sup> population of cells from a range of human brain tumours including medulloblastomas, pilocytic astrocytoma, glioblastoma and anaplastic ependymoma that *in vitro* exhibited stem cell properties and gave rise to heterogeneous cell populations with the same phenotype as the original tumour cells. Upon transplantation of as few as 100 CD133<sup>+</sup> glioma cells into the frontal lobes of NOD/SCID mice, serially transplantable tumours were initiated that mirrored the original tumour phenotype, whereas no tumours developed after injection of a much larger number of CD133<sup>-</sup> cells from the same tumour (Singh *et al.*, 2003; Singh *et al.*, 2004).

#### 4.2.3 Liver cancer stem cells

Only very recently have liver CSCs been described. However the mounting evidence is compelling and ever more markers are suggested to describe the population of cells that may be responsible for liver cancer initiation, maintenance and potentially tumour recurrence after HCC resection, as described below.

##### 4.2.3.1 Side population (Hoechst 33342 dye efflux)

The first evidence for the existence of liver CSCs came from Haraguchi and colleagues who performed Hoechst 33342 side population (SP) analyses of various human gastrointestinal cell lines and identified a subpopulation of cells with CSC properties. The SP approach is based on the finding that cells without stem cell characteristics accumulate the fluorescent nucleic acid-binding dye Hoechst 33342, whereas stem cells and CSCs do not as they are capable of effectively effluxing the dye through high activity of adenosine triphosphate (ATP)-binding cassette (ABC) transporters such as the multidrug resistance transporter 1 (MDR1) or breast cancer resistance protein (BCRP, also known as ABCG2). These ABC transporters employ ATP hydrolysis to facilitate substrate export across membranes against steep concentration gradients and thereby protect cells from cytotoxic agents and importantly from chemotherapeutic drugs such as cisplatin and doxorubicin. The authors report that the HCC lines HuH7 and Hep3B contained 0.9% to 1.8% SP cells with CSC properties, respectively, whereas no SP cells could be purified from the less aggressive hepatoma cell line HepG2 (Haraguchi *et al.*, 2006). These results were confirmed shortly after by Chiba and colleagues who identified SP cells in some human liver cell lines, which

successfully induced xenograft tumours in NOD/SCID mice upon transplantation of as few as 1000 SP cells, while attempts to produce tumours with  $1 \times 10^6$  non-SP cells failed consistently (Chiba *et al.*, 2006).

#### 4.2.3.2 CD133 (Prominin 1)

Several recent studies have used this glycoprotein initially identified as a marker for CD34<sup>+</sup> haematopoietic stem cells and later as a marker of LPCs for the isolation of liver CSCs. Suetsugu *et al.* reported that both the hepatoblastoma cell line HepG2 as well as the human fetal hepatoblast cell line Hc lacked CD133 expression, and that CD133<sup>+</sup> cells could only be demonstrated in the human HCC line Huh7. CD133<sup>+</sup> cells showed a higher proliferative potential in culture but also a greater ability to initiate tumour growth *in vivo* compared to the CD133<sup>-</sup> population (Suetsugu *et al.*, 2006). Hepatic cells with a CD133 phenotype have been shown to be more resistant to chemotherapeutic drugs such as doxorubicin and 5-fluorouracil than their CD133<sup>-</sup> counterparts through preferential activation of the Akt/protein kinase B and Bcl-2 cell survival pathways. Furthermore, resistance of normal stem cells to cyclophosphamide is facilitated by the differentially expressed marker ALDH. Studies on ALDH and CD133<sup>+</sup> cells found ALDH expression only in the CD133<sup>+</sup> subpopulation and suggested a hierarchical cell organisation with regard to tumorigenicity in the order CD133<sup>+</sup>ALDH<sup>+</sup> > CD133<sup>+</sup>ALDH<sup>-</sup> > CD133<sup>-</sup>ALDH<sup>-</sup>, which suggests ALDH as an additional marker useful for liver CSC identification (Ma *et al.*, 2007, Ma *et al.*, 2008). In addition, it has been demonstrated that TGF $\beta$  signalling can induce CD133 expression in the HCC cell line Huh7 through epigenetic regulation, which results in a significant increase in tumour initiation capacity in these cells compared to CD133<sup>-</sup> Huh7 cells (You *et al.*, 2010).

#### 4.2.3.3 Epithelial cell adhesion molecule (EpCAM)

Myajima and colleagues identified EpCAM as a biliary and LPC marker, which is expressed in biliary epithelial cells and becomes upregulated in liver upon 2-AAF/PHx and DDC treatment (Okabe *et al.*, 2009). Since EpCAM expression has been reported in many normal epithelial as well as in tumour cells, it is not surprising that it has been suggested as a useful CSC marker. EpCAM<sup>+</sup> cells isolated from human HCC tissues were shown to be more tumorigenic and invasive than EpCAM<sup>-</sup> cells and consistently formed invasive tumours in NOD/SCID mice, even after serial transplantation, whereas the EpCAM<sup>-</sup> population did not (Yamashita *et al.*, 2009). EpCAM is a direct transcriptional target of Wnt/ $\beta$ -catenin signalling, which has been implicated as a CSC self-renewal pathway (Yamashita *et al.*, 2007). Activation of the Wnt/ $\beta$ -catenin pathway increased the EpCAM<sup>+</sup> cell population, whereas knockdown of EpCAM resulted in decreased proliferation, colony formation, migration and drug resistance (Yamashita *et al.*, 2009).

## 5. Hepatocellular carcinoma

Mortality from chronic liver disease is the most rapidly increasing cause of death in many western nations. The commonest aetiologies contributing to this escalation are chronic viral hepatitis C or B infection, alcoholic and non-alcoholic fatty liver disease. All these conditions can cause fibrosis and, subsequently, cirrhosis and HCC. Much evidence has been gathered demonstrating that HCC can arise from deregulated LPC proliferation and maturation during chronic liver injury in humans and in animal models of liver disease and carcinogenesis.

### 5.1 HCC: A clinically important end-stage complication of chronic liver disease

End-stage complications of chronic liver disease (cirrhosis and HCC) are the 9<sup>th</sup> commonest global cause of death and will remain so for at least the next 20 years. Of great concern is the prediction by the World Health Organisation that by 2030, deaths from HCC will for the first time exceed those from non-malignant complications of cirrhosis, such as liver failure and portal hypertension (Mathers *et al.*, 2006).

Most cases of HCC in the western world arise in the setting of established cirrhosis (Bruix & Sherman, 2005; Olsen *et al.*, 2010; Sherman, 2011). The median survival of untreated HCC is in the order of 6-16 months. In view of the poor survival in the absence of therapy, strategies have been implemented to reduce the incidence of HCC through immunisation to prevent chronic HBV infection and screening of high-risk groups (i.e. those with cirrhosis). Despite these approaches, we are still faced with an escalation in the number of cases and requirement for treatment (El-Serag & Mason, 1999; Bruix & Sherman, 2005; Llovet *et al.*, 2005; Mathers & Loncar, 2006; Llovet *et al.*, 2008).

Presently the treatment of choice for HCC is liver resection or orthotopic liver transplantation (OLT), either with or without adjunctive chemotherapy or non-surgical ablative therapy. Liver resection is the treatment of choice for HCC in non-cirrhotic livers, and accounts for 5% of HCC cases in western countries and 40% of cases in non-western countries. Patients with well compensated cirrhosis and who do not have portal hypertension may also be considered for resection, provided that lesions are confined to the liver and enough "functional reserve" of liver is retained to ensure survival of the patient (Bruix & Sherman, 2005; Llovet *et al.*, 2005). Currently, tumour size, number and vascular invasion are still the strongest predictors of survival with up to 70% of subjects surviving five years. Tumour recurrence complicates 70% of cases at five years, reflecting either intrahepatic metastases (true recurrences) or the development of *de novo* tumours (Llovet *et al.*, 2005). Based on comparative genomic hybridisation, DNA fingerprinting using loss of heterozygosity assays, or DNA microarray studies, it is estimated that just over half of recurrences correspond to intrahepatic metastases undetected by the time of resection, whereas less than half are *de novo* HCCs (Chen *et al.*, 2000; Finkelstein *et al.*, 2003; Ng *et al.*, 2003). OLT is indicated in individuals who fulfil the "Milan criteria": patients with a single HCC of up to five centimetres in size or up to three nodules not larger than three centimetres each. Strict adherence to these criteria results in 5-year survival of up to 70% with recurrence rates usually less than 15% (Bismuth *et al.*, 1993; Mazzaferro *et al.*, 1996; Llovet *et al.*, 2005; Mazzaferro *et al.*, 2009).

### 5.2 Pathogenesis of HCC: The emerging role of LPCs and CSCs

The activation and proliferation of LPCs during chronic liver injury is associated with an inflammatory response that involves activation of resident and recruited inflammatory cells (Fig. 4). These inflammatory cells initiate tissue regeneration by promoting the removal of cellular debris and by stimulating LPCs to proliferate through release of mitogenic growth factors and cytokines (Lowe *et al.*, 2003; Knight *et al.*, 2005). Whilst LPCs play an important role in normal liver repair processes, dysregulation of their proliferation and differentiation has been linked to fibrogenesis and carcinogenesis (Lowe *et al.*, 1999; Clouston *et al.*, 2005; Knight *et al.*, 2008; Ruddell *et al.*, 2009; Tirnitz-Parker *et al.*, 2010). Clear demonstration of a role for LPCs, and possibly CSCs, in HCC development was reported by Shachaf and

colleagues (Shachaf *et al.*, 2004). Inactivation of the Myc oncogene was sufficient to induce sustained regression of invasive HCC in a murine model. Tumour cells differentiated into hepatocytes and biliary epithelial cells. This process was associated with rapid loss of expression of the tumour marker  $\alpha$ -fetoprotein, increase in expression of liver cell markers CK8 and carcinoembryonic antigen, and in some cells the biliary LPC marker CK19. Many of the “reverted” tumour cells remained dormant as long as Myc remained inactivated; however, Myc reactivation immediately restored their neoplastic features. Using array comparative genomic hybridisation, Shachaf and coworkers confirmed that the dormant liver cells and the restored tumour retained the identical molecular signature and hence were clonally derived from the tumour cells. Thus, tumours have pluripotent capacity to differentiate into normal cellular lineages and tissue structures, while retaining their latent potential to become cancerous

Several other studies have confirmed a LPC phenotype in a substantial number of HCCs. Detailed immunophenotyping revealed that 28–50% of HCCs express markers of LPCs, such as CK7 and CK19. Histologically, these tumours consist of cells that have an intermediate phenotype between LPCs and mature hepatocytes. Furthermore, HCCs that express both hepatocyte and biliary cell markers such as albumin, CK7 and CK19, carry a significantly poorer prognosis and higher recurrence after surgical resection and liver transplantation (Roskams, 2006; Yao & Mishra, 2009). The “precursor-product” relationship between LPCs, CSCs and HCC is further strengthened by the observation that 55% of small dysplastic foci, which represent the earliest premalignant lesions, are comprised of LPCs and intermediate hepatocytes (Weinstein *et al.*, 2001). Finally, inhibition of the LPC response to liver injury using a broad range of pharmacological therapies such as interferon alpha 2b (Lim *et al.*, 2006), COX-II inhibitors (Davies *et al.*, 2006), or tyrosine kinase inhibitors (Knight *et al.*, 2008) is associated with a reduction in the severity of hepatic fibrosis and incidence of HCC. These observations provide more evidence in support of a critical role for LPCs and CSCs in the carcinogenic process. Collectively these studies suggest that anti-inflammatory agents may be useful therapeutically in reducing the incidence of liver cancer among patients with chronic liver pathologies.

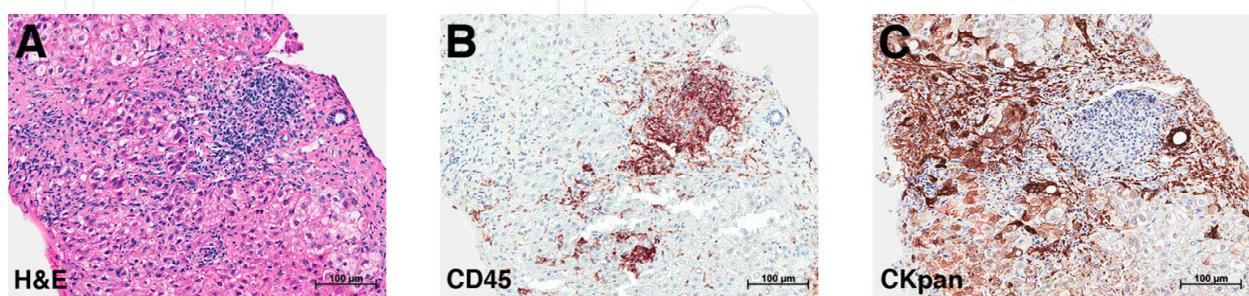


Fig. 4. Co-regulation of inflammatory response and LPC proliferation in hepatitis C patients. Haematoxylin and eosin (H&E) staining of a liver section from a hepatitis C virus-infected patient demonstrates disrupted liver architecture through infiltration and proliferation of small basophilic cells as well as steatotic changes in hepatocytes (A). Staining for the common leukocyte marker CD45 (B) and the biliary LPC marker CKpan (C) suggests co-regulation of the inflammatory response with the Ductular Reaction.

## 6. Conclusion

The cellular target of transformation leading to HCC is currently undefined. Potential candidates include the hepatocyte and the LPC and they need not be mutually exclusive. However, there is substantial circumstantial as well as some direct evidence implicating LPCs. This view would also be compatible with the increasingly popular theory of the stem cell basis of cancer. In the context of HCC, a variety of animal models, which induce chronic liver injury ultimately produce liver cancers and most of these pathologies display increased proliferation of LPCs. To conform to current views on carcinogenesis i.e. it is a rare event that affects a few cells and there are multiple stages in the process, it is necessary to hypothesise that a minority of LPCs are tumorigenic and that these have incurred the early genetic alterations that have initiated their progression to cancer. The challenge for future strategies to treat liver cancer is to identify these initiated LPCs and to show their direct link to HCC. This should be followed up with studies to elucidate progressive changes at the molecular level, which govern their behaviour and to exploit their vulnerability. Such knowledge will facilitate better diagnosis as well as treatment and prevention of HCC.

## 7. Acknowledgement

The authors would like to thank Ian Dickson for technical assistance with preparation of the figures.

## 8. References

- Akhurst, B., Croager, E. J., Farley-Roche, C. A., Ong, J. K., Dumble, M. L., Knight, B. & Yeoh, G. C. (2001). A modified choline-deficient, ethionine-supplemented diet protocol effectively induces oval cells in mouse liver. *Hepatology*, 34, 519-522.
- Akhurst, B., Matthews, V., Husk, K., Smyth, M. J., Abraham, L. J. & Yeoh, G. C. (2005). Differential lymphotoxin-beta and interferon gamma signaling during mouse liver regeneration induced by chronic and acute injury. *Hepatology*, 41, 327-335.
- Al-Hajj, M., Wicha, M. S., Benito-Hernandez, A., Morrison, S. J. & Clarke, M. F. (2003). Prospective identification of tumorigenic breast cancer cells. *Proceedings of the National Academy of Sciences U S A*, 100, 3983-3988.
- Alison, M., Golding, M., Lalani, E. N., Nagy, P., Thorgeirsson, S. & Sarraf, C. (1997). Wholesale hepatocytic differentiation in the rat from ductular oval cells, the progeny of biliary stem cells. *Journal of Hepatology*, 26, 343-352.
- Arber, N., Zajicek, G. & Ariel, I. (1988). The streaming liver. II. Hepatocyte life history. *Liver*, 8, 80-87.
- Bisgaard, H. C., Parmelee, D. C., Dunsford, H. A., Sechi, S. & Thorgeirsson, S. S. (1993). Keratin 14 protein in cultured nonparenchymal rat hepatic epithelial cells: characterization of keratin 14 and keratin 19 as antigens for the commonly used mouse monoclonal antibody OV-6. *Molecular Carcinogenesis*, 7, 60-66.
- Bismuth, H., Chiche, L., Adam, R., Castaing, D., Diamond, T. & Dennison, A. (1993). Liver resection versus transplantation for hepatocellular carcinoma in cirrhotic patients. *Annals of Surgery*, 218, 145-151.
- Bonnet, D. & Dick, J. E. (1997). Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nature Medicine*, 3, 730-737.

- Bralet, M. P., Branchereau, S., Brechot, C. & Ferry, N. (1994). Cell lineage study in the liver using retroviral mediated gene transfer. Evidence against the streaming of hepatocytes in normal liver. *American Journal of Pathology*, 144, 896-905.
- Bruix, J. & Sherman, M. (2005). Management of hepatocellular carcinoma. *Hepatology*, 42, 1208-1236.
- Bucher, N. L. R. & Malt, R. A. (1971) *Regeneration of the liver and kidney*. Little, Brown and Company, Boston.
- Cao, L., Zhou, Y., Zhai, B., Liao, J., Xu, W., Zhang, R., Li, J., Zhang, Y., Chen, L., Qian, H., Wu, M. & Yin, Z. (2011). Sphere-forming cell subpopulations with cancer stem cell properties in human hepatoma cell lines. *BMC Gastroenterology*, 11, 71.
- Chen, Y. J., Yeh, S. H., Chen, J. T., Wu, C. C., Hsu, M. T., Tsai, S. F., Chen, P. J. & Lin, C. H. (2000). Chromosomal changes and clonality relationship between primary and recurrent hepatocellular carcinoma. *Gastroenterology*, 119, 431-440.
- Chiba, T., Kita, K., Zheng, Y. W., Yokosuka, O., Saisho, H., Iwama, A., Nakauchi, H. & Taniguchi, H. (2006). Side population purified from hepatocellular carcinoma cells harbors cancer stem cell-like properties. *Hepatology*, 44, 240-251.
- Clouston, A. D., Powell, E. E., Walsh, M. J., Richardson, M. M., Demetris, A. J. & Jonsson, J. R. (2005). Fibrosis correlates with a ductular reaction in hepatitis C: roles of impaired replication, progenitor cells and steatosis. *Hepatology*, 41, 809-818.
- Colombo, F., Baldan, F., Mazzucchelli, S., Martin-Padura, I., Marighetti, P., Cattaneo, A., Foglieni, B., Spreafico, M., Gueneri, S., Baccarin, M., Bertolini, F., Rossi, G., Mazzaferro, V., Cadamuro, M., Maggioni, M., Agnelli, L., Rebulla, P., Prati, D. & Porretti, L. (2011). Evidence of distinct tumour-propagating cell populations with different properties in primary human hepatocellular carcinoma. *PLoS One*, 6, e21369.
- Cox, C. V., Evely, R. S., Oakhill, A., Pamphilon, D. H., Goulden, N. J. & Blair, A. (2004). Characterization of acute lymphoblastic leukemia progenitor cells. *Blood*, 104, 2919-2925.
- Cox, C. V., Martin, H. M., Kearns, P. R., Virgo, P., Evely, R. S. & Blair, A. (2007). Characterization of a progenitor cell population in childhood T-cell acute lymphoblastic leukemia. *Blood*, 109, 674-682.
- Dabeva, M. D. & Shafritz, D. A. (1993). Activation, proliferation, and differentiation of progenitor cells into hepatocytes in the D-galactosamine model of liver regeneration. *American Journal of Pathology*, 143, 1606-1620.
- Davies, R. A., Knight, B., Tian, Y. W., Yeoh, G. C. & Olynyk, J. K. (2006). Hepatic oval cell response to the choline-deficient, ethionine supplemented model of murine liver injury is attenuated by the administration of a cyclo-oxygenase 2 inhibitor. *Carcinogenesis*, 27, 1607-1616.
- Decker, K. & Keppler, D. (1972). Galactosamine induced liver injury. *Progress in Liver Diseases*, 4, 183-199.
- Dunsford, H. A., Karnasuta, C., Hunt, J. M. & Sell, S. (1989). Different lineages of chemically induced hepatocellular carcinoma in rats defined by monoclonal antibodies. *Cancer Research*, 49, 4894-4900.
- El-Serag, H. B. & Mason, A. C. (1999). Rising incidence of hepatocellular carcinoma in the United States. *New England Journal of Medicine*, 340, 745-750.

- Engelhardt, N. V., Factor, V. M., Medvinsky, A. L., Baranov, V. N., Lazareva, M. N. & Poltoranina, V. S. (1993). Common antigen of oval and biliary epithelial cells (A6) is a differentiation marker of epithelial and erythroid cell lineages in early development of the mouse. *Differentiation*, 55, 19-26.
- Evarts, R. P., Nagy, P., Marsden, E. & Thorgeirsson, S. S. (1987). In situ hybridization studies on expression of albumin and alpha-fetoprotein during the early stage of neoplastic transformation in rat liver. *Cancer Research*, 47, 5469-5475.
- Falkowski, O., An, H. J., Ianus, I. A., Chiriboga, L., Yee, H., West, A. B. & Theise, N. D. (2003). Regeneration of hepatocyte 'buds' in cirrhosis from intrabiliary stem cells. *Journal of Hepatology*, 39, 357-364.
- Farber, E. (1956a). Carcinoma of the liver in rats fed ethionine. *American Medical Association Archives of Pathology*, 62, 445-453.
- Farber, E. (1956b). Similarities in the sequence of early histological changes induced in the liver of the rat by ethionine, 2-acetyl-amino-fluorene, and 3'-methyl-4-dimethylaminoazobenzene. *Cancer Research*, 16, 142-148.
- Farber, E. (1967). Ethionine fatty liver. *Advances in Lipid Research*, 5, 119-183.
- Fausto, N. & Campbell, J. S. (2003). The role of hepatocytes and oval cells in liver regeneration and repopulation. *Mechanisms of Development*, 120, 117-130.
- Fausto, N., Campbell, J. S. & Riehle, K. J. (2006). Liver regeneration. *Hepatology*, 43, S45-53.
- Finkelstein, S. D., Marsh, W., Demetris, A. J., Swalsky, P. A., Sasatomi, E., Bonham, A., Subotin, M. & Dvorchik, I. (2003). Microdissection-based allelotyping discriminates de novo tumor from intrahepatic spread in hepatocellular carcinoma. *Hepatology*, 37, 871-879.
- Forbes, S. J., Russo, F. P., Rey, V., Burra, P., Ruge, M., Wright, N. A. & Alison, M. R. (2004). A significant proportion of myofibroblasts are of bone marrow origin in human liver fibrosis. *Gastroenterology*, 126, 955-963.
- Fujio, K., Evarts, R. P., Hu, Z., Marsden, E. R. & Thorgeirsson, S. S. (1994). Expression of stem cell factor and its receptor, c-kit, during liver regeneration from putative stem cells in adult rat. *Laboratory Investigation*, 70, 511-516.
- Fujio, K., Hu, Z., Evarts, R. P., Marsden, E. R., Niu, C. H. & Thorgeirsson, S. S. (1996). Coexpression of stem cell factor and c-kit in embryonic and adult liver. *Experimental Cell Research*, 224, 243-250.
- Furuyama, K., Kawaguchi, Y., Akiyama, H., Horiguchi, M., Kodama, S., Kuhara, T., Hosokawa, S., Elbahrawy, A., Soeda, T., Koizumi, M., Masui, T., Kawaguchi, M., Takaori, K., Doi, R., Nishi, E., Kakinoki, R., Deng, J. M., Behringer, R. R., Nakamura, T. & Uemoto, S. (2011). Continuous cell supply from a Sox9-expressing progenitor zone in adult liver, exocrine pancreas and intestine. *Nature Genetics*, 43, 34-41.
- Gebhardt, R. (1988). Different proliferative activity in vitro of periportal and perivenous hepatocytes. *Scandinavian Journal of Gastroenterology Supplement*, 151, 8-18.
- Ghoshal, A. K. & Farber, E. (1984). The induction of liver cancer by dietary deficiency of choline and methionine without added carcinogens. *Carcinogenesis*, 5, 1367-1370.
- Ginestier, C., Hur, M. H., Charafe-Jauffret, E., Monville, F., Dutcher, J., Brown, M., Jacquemier, J., Viens, P., Kleer, C. G., Liu, S., Schott, A., Hayes, D., Birnbaum, D., Wicha, M. S. & Dontu, G. (2007). ALDH1 is a marker of normal and malignant

- human mammary stem cells and a predictor of poor clinical outcome. *Cell Stem Cell*, 1, 555-567.
- Golding, M., Sarraf, C. E., Lalani, E. N., Anilkumar, T. V., Edwards, R. J., Nagy, P., Thorgeirsson, S. S. & Alison, M. R. (1995). Oval cell differentiation into hepatocytes in the acetylaminofluorene-treated regenerating rat liver. *Hepatology*, 22, 1243-1253.
- Grisham, J. W. (1962). A morphologic study of deoxyribonucleic acid synthesis and cell proliferation in regenerating rat liver; autoradiography with thymidine-H3. *Cancer Research*, 22, 842-849.
- Grisham, J. W. & Porta, E. A. (1964). Origin and Fate of Proliferated Hepatic Ductal Cells in the Rat: Electron Microscopic and Autoradiographic Studies. *Experimental and Molecular Pathology*, 86, 242-261.
- Haque, S., Haruna, Y., Saito, K., Nalesnik, M. A., Atillasoy, E., Thung, S. N. & Gerber, M. A. (1996). Identification of bipotential progenitor cells in human liver regeneration. *Laboratory Investigation*, 75, 699-705.
- Haraguchi, N., Utsunomiya, T., Inoue, H., Tanaka, F., Mimori, K., Barnard, G. F. & Mori, M. (2006). Characterization of a side population of cancer cells from human gastrointestinal system. *Stem Cells*, 24, 506-513.
- Higgins, G. M. & Anderson, R. M. (1931). Experimental pathology of the liver. I. Restoration of the liver of the white rat following partial surgical removal. *Archives of Pathology*, 12, 186-262.
- Holic, N., Suzuki, T., Corlu, A., Couchie, D., Chobert, M. N., Guguen-Guillouzo, C. & Laperche, Y. (2000). Differential expression of the rat gamma-glutamyl transpeptidase gene promoters along with differentiation of hepatoblasts into biliary or hepatocytic lineage. *American Journal of Pathology*, 157, 537-548.
- Ishii, T., Yasuchika, K., Suemori, H., Nakatsuji, N., Ikai, I. & Uemoto, S. (2010). Alpha-fetoprotein producing cells act as cancer progenitor cells in human cholangiocarcinoma. *Cancer Letters*, 294, 25-34.
- Jakubowski, A., Ambrose, C., Parr, M., Lincecum, J. M., Wang, M. Z., Zheng, T. S., Browning, B., Michaelson, J. S., Baetscher, M., Wang, B., Bissell, D. M. & Burkly, L. C. (2005). TWEAK induces liver progenitor cell proliferation. *Journal of Clinical Investigation*, 115, 2330-2340.
- Jang, Y. Y., Collector, M. I., Baylin, S. B., Diehl, A. M. & Sharkis, S. J. (2004). Hematopoietic stem cells convert into liver cells within days without fusion. *Nature Cell Biology*, 6, 532-539.
- Jensen, C. H., Jauho, E. I., Santoni-Rugiu, E., Holmskov, U., Teisner, B., Tygstrup, N. & Bisgaard, H. C. (2004). Transit-amplifying ductular (oval) cells and their hepatocytic progeny are characterized by a novel and distinctive expression of delta-like protein/preadipocyte factor 1/fetal antigen 1. *American Journal of Pathology*, 164, 1347-1359.
- Jiang, Y., Jahagirdar, B. N., Reinhardt, R. L., Schwartz, R. E., Keene, C. D., Ortiz-Gonzalez, X. R., Reyes, M., Lenvik, T., Lund, T., Blackstad, M., Du, J., Aldrich, S., Lisberg, A., Low, W. C., Largaespada, D. A. & Verfaillie, C. M. (2002). Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature*, 418, 41-49.
- Kawasaki, S., Makuuchi, M., Ishizone, S., Matsunami, H., Terada, M. & Kawarazaki, H. (1992). Liver regeneration in recipients and donors after transplantation. *Lancet*, 339, 580-581.

- Kawasaki, Y., Omori, Y., Li, Q., Nishikawa, Y., Yoshioka, T., Yoshida, M., Ishikawa, K. & Enomoto, K. (2011). Cytoplasmic accumulation of connexin32 expands cancer stem cell population in human HuH7 hepatoma cells by enhancing its self-renewal. *International Journal of Cancer*, 128, 51-62.
- Kennedy, S., Rettinger, S., Flye, M. W. & Ponder, K. P. (1995). Experiments in transgenic mice show that hepatocytes are the source for postnatal liver growth and do not stream. *Hepatology*, 22, 160-168.
- Kinosita, R. (1937). Studies on the cancerogenic chemical substances. *Transactions of the Japanese Society for Pathology*, 27, 665-727.
- Knight, B., Yeoh, G. C., Husk, K. L., Ly, T., Abraham, L. J., Yu, C., Rhim, J. A. & Fausto, N. (2000). Impaired preneoplastic changes and liver tumor formation in tumor necrosis factor receptor type 1 knockout mice. *Journal of Experimental Medicine*, 192, 1809-1818.
- Knight, B., Matthews, V. B., Akhurst, B., Croager, E. J., Klinken, E., Abraham, L. J., Olynyk, J. K. & Yeoh, G. (2005). Liver inflammation and cytokine production, but not acute phase protein synthesis, accompany the adult liver progenitor (oval) cell response to chronic liver injury. *Immunology and Cell Biology*, 83, 364-374.
- Knight, B. & Yeoh, G. C. (2005). TNF/LTalpha double knockout mice display abnormal inflammatory and regenerative responses to acute and chronic liver injury. *Cell and Tissue Research*, 319, 61-70.
- Knight, B., Lim, R., Yeoh, G. C. & Olynyk, J. K. (2007). Interferon-gamma exacerbates liver damage, the hepatic progenitor cell response and fibrosis in a mouse model of chronic liver injury. *Journal of Hepatology*, 47, 826-833.
- Knight, B., Tirnitz-Parker, J. E. & Olynyk, J. K. (2008). C-kit inhibition by imatinib mesylate attenuates progenitor cell expansion and inhibits liver tumor formation in mice. *Gastroenterology*, 135, 969-979, 979 e961.
- Krause, D. S., Theise, N. D., Collector, M. I., Henegariu, O., Hwang, S., Gardner, R., Neutzel, S. & Sharkis, S. J. (2001). Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell*, 105, 369-377.
- Lagasse, E., Connors, H., Al-Dhalimy, M., Reitsma, M., Dohse, M., Osborne, L., Wang, X., Finegold, M., Weissman, I. L. & Grompe, M. (2000). Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. *Nature Medicine*, 6, 1229-1234.
- Lee, K. P., Lee, J. H., Kim, T. S., Kim, T. H., Park, H. D., Byun, J. S., Kim, M. C., Jeong, W. I., Calvisi, D. F., Kim, J. M. & Lim, D. S. (2010). The Hippo-Salvador pathway restrains hepatic oval cell proliferation, liver size, and liver tumorigenesis. *Proceedings of the National Academy of Sciences U S A*, 107, 8248-8253.
- Lee, T. K., Castilho, A., Cheung, V. C., Tang, K. H., Ma, S. & Ng, I. O. (2011). CD24(+) liver tumor-initiating cells drive self-renewal and tumor initiation through STAT3-mediated NANOG regulation. *Cell Stem Cell*, 9, 50-63.
- Leite, A. R., Correa-Giannella, M. L., D'Agli, M. L., Fortes, M. A., Vegas, V. M. & Giannella-Neto, D. (2007). Fibronectin and laminin induce expression of islet cell markers in hepatic oval cells in culture. *Cell and Tissue Research*, 327, 529-537.
- Lemire, J. M., Shiojiri, N. & Fausto, N. (1991). Oval cell proliferation and the origin of small hepatocytes in liver injury induced by D-galactosamine. *American Journal of Pathology*, 139, 535-552.

- Lenzi, R., Liu, M. H., Tarsetti, F., Slott, P. A., Alpini, G., Zhai, W. R., Paronetto, F., Lenzen, R. & Tavoloni, N. (1992). Histogenesis of bile duct-like cells proliferating during ethionine hepatocarcinogenesis. Evidence for a biliary epithelial nature of oval cells. *Laboratory Investigation*, 66, 390-402.
- Lim, R., Knight, B., Patel, K., McHutchison, J. G., Yeoh, G. C. & Olynyk, J. K. (2006). Antiproliferative effects of interferon alpha on hepatic progenitor cells in vitro and in vivo. *Hepatology*, 43, 1074-1083.
- Llovet, J. M., Schwartz, M. & Mazzaferro, V. (2005). Resection and liver transplantation for hepatocellular carcinoma. *Seminars in Liver Disease*, 25, 181-200.
- Llovet, J. M., Ricci, S., Mazzaferro, V., Hilgard, P., Gane, E., Blanc, J. F., de Oliveira, A. C., Santoro, A., Raoul, J. L., Forner, A., Schwartz, M., Porta, C., Zeuzem, S., Bolondi, L., Greten, T. F., Galle, P. R., Seitz, J. F., Borbath, I., Haussinger, D., Giannaris, T., Shan, M., Moscovici, M., Voliotis, D. & Bruix, J. (2008). Sorafenib in advanced hepatocellular carcinoma. *New England Journal of Medicine*, 359, 378-390.
- Locker, J., Reddy, T. V. & Lombardi, B. (1986). DNA methylation and hepatocarcinogenesis in rats fed a choline-devoid diet. *Carcinogenesis*, 7, 1309-1312.
- Lombardi, B., Ugazio, G. & Raick, A. N. (1966). Choline-deficiency fatty liver: relation of plasma phospholipids to liver triglycerides. *American Journal of Physiology*, 210, 31-36.
- Lombardi, B., Pani, P. & Schlunk, F. F. (1968). Choline-deficiency fatty liver: impaired release of hepatic triglycerides. *Journal of Lipid Research*, 9, 437-446.
- Lowes, K. N., Brennan, B. A., Yeoh, G. C. & Olynyk, J. K. (1999). Oval cell numbers in human chronic liver diseases are directly related to disease severity. *American Journal of Pathology*, 154, 537-541.
- Lowes, K. N., Croager, E. J., Olynyk, J. K., Abraham, L. J. & Yeoh, G. C. (2003). Oval cell-mediated liver regeneration: Role of cytokines and growth factors. *Journal of Gastroenterology and Hepatology*, 18, 4-12.
- Ma, S., Chan, K. W., Hu, L., Lee, T. K., Wo, J. Y., Ng, I. O., Zheng, B. J. & Guan, X. Y. (2007). Identification and characterization of tumorigenic liver cancer stem/progenitor cells. *Gastroenterology*, 132, 2542-2556.
- Ma, S., Chan, K. W., Lee, T. K., Tang, K. H., Wo, J. Y., Zheng, B. J. & Guan, X. Y. (2008). Aldehyde dehydrogenase discriminates the CD133 liver cancer stem cell populations. *Molecular Cancer Research*, 6, 1146-1153.
- Marquardt, J. U., Raggi, C., Andersen, J. B., Seo, D., Avital, I., Geller, D., Lee, Y. H., Kitade, M., Holczbauer, A., Gillen, M. C., Conner, E. A., Factor, V. M. & Thorgeirsson, S. S. (2011). Human hepatic cancer stem cells are characterized by common stemness traits and diverse oncogenic pathways. *Hepatology*, 54, 1031-1042.
- Mathers, C. D. & Loncar, D. (2006). Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Medicine*, 3, e442.
- Mazzaferro, V., Regalia, E., Doci, R., Andreola, S., Pulvirenti, A., Bozzetti, F., Montalto, F., Ammatuna, M., Morabito, A. & Gennari, L. (1996). Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *New England Journal of Medicine*, 334, 693-699.
- Mazzaferro, V., Llovet, J. M., Miceli, R., Bhoori, S., Schiavo, M., Mariani, L., Camerini, T., Roayaie, S., Schwartz, M. E., Grazi, G. L., Adam, R., Neuhaus, P., Salizzoni, M., Bruix, J., Forner, A., De Carlis, L., Cillo, U., Burroughs, A. K., Troisi, R., Rossi, M.,

- Gerunda, G. E., Lerut, J., Belghiti, J., Boin, I., Gugenheim, J., Rochling, F., Van Hoek, B. & Majno, P. (2009). Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. *Lancet Oncology*, 10, 35-43.
- Michalopoulos, G. K. & DeFrances, M. C. (1997). Liver regeneration. *Science*, 276, 60-66.
- Ng, I. O., Guan, X. Y., Poon, R. T., Fan, S. T. & Lee, J. M. (2003). Determination of the molecular relationship between multiple tumour nodules in hepatocellular carcinoma differentiates multicentric origin from intrahepatic metastasis. *Journal of Pathology*, 199, 345-353.
- Ng, Y. K. & Iannaccone, P. M. (1992). Fractal geometry of mosaic pattern demonstrates liver regeneration is a self-similar process. *Developmental Biology*, 151, 419-430.
- Okabe, M., Tsukahara, Y., Tanaka, M., Suzuki, K., Saito, S., Kamiya, Y., Tsujimura, T., Nakamura, K. & Miyajima, A. (2009). Potential hepatic stem cells reside in EpCAM+ cells of normal and injured mouse liver. *Development*, 136, 1951-1960.
- Oliva, J., French, B. A., Qing, X. & French, S. W. (2010). The identification of stem cells in human liver diseases and hepatocellular carcinoma. *Experimental and Molecular Pathology*, 88, 331-340.
- Olsen, S. K., Brown, R. S. & Siegel, A. B. (2010). Hepatocellular carcinoma: review of current treatment with a focus on targeted molecular therapies. *Therapeutic Advances in Gastroenterology*, 3, 55-66.
- Omori, N., Omori, M., Evarts, R. P., Teramoto, T., Miller, M. J., Hoang, T. N. & Thorgeirsson, S. S. (1997). Partial cloning of rat CD34 cDNA and expression during stem cell-dependent liver regeneration in the adult rat. *Hepatology*, 26, 720-727.
- Overturf, K., al-Dhalimy, M., Ou, C. N., Finegold, M. & Grompe, M. (1997). Serial transplantation reveals the stem-cell-like regenerative potential of adult mouse hepatocytes. *American Journal of Pathology*, 151, 1273-1280.
- Paku, S., Schnur, J., Nagy, P. & Thorgeirsson, S. S. (2001). Origin and structural evolution of the early proliferating oval cells in rat liver. *American Journal of Pathology*, 158, 1313-1323.
- Paku, S., Nagy, P., Kopper, L. & Thorgeirsson, S. S. (2004). 2-acetylaminofluorene dose-dependent differentiation of rat oval cells into hepatocytes: confocal and electron microscopic studies. *Hepatology*, 39, 1353-1361.
- Paradis, V., Youssef, N., Dargere, D., Ba, N., Bonvoust, F., Deschatrette, J. & Bedossa, P. (2001). Replicative senescence in normal liver, chronic hepatitis C, and hepatocellular carcinomas. *Human Pathology*, 32, 327-332.
- Petersen, B. E., Goff, J. P., Greenberger, J. S. & Michalopoulos, G. K. (1998a). Hepatic oval cells express the hematopoietic stem cell marker Thy-1 in the rat. *Hepatology*, 27, 433-445.
- Petersen, B. E., Zajac, V. F. & Michalopoulos, G. K. (1998b). Hepatic oval cell activation in response to injury following chemically induced periportal or pericentral damage in rats. *Hepatology*, 27, 1030-1038.
- Petersen, B. E., Bowen, W. C., Patrene, K. D., Mars, W. M., Sullivan, A. K., Murase, N., Boggs, S. S., Greenberger, J. S. & Goff, J. P. (1999). Bone marrow as a potential source of hepatic oval cells. *Science*, 284, 1168-1170.

- Petersen, B. E., Grossbard, B., Hatch, H., Pi, L., Deng, J. & Scott, E. W. (2003). Mouse A6-positive hepatic oval cells also express several hematopoietic stem cell markers. *Hepatology*, 37, 632-640.
- Ponder, K. P. (1996). Analysis of liver development, regeneration, and carcinogenesis by genetic marking studies. *Faseb Journal*, 10, 673-682.
- Qiu, Q., Hernandez, J. C., Dean, A. M., Rao, P. H. & Darlington, G. J. (2011). CD24-positive cells from normal adult mouse liver are hepatocyte progenitor cells. *Stem Cells and Development*, 20, 2177-2188.
- Rabes, H. M. (1976). Kinetics of hepatocellular proliferation after partial resection of the liver. *Progress in Liver Diseases*, 5, 83-99.
- Rhim, J. A., Sandgren, E. P., Degen, J. L., Palmiter, R. D. & Brinster, R. L. (1994). Replacement of diseased mouse liver by hepatic cell transplantation. *Science*, 263, 1149-1152.
- Rhim, J. A., Sandgren, E. P., Palmiter, R. D. & Brinster, R. L. (1995). Complete reconstitution of mouse liver with xenogeneic hepatocytes. *Proceedings of the National Academy of Sciences U S A*, 92, 4942-4946.
- Rogler, L. E. (1997). Selective bipotential differentiation of mouse embryonic hepatoblasts in vitro. *American Journal of Pathology*, 150, 591-602.
- Roskams, T. & Desmet, V. (1998). Ductular reaction and its diagnostic significance. *Seminars in Diagnostic Pathology*, 15, 259-269.
- Roskams, T., Yang, S. Q., Koteish, A., Durnez, A., DeVos, R., Huang, X., Achten, R., Verslype, C. & Diehl, A. M. (2003a). Oxidative stress and oval cell accumulation in mice and humans with alcoholic and nonalcoholic fatty liver disease. *American Journal of Pathology*, 163, 1301-1311.
- Roskams, T. A., Libbrecht, L. & Desmet, V. J. (2003b). Progenitor cells in diseased human liver. *Seminars in Liver Disease*, 23, 385-396.
- Roskams, T. (2006). Liver stem cells and their implication in hepatocellular and cholangiocarcinoma. *Oncogene*, 25, 3818-3822.
- Rountree, C. B., Mishra, L. & Willenbring, H. (2012). Stem cells in liver diseases and cancer: Recent advances on the path to new therapies. *Hepatology*, 55, 298-306.
- Ruddell, R. G., Knight, B., Tirnitz-Parker, J. E., Akhurst, B., Summerville, L., Subramaniam, V. N., Olynyk, J. K. & Ramm, G. A. (2009). Lymphotoxin-beta receptor signaling regulates hepatic stellate cell function and wound healing in a murine model of chronic liver injury. *Hepatology*, 49, 227-239.
- Sarraf, C., Lalani, E. N., Golding, M., Anilkumar, T. V., Poulsom, R. & Alison, M. (1994). Cell behavior in the acetylaminofluorene-treated regenerating rat liver. Light and electron microscopic observations. *American Journal of Pathology*, 145, 1114-1126.
- Sasaki, K., Kon, J., Mizuguchi, T., Chen, Q., Ooe, H., Oshima, H., Hirata, K. & Mitaka, T. (2008). Proliferation of hepatocyte progenitor cells isolated from adult human livers in serum-free medium. *Cell Transplantation*, 17, 1221-1230.
- Schmelzer, E., Zhang, L., Bruce, A., Wauthier, E., Ludlow, J., Yao, H. L., Moss, N., Melhem, A., McClelland, R., Turner, W., Kulik, M., Sherwood, S., Tallheden, T., Cheng, N., Furth, M. E. & Reid, L. M. (2007). Human hepatic stem cells from fetal and postnatal donors. *Journal of Experimental Medicine*, 204, 1973-1987.

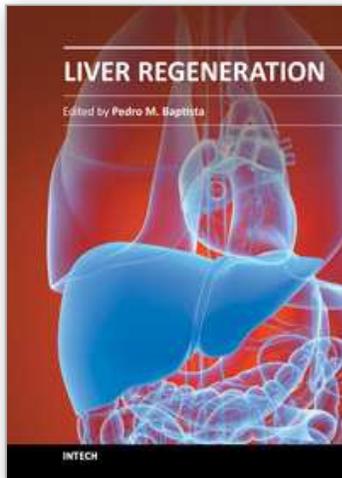
- Schwartz, R. E., Reyes, M., Koodie, L., Jiang, Y., Blackstad, M., Lund, T., Lenvik, T., Johnson, S., Hu, W. S. & Verfaillie, C. M. (2002). Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. *Journal of Clinical Investigation*, 109, 1291-1302.
- Sell, S. (1978). Distribution of alpha-fetoprotein- and albumin-containing cells in the livers of Fischer rats fed four cycles of N-2-fluorenylacetamide. *Cancer Research*, 38, 3107-3113.
- Sell, S. (2001). Heterogeneity and plasticity of hepatocyte lineage cells. *Hepatology*, 33, 738-750.
- Shachaf, C. M., Kopelman, A. M., Arvanitis, C., Karlsson, A., Beer, S., Mandl, S., Bachmann, M. H., Borowsky, A. D., Ruebner, B., Cardiff, R. D., Yang, Q., Bishop, J. M., Contag, C. H. & Felsher, D. W. (2004). MYC inactivation uncovers pluripotent differentiation and tumour dormancy in hepatocellular cancer. *Nature*, 431, 1112-1117.
- Sherman, M. (2011). Modern approach to hepatocellular carcinoma. *Current Gastroenterology Reports*, 13, 49-55.
- Shinozuka, H., Lombardi, B., Sell, S. & Iammarino, R. M. (1978a). Enhancement of DL-ethionine-induced liver carcinogenesis in rats fed a choline-devoid diet. *Journal of the National Cancer Institute*, 61, 813-817.
- Shinozuka, H., Lombardi, B., Sell, S. & Iammarino, R. M. (1978b). Early histological and functional alterations of ethionine liver carcinogenesis in rats fed a choline-deficient diet. *Cancer Research*, 38, 1092-1098.
- Sidransky, H. & Verney, E. (1969). Influence of ethionine on choline-deficiency fatty liver. *Journal of Nutrition*, 97, 419-430.
- Sigal, S. H., Brill, S., Fiorino, A. S. & Reid, L. M. (1992). The liver as a stem cell and lineage system. *American Journal of Physiology*, 263, G139-148.
- Simpson, G. E. & Finck, E. S. (1963). The Pattern of Regeneration of Rat Liver after Repeated Partial Hepatectomies. *Journal of Pathology and Bacteriology*, 86, 361-370.
- Singh, S. K., Clarke, I. D., Terasaki, M., Bonn, V. E., Hawkins, C., Squire, J. & Dirks, P. B. (2003). Identification of a cancer stem cell in human brain tumours. *Cancer Research* 63, 5821-5828.
- Singh, S. K., Hawkins, C., Clarke, I. D., Squire, J. A., Bayani, J., Hide, T., Henkelman, R. M., Cusimano, M. D. & Dirks, P. B. (2004). Identification of human brain tumour initiating cells. *Nature*, 432, 396-401.
- Smith, P. G., Tee, L. B. & Yeoh, G. C. (1996). Appearance of oval cells in the liver of rats after long-term exposure to ethanol. *Hepatology*, 23, 145-154.
- Solt, D. & Farber, E. (1976). New principle for the analysis of chemical carcinogenesis. *Nature*, 263, 701-703.
- Starzl, T. E., Fung, J., Tzakis, A., Todo, S., Demetris, A. J., Marino, I. R., Doyle, H., Zeevi, A., Warty, V., Michaels, M., Kusne, S., Rudert, W. A., & Trucco, M. (1993). Baboon-to-human liver transplantation. *Lancet*, 341, 65-71.
- Suetsugu, A., Nagaki, M., Aoki, H., Motohashi, T., Kunisada, T. & Moriwaki, H. (2006). Characterization of CD133+ hepatocellular carcinoma cells as cancer stem/progenitor cells. *Biochemical and Biophysical Research Communications*, 351, 820-824.

- Suzuki, A., Sekiya, S., Onishi, M., Oshima, N., Kiyonari, H., Nakauchi, H. & Taniguchi, H. (2008). Flow cytometric isolation and clonal identification of self-renewing bipotent hepatic progenitor cells in adult mouse liver. *Hepatology*, 48, 1964-1978.
- Tatematsu, M., Ho, R. H., Kaku, T., Ekem, J. K. & Farber, E. (1984). Studies on the proliferation and fate of oval cells in the liver of rats treated with 2-acetylaminofluorene and partial hepatectomy. *American Journal of Pathology*, 114, 418-430.
- Tatematsu, M., Kaku, T., Medline, A. & Farber, E. (1985). Intestinal metaplasia as a common option of oval cells in relation to cholangiofibrosis in liver of rats exposed to 2-acetylaminofluorene. *Laboratory Investigation*, 52, 354-362.
- Tee, L. B., Smith, P. G. & Yeoh, G. C. (1992). Expression of alpha, mu and pi class glutathione S-transferases in oval and ductal cells in liver of rats placed on a choline-deficient, ethionine-supplemented diet. *Carcinogenesis*, 13, 1879-1885.
- Theise, N. D., Saxena, R., Portmann, B. C., Thung, S. N., Yee, H., Chiriboga, L., Kumar, A. & Crawford, J. M. (1999). The canals of Hering and hepatic stem cells in humans. *Hepatology*, 30, 1425-1433.
- Theise, N. D., Badve, S., Saxena, R., Henegariu, O., Sell, S., Crawford, J. M. & Krause, D. S. (2000a). Derivation of hepatocytes from bone marrow cells in mice after radiation-induced myeloablation. *Hepatology*, 31, 235-240.
- Theise, N. D., Nimmakayalu, M., Gardner, R., Illei, P. B., Morgan, G., Teperman, L., Henegariu, O. & Krause, D. S. (2000b). Liver from bone marrow in humans. *Hepatology*, 32, 11-16.
- Thorgeirsson, S. S. & Grisham, J. W. (2006). Hematopoietic cells as hepatocyte stem cells: a critical review of the evidence. *Hepatology*, 43, 2-8.
- Thurman, R. G. & Kauffman, F. C. (1985). Sublobular compartmentation of pharmacologic events (SCOPE): metabolic fluxes in periportal and pericentral regions of the liver lobule. *Hepatology*, 5, 144-151.
- Tian, Y. W., Smith, P. G. & Yeoh, G. C. (1997). The oval-shaped cell as a candidate for a liver stem cell in embryonic, neonatal and precancerous liver: identification based on morphology and immunohistochemical staining for albumin and pyruvate kinase isoenzyme expression. *Histochemistry and Cell Biology*, 107, 243-250.
- Tirnitz-Parker, J. E., Tonkin, J. N., Knight, B., Olynyk, J. K. & Yeoh, G. C. (2007). Isolation, culture and immortalisation of hepatic oval cells from adult mice fed a choline-deficient, ethionine-supplemented diet. *International Journal of Biochemistry & Cell Biology*, 39, 2226-2239.
- Tirnitz-Parker, J. E., Viebahn, C. S., Jakubowski, A., Klopčič, B. R., Olynyk, J. K., Yeoh, G. C. & Knight, B. (2010). Tumor necrosis factor-like weak inducer of apoptosis is a mitogen for liver progenitor cells. *Hepatology*, 52, 291-302.
- Tonkin, J. N., Knight, B., Curtis, D., Abraham, L. J. & Yeoh, G. C. (2008). Bone marrow cells play only a very minor role in chronic liver regeneration induced by a choline-deficient, ethionine-supplemented diet. *Stem Cell Research*, 1, 195-204.
- Ueberham, E., Aigner, T., Ueberham, U. & Gebhardt, R. (2007). E-cadherin as a reliable cell surface marker for the identification of liver specific stem cells. *Journal of Molecular Histology*, 38, 359-368.
- Van Hul, N. K., Abarca-Quinones, J., Sempoux, C., Horsmans, Y. & Leclercq, I. A. (2009). Relation between liver progenitor cell expansion and extracellular matrix

- deposition in a CDE-induced murine model of chronic liver injury. *Hepatology*, 49, 1625-1635.
- Vassilopoulos, G., Wang, P. R. & Russell, D. W. (2003). Transplanted bone marrow regenerates liver by cell fusion. *Nature*, 422, 901-904.
- Wang, C., Yang, W., Yan, H. X., Luo, T., Zhang, J., Tang, L., Wu, F. Q., Zhang, H. L., Yu, L. X., Zheng, L. Y., Li, Y. Q., Dong, W., He, Y. Q., Liu, Q., Zou, S. S., Lin, Y., Hu, L., Li, Z., Wu, M. C. & Wang, H. Y. (2012). Hepatitis B virus X (HBx) induces tumorigenicity of hepatic progenitor cells in 3,5-diethoxycarbonyl-1,4-dihydrocollidine-treated HBx transgenic mice. *Hepatology*, 55, 108-120.
- Wang, X., Montini, E., Al-Dhalimy, M., Lagasse, E., Finegold, M. & Grompe, M. (2002). Kinetics of liver repopulation after bone marrow transplantation. *American Journal of Pathology*, 161, 565-574.
- Wang, X., Foster, M., Al-Dhalimy, M., Lagasse, E., Finegold, M. & Grompe, M. (2003a). The origin and liver repopulating capacity of murine oval cells. *Proceedings of the National Academy of Sciences U S A*, 100 Suppl 1, 11881-11888.
- Wang, X., Willenbring, H., Akkari, Y., Torimaru, Y., Foster, M., Al-Dhalimy, M., Lagasse, E., Finegold, M., Olson, S. & Grompe, M. (2003b). Cell fusion is the principal source of bone-marrow-derived hepatocytes. *Nature*, 422, 897-901.
- Weinstein, M., Monga, S. P., Liu, Y., Brodie, S. G., Tang, Y., Li, C., Mishra, L. & Deng, C. X. (2001). Smad proteins and hepatocyte growth factor control parallel regulatory pathways that converge on beta1-integrin to promote normal liver development. *Molecular and Cell Biology*, 21, 5122-5131.
- Wiemann, S. U., Satyanarayana, A., Tsahuridu, M., Tillmann, H. L., Zender, L., Klempnauer, J., Flemming, P., Franco, S., Blasco, M. A., Manns, M. P. & Rudolph, K. L. (2002). Hepatocyte telomere shortening and senescence are general markers of human liver cirrhosis. *Faseb Journal*, 16, 935-942.
- Wilson, J. W. & Leduc, E. H. (1958). Role of cholangioles in restoration of the liver of the mouse after dietary injury. *Journal of Pathology and Bacteriology*, 76, 441-449.
- Yamashita, T., Budhu, A., Forgues, M. & Wang, X. W. (2007). Activation of hepatic stem cell marker EpCAM by Wnt-beta-catenin signaling in hepatocellular carcinoma. *Cancer Research*, 67, 10831-10839.
- Yamashita, T., Ji, J., Budhu, A., Forgues, M., Yang, W., Wang, H. Y., Jia, H., Ye, Q., Qin, L. X., Wauthier, E., Reid, L. M., Minato, H., Honda, M., Kaneko, S., Tang, Z. Y. & Wang, X. W. (2009). EpCAM-positive hepatocellular carcinoma cells are tumor-initiating cells with stem/progenitor cell features. *Gastroenterology*, 136, 1012-1024.
- Yamashita, T., Honda, M., Nio, K., Nakamoto, Y., Yamashita, T., Takamura, H., Tani, T., Zen, Y. & Kaneko, S. (2010). Oncostatin m renders epithelial cell adhesion molecule-positive liver cancer stem cells sensitive to 5-Fluorouracil by inducing hepatocytic differentiation. *Cancer Research*, 70, 4687-4697.
- Yanai, H., Nakamura, K., Hijioka, S., Kamei, A., Ikari, T., Ishikawa, Y., Shinozaki, E., Mizunuma, N., Hatake, K. & Miyajima, A. (2010). Dlk-1, a cell surface antigen on foetal hepatic stem/progenitor cells, is expressed in hepatocellular, colon, pancreas and breast carcinomas at a high frequency. *Journal of Biochemistry*, 148, 85-92.
- Yang, L., Li, S., Hatch, H., Ahrens, K., Cornelius, J. G., Petersen, B. E. & Peck, A. B. (2002). In vitro trans-differentiation of adult hepatic stem cells into pancreatic endocrine

- hormone-producing cells. *Proceedings of the National Academy of Sciences U S A*, 99, 8078-8083.
- Yao, Z. & Mishra, L. (2009). Cancer stem cells and hepatocellular carcinoma. *Cancer Biology & Therapy*, 8, 1691-1698.
- Yokoyama, S., Sells, M. A., Reddy, T. V. & Lombardi, B. (1985). Hepatocarcinogenic and promoting action of a choline-devoid diet in the rat. *Cancer Research*, 45, 2834-2842.
- Yoon, S. M., Gerasimidou, D., Kuwahara, R., Hytioglou, P., Yoo, J. E., Park, Y. N. & Theise, N. D. (2011). Epithelial cell adhesion molecule (EpCAM) marks hepatocytes newly derived from stem/progenitor cells in humans. *Hepatology*, 53, 964-973.
- You, H., Ding, W. & Rountree, C. B. (2010). Epigenetic regulation of cancer stem cell marker CD133 by transforming growth factor-beta. *Hepatology*, 51, 1635-1644.
- Zajicek, G., Oren, R. & Weinreb, M., Jr. (1985). The streaming liver. *Liver*, 5, 293-300.
- Zhang, A., London, R., Schulz, F. M., Giguere-Simmonds, P. W., Delriviere, L., Chandraratana, H., Hardy, K., Zheng, S., Olynyk, J. K. & Yeoh, G. (2010). Human liver progenitor cell lines are readily established from non-tumorous tissue adjacent to hepatocellular carcinoma. *Stem Cells and Development*, 19, 1277-1284.
- Zhang, M. & Thorgeirsson, S. S. (1994). Modulation of connexins during differentiation of oval cells into hepatocytes. *Experimental Cell Research*, 213, 37-42.

IntechOpen



## **Liver Regeneration**

Edited by PhD. Pedro Baptista

ISBN 978-953-51-0622-7

Hard cover, 252 pages

**Publisher** InTech

**Published online** 16, May, 2012

**Published in print edition** May, 2012

Doctors and scientists have been aware of the "phenomenon" 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.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Janina E.E. Tirnitz-Parker, George C.T. Yeoh and John K. Olynyk (2012). Liver Progenitor Cells, Cancer Stem Cells and Hepatocellular Carcinoma, Liver Regeneration, PhD. Pedro Baptista (Ed.), ISBN: 978-953-51-0622-7, InTech, Available from: <http://www.intechopen.com/books/liver-regeneration/liver-progenitor-cells-cancer-stem-cells-and-hepatocellular-carcinoma>

**INTECH**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen