

LIVER CARCINOGENESIS

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Abstract

Hepatocellular carcinoma occurs most commonly in the setting of cirrhosis, where the annual rate of cancer development approximates 3-7%. Most cases arise in the setting of impaired liver regeneration combined with chronic inflammation and fibrosis. Liver progenitor cells play an important role in cell renewal processes in the liver in the setting of chronic injury and have recently emerged as potential candidates in the carcinogenic pathway. There are two main hypotheses which have been proposed to explain hepatocellular carcinogenesis, namely the de-differentiation and the maturation arrest hypotheses. Understanding the carcinogenic pathways and the role of liver progenitor cells will provide greater understanding and novel approaches to preventative strategies.

Hepatic tissue renewal

Compared to intestine and skin, where tissue is renewed within days or weeks respectively, the healthy liver has a very slow cell turnover rate and hepatocytes are considered to be in the quiescent, non-proliferative G₀ phase of the cell cycle. It has been estimated that only one in 20,000 to 40,000 cells divides at any time with an average hepatocyte life span of 200 to 300 days.¹ However, in response to injury, the liver has a remarkable potential to regenerate itself. Replication of the remaining healthy hepatocytes is the most efficient way to restore liver mass during normal tissue renewal and repair. If this process is impaired due to chronic liver injury, such as occurs in most chronic liver diseases, the liver relies on restoration of cellular mass through the activation, expansion and differentiation of stem-like cells termed liver progenitor cells (LPCs).²⁻⁵

Liver progenitor cells

Early animal studies identified small ovoid cells, which appeared periportal and proliferated readily following chronic or carcinogenic injury.⁶ Many experimental models involving toxins and carcinogens, alone or in combination with other surgical or dietary regimes,⁷⁻¹⁰ have since facilitated the study of these cells, which are now widely accepted to represent adult liver progenitor cells, the progeny of hepatic stem cells.¹¹ Evidence from experiments showing that LPCs always emerge from periportal liver zones and the fact that selective periportal damage inhibits the LPC response, has led to the conclusion that the precursor cell likely resides somewhere in the vicinity of the canal of Hering.¹² The canal of Hering is a channel partly lined by hepatocytes and partly by cholangiocytes. It represents the anatomic and physiological link between the intralobular canalicular system and the biliary tree.^{13,14} Undetectable in healthy tissue, LPCs are detected periportal following chronic insult. They proliferate and migrate into the parenchyma and eventually differentiate into cholangiocytes and hepatocytes to restore liver mass, morphology and function (figure 1). The LPC response is most evident in

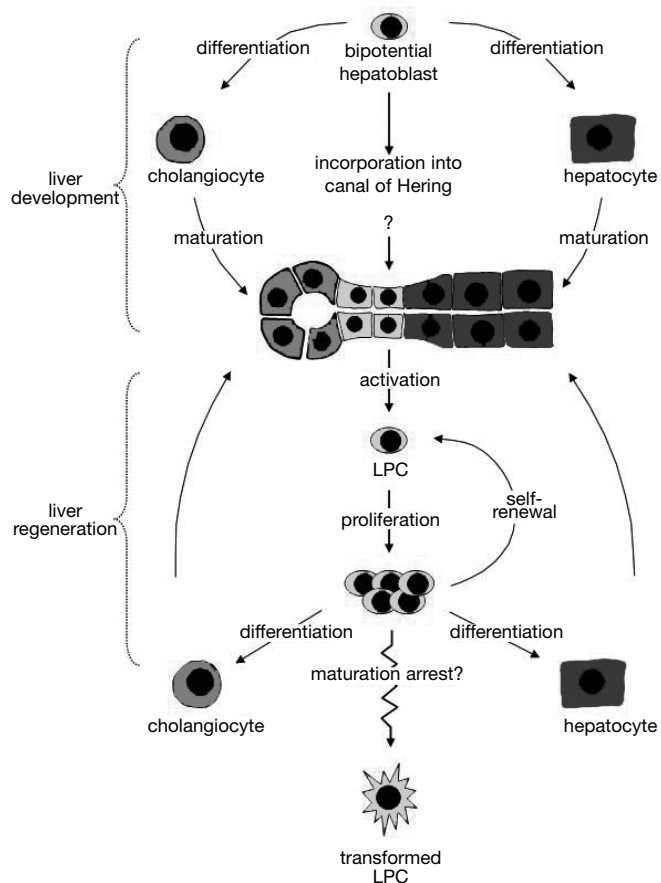


Figure 1. Liver progenitor cell (LPC) ontogeny and potential role in carcinogenesis

During liver development, hepatoblasts differentiate into cholangiocytes and hepatocytes and may be incorporated into the canals of Hering to serve as an immature precursor or stem cell compartment during chronic liver injury. Activated LPCs that 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 subsequent hepatic tumour formation.

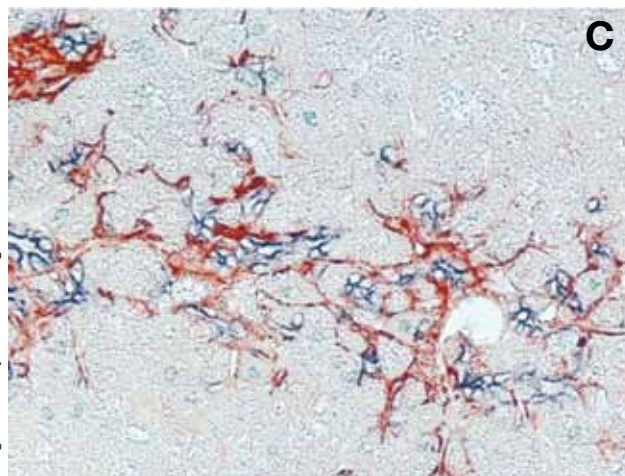
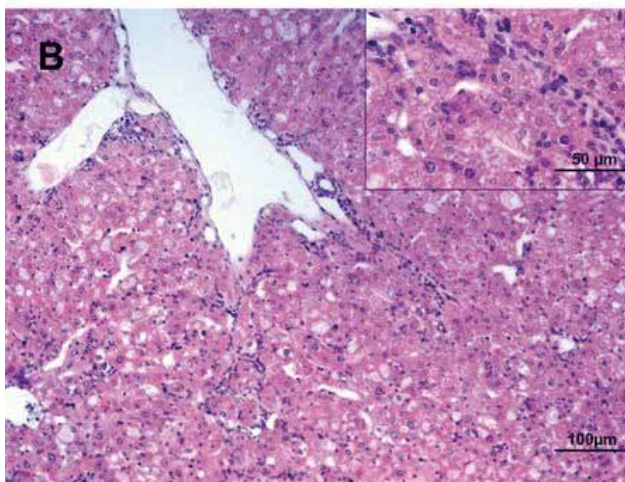
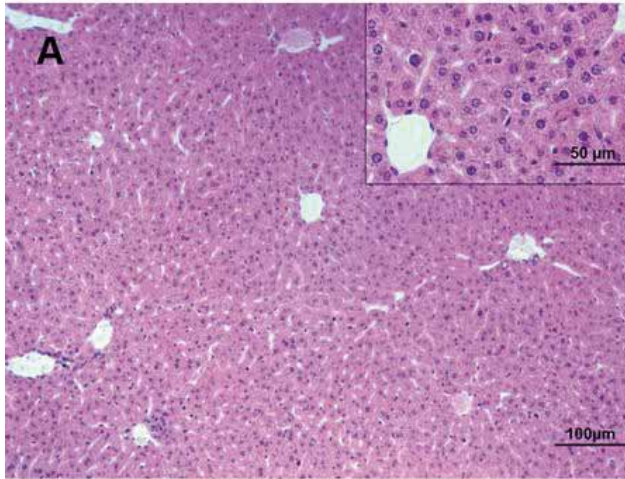


Image courtesy, Belinda Knight

Figures 2a, 2b, 2c. Haematoxylin and Eosin staining of healthy and three week chronically injured liver.

Adult mice on a control diet display normal liver architecture with cords of hepatocytes and sinusoidal structures in between the plates (A). On day 21 of feeding a choline-deficient, ethionine-supplemented diet that induces chronic liver damage, the liver architecture is highly disrupted by steatosis and scattered aggregates of liver progenitor cells and infiltrating inflammatory cells (B). Immunohistochemistry of LPCs (blue, CK19 antibody) and activated hepatic stellate cells (red, alpha smooth muscle actin antibody) in chronic liver injury. LPCs co-localise with hepatic stellate cells during chronic liver injury.

chronic liver diseases which predispose to hepatocellular carcinoma and their high proliferative potential makes them possible targets for transformation, associations that overshadow their restorative capability.^{11,14} These features mandate that we continue to investigate factors that govern their activation, proliferation and differentiation into mature, functional cells, so that in the future we can direct LPCs towards regeneration as opposed to carcinogenesis.

Liver progenitor cells in human pathologies

It is now generally accepted that LPCs exist in human liver and are activated like their rodent counterparts to regenerate chronically injured liver.^{11,14-16} Like the so-called 'oval cells' in rodents, human LPCs are usually associated with hepatocellular necrosis.^{4,5,17-20} Their proliferation is frequently seen in patients with hereditary haemochromatosis, alcoholic liver disease and chronic hepatitis B or C infection.^{4,5} They also proliferate in non-alcoholic fatty liver disease when hepatocytes are injured by oxidative stress.²¹ The number of LPCs induced in these pathologies is directly proportional to the severity of the underlying liver fibrosis.^{4,5} Furthermore, inhibition of the LPC response in chronically injured liver results in reduced formation of cancerous lesions, strongly supporting the association between LPCs and hepatocarcinogenesis.²²⁻²⁶ Therapy of human chronic liver disease, which reduces the risk of hepatocellular carcinoma, has been shown to also reduce the number of LPCs and promote their differentiation, again supporting a role for these cells in carcinogenesis.²⁷

Liver progenitor cell involvement in multistep hepatocarcinogenesis

LPC activation and proliferation during chronic liver injury is associated with an inflammatory response that involves activation of resident as well as recruited immune cells. These inflammatory cells initiate tissue regeneration by promoting the removal of cellular debris and by directly stimulating LPCs to proliferate through release

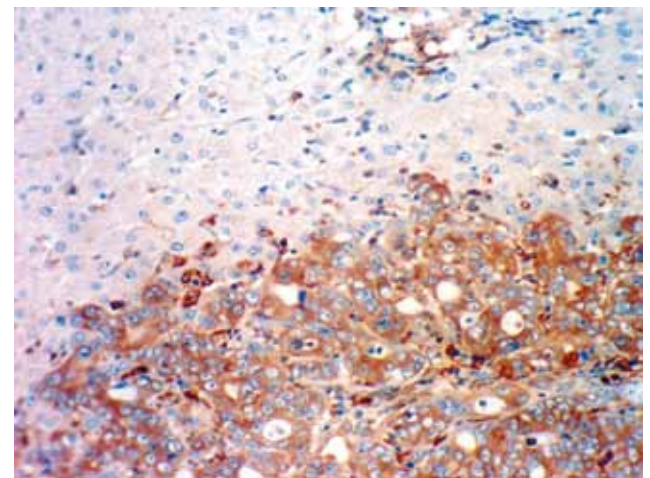


Figure 3 Histological section demonstrating human hepatocellular carcinoma staining positively with antibody to the LPC marker M-pyruvate kinase (brown). Note that the non-cancerous surrounding liver tissue does not stain for the LPC marker.

of mitogenic growth factors and cytokines.^{11,20,28} For periportal induced LPCs to regenerate the liver in pericentral areas, they need to migrate through the liver parenchyma. It is not surprising that LPCs are usually seen in close spatial organisation with hepatic stellate cells (HSCs) that become activated into myofibroblasts to release tissue-degrading matrix metalloproteinases and secrete tissue-remodelling extracellular matrix components (figure 2). HSCs are key mediators of the fibrotic process that accompanies the wound healing process. Fibrosis is characterised by accumulation of proteins such as collagen types I and II, proteoglycans, fibronectin and lamin, providing the scaffold for migrating cells.²⁹ Recent work even suggests that HSCs are a type of LPC that can transition through an LPC intermediary into hepatocytes.³⁰ LPCs and HSCs have been reported to influence each other's behaviour through paracrine signalling.³¹ LPCs produce a range of cytokines, including lymphotoxin- β (LT- β). LT- β signals via the LT- β receptor on HSCs to activate the NF- κ B pathway, which results in production of intercellular adhesion molecule 1 and regulated upon activation, normal T-cell expressed and secreted (RANTES). These act as chemotactic agents for LPCs and inflammatory cells, which are involved in the wound healing response to liver injury.³¹ Abrogation of the LT- β pathway inhibits the LPC response to injury and prevents liver fibrosis in animal models.^{22,23,27}

Hepatocellular carcinoma

De-differentiation or maturation arrest?

Most cases of hepatocellular carcinoma arise in the setting of impaired liver regeneration combined with chronic inflammation and cirrhosis. Cancer is typically caused by accumulated mutations in genes critical for cell cycle control, self-renewal, cell proliferation and differentiation and it has been postulated that three to six of these genetic aberrant alterations are necessary to transform a normal cell into a cancerous cell.^{32,33} This makes rapidly replicating cells, such as the progeny of stem cells and LPCs, obvious targets for transformation events. *In vitro* studies confirm that LPCs are easily transformed in culture into malignant cells^{2,34} and tissue-based studies demonstrate that hepatocellular carcinomas often express LPC immunochemical markers, supporting the role of LPCs as targets for malignant transformation in chronic liver injury (figure 3).³⁵⁻³⁸ This concept has recently been confirmed for various tissue-specific stem cells, including those shown to be involved in the formation of breast cancer.^{39,40} In the context of the liver, it remains controversial as not one cell type, but several cell populations, in addition to hepatic stem cells, are capable of responding to the demand for cell proliferation (and in the case of LPCs, differentiation) to restore dysfunctional liver mass. In general, two main hypotheses have been commonly proposed to explain the cellular origin of hepatocellular carcinoma, and derive from the fact that carcinogenesis always involves proliferation of immature, less differentiated cells – the *de-differentiation* and the *maturation-arrest* hypotheses.

De-differentiation hypothesis

Exposure to some hepatocarcinogens leads to the development of pre-malignant foci that arise by clonal proliferation of hepatocytes.⁴¹⁻⁴³ These “enzyme-altered” lesions are believed to sequentially give rise to larger nodules that displace normal hepatic tissue and ultimately evolve into liver tumours.⁴⁴ The progressive morphological and enzymatic changes from foci to nodules and the formation of cancer have led to the hypothesis that mature, “initiated” hepatocytes de-differentiate to an immature phenotype to obtain a high proliferate capacity. It is possible that the observations supported by this hypothesis can also be explained by LPC proliferation during the early stages of hepatocarcinogenesis, when the designated preneoplastic changes occur.¹¹

Maturation arrest hypothesis

A more accepted hypothesis of tumour formation was first proposed by Potter and has been referred to as the maturation arrest or blocked ontogeny hypothesis.⁴⁵ It postulates that tumours arise when tissue-specific or determined stem cells are blocked from terminally differentiating without undergoing apoptosis. Thereby, a cell mass accumulates with maturation-arrested cells displaying an immature phenotype, which may acquire genetic alterations resulting in carcinogenesis.

Numerous studies provide evidence in support of this hypothesis. Not only are LPCs seen during the early stages of hepatocarcinogenesis, it has also been demonstrated that LPCs are cellular sources of hepatocellular carcinoma in animal models.^{2,34,46} Additionally, it has been shown that a proportion of precursor lesions and hepatocellular carcinomas express markers that are not present in mature hepatocytes. About half of the small cell dysplastic foci, the earliest pre-malignant lesions in human hepatocellular carcinoma, have been shown to be LPC-derived as judged by expression of markers such as CK7, C19 and OV-6.⁴⁷ Furthermore, inactivation of the MYC oncogene in a murine model of hepatocellular carcinoma triggered their differentiation into normal hepatic lineages, including hepatocytes and biliary cells. Reactivation of the MYC oncogene resulted in hepatocytes and LPC transforming back to hepatocellular carcinoma cells, revealing their pluripotency and supporting the concept that hepatocellular carcinoma may originate from the maturation arrest of LPCs.⁴⁸

Zender and co-workers recently strengthened the hypothesised relationship between tissue-specific stem or progenitor cells and hepatocellular carcinoma by demonstrating that LPCs, which had been genetically manipulated *ex vivo* by retroviral gene transfer of oncogenes, rapidly produced liver tumours upon transplantation into conditioned recipient mice, which histopathologically resembled human hepatocellular carcinoma.⁴⁹ While it has been very difficult to determine the exact origin of any specific hepatocellular carcinoma, there is likely more than one potential target cell for transformation and hepatocarcinogenesis. The available data suggest that poorly differentiated hepatocellular carcinomas most likely originate from LPCs and have a poorer, more aggressive progression than well-differentiated cancers, which might be derived from

mature hepatocytes.^{50,51} Furthermore, a side population of cells in human hepatocellular carcinoma cell lines, which show both biliary and hepatocytic characteristics, was highly proliferative and found to give rise to persistently aggressive tumours on serial transplantation into immunodeficient non-obese diabetic/severe combined immunodeficient mice.⁵²

Conclusion

Much evidence has been gathered demonstrating that hepatocellular carcinoma can arise from dysregulated LPC maturation and proliferation during chronic liver injury in humans and in animal models of liver disease and carcinogenesis. The carcinogenic and fibrogenic processes are amenable to manipulation by agents which interfere with LPC proliferation and differentiation. These approaches may be useful for future therapeutic approaches for the prevention of hepatocellular carcinoma.

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