

Role of liver stem cells in hepatocarcinogenesis

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Abstract

Liver cancer is an aggressive disease with a high mortality rate. Management of liver cancer is strongly dependent on the tumor stage and underlying liver disease. Unfortunately, most cases are discovered when the cancer is already advanced, missing the opportunity for surgical resection. Thus, an improved understanding of the mechanisms responsible for liver cancer initiation and progression will facilitate the detection of more reliable tumor markers and the development of new small molecules for targeted therapy of liver cancer. Recently, there is increasing evidence for the "cancer stem cell hypothesis", which postulates that liver cancer originates from the malignant transformation of liver stem/progenitor cells (liver cancer stem cells). This cancer stem cell model has important significance for understanding the basic biology of liver cancer and has profound importance for the development of new strategies for cancer prevention and treatment. In this review, we highlight recent advances in the role of liver stem cells in hepatocarcinogenesis. Our review of the literature shows that identification of the

cellular origin and the signaling pathways involved is challenging issues in liver cancer with pivotal implications in therapeutic perspectives. Although the dedifferentiation of mature hepatocytes/cholangiocytes in hepatocarcinogenesis cannot be excluded, neoplastic transformation of a stem cell subpopulation more easily explains hepatocarcinogenesis. Elimination of liver cancer stem cells in liver cancer could result in the degeneration of downstream cells, which makes them potential targets for liver cancer therapies. Therefore, liver stem cells could represent a new target for therapeutic approaches to liver cancer in the near future.

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Key words: Liver cancer; Liver stem cells; Hepatocarcinogenesis; Tumorigenic transformation; Transdifferentiation

Core tip: Liver cancer is an aggressive disease with a high mortality rate. However, the concept of liver cancer origin is controversial. Recently, there is increasing evidence for the "cancer stem cell hypothesis", which proposes that liver cancer originates from the malignant transformation of liver stem/progenitor cells (liver cancer stem cells). This cancer stem cell model has important significance for understanding the basic biology of liver cancer and has profound importance for the development of new strategies for cancer prevention and treatment. This review discusses current knowledge concerning the role of liver stem cells in the hepatocarcinogenesis of primary liver cancer.

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INTRODUCTION

Liver cancer is one of the most common tumors and

represents the second leading cause of cancer-related death worldwide. Its incidence continues to increase while the prognosis remains gloomy^[1]. Management of liver cancer is strongly dependent on the tumor stage and underlying liver disease. Unfortunately, most cases are discovered when the cancer is already advanced, missing the opportunity for surgical resection. For patients with unresectable or metastatic disease, however, no systemic treatment has been found to prolong survival in randomized studies and no systemic chemotherapy provides a sustained remission^[2]. Although Llovet *et al.*^[3] showed that sorafenib, an oral multikinase inhibitor, prolonged the median survival and the time to progression in patients with advanced hepatocellular carcinoma (HCC), most of the recent phase III trials of multi-targeted tyrosine kinase inhibitors (TKIs) have obtained disappointing results^[4-6]. Thus, an improved understanding of the mechanisms responsible for liver cancer initiation and progression will facilitate the detection of more reliable tumor markers and the development of new small molecules for targeted therapy of liver cancer^[3].

Primary liver cancer (PLC) is a form of liver cancer that begins in the liver. The molecular mechanism associated with initiation and progression of PLC remains obscure. HCC is the most common type of PLC, representing more than 80% of the cases of PLC. Cholangiocellular carcinoma (CCC), the second most common PLC, accounts for approximately 15% of PLC cases worldwide^[7]. Combined HCC and cholangiocarcinoma (cHCC-CC) is an uncommon subtype of PLC that displays components of both HCC and CCC and now accounts for 0.4% to 14.2% of all PLC cases, with significant variations from country to country^[8-10]. Although all three subtypes of PLC begin in the liver, they show very different biological characteristics that have remained unexplained until now.

Stem cells are undifferentiated biological cells with the capacity to undergo extended self-renewal through mitotic division (to produce more stem cells) and to differentiate into mature cells. There are two broad types of stem cells in mammals: embryonic stem (ES) cells that are found in the inner cell mass of blastocysts, and adult stem cells that are found in various adult tissues. In adult organisms, stem cells are responsible for tissue renewal and repair, replenishing aged or damaged tissues^[11]. Fifty-six years ago, Wilson and Leduc suggested that liver stem cells (LSCs) are present in the adult liver^[12]. Later, accumulating evidence suggested that LSCs play a pivotal role in the initiation and progression of PLC. This review summarizes and discusses current knowledge regarding the role of LSCs in the hepatocarcinogenesis of PLC.

LSC CANDIDATES

The liver is known to comprise two epithelial cell lineages, hepatocytes and cholangiocytes, which are known to originate from hepatoblasts during embryonic development. LSCs are bi-potential stem cells that are able to dif-

ferentiate towards the hepatocyte and the biliary lineages. Under normal physiologic conditions, LSCs are quiescent stem cells with a low proliferating rate, representing a reserve compartment^[13]. Upon acute injury, the mature hepatocytes and cholangiocytes, which can be considered conceptually as unipotent stem cells, acquire unexpected plasticity by direct dedifferentiation into LSCs, compensating for the loss^[14,15]. However, when the mature epithelial cells of the liver are continuously damaged or in cases of severe cell loss, LSCs are activated as a consequence and contribute to liver regeneration^[13]. There are two possible sources of liver stem cells: endogenous or intrahepatic LSCs and exogenous or extrahepatic LSCs (Figure 1)^[13,16].

Intrahepatic LSCs

Included in the intrahepatic LSC compartment are the adult liver stem/progenitor cells (referred to as oval cells), which are present in great numbers but with a short term proliferation capacity. In 1956, the term oval cell was first assigned by Farber^[17], who observed a population of nonparenchymal cells in the portal area of the rat liver after being fed ethionine, and described them as small oval cells with scanty, lightly basophilic cytoplasm and pale blue-staining nuclei. Over the past several decades, oval cells have been shown to be localized within the canals of Hering (the most peripheral branches of the intrahepatic biliary tree)^[18,19], interlobular bile ducts^[20], or in the periductular/intraportal zone of the liver^[21]. These cells are called into action when hepatocytes/cholangiocytes are insufficient or unable to respond. Numerous investigators have concluded that oval cell activation was the first step in liver regeneration in response to certain types of injury^[18,22,23].

In addition, it has been reported that mature hepatocytes have the capacity to dedifferentiate into LSCs through a transient oval cell-like stage both *in vitro* and *in vivo*, which indicates that mature hepatocytes are direct contributors to the LSC pool^[14]. Moreover, some investigators observed that liver regeneration also can proceed from a novel cell type, the small hepatocyte-like progenitor cells (SHPCs), which are phenotypically distinct from fully differentiated hepatocytes/cholangiocytes and oval cells^[24,25]. However, some other researchers suggest that SHPCs may represent an intermediate cell type between mature hepatic parenchymal cells and oval cells rather than a distinct stem/progenitor cell population^[26,27]. Thus, further studies are required to better understand this phenomenon.

Extrahepatic LSCs

Extrahepatic LSCs comprise ES cells and bone marrow stem cells (BMSCs), which are usually present in small numbers but have a long-term proliferation capacity. These cells have been reported to be capable of self-renewal, giving rise to oval cells and mature, fully functioning liver cells both *in vitro* and *in vivo*^[22,28,29].

ES cells, continuously growing pluripotent stem cells

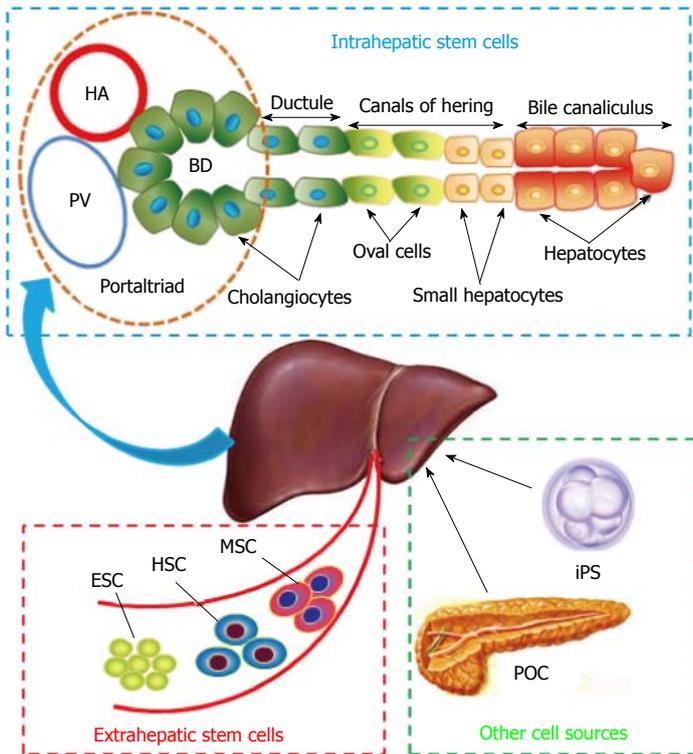


Figure 1 A schematic representation of various sources of liver stem cells^[13,16]. HA: Hepatic artery; PV: Portal vein; BD: Bile duct; ESC: Embryonic stem cell; HSC: Hematopoietic stem cell; MSC: Mesenchymal stem cell; POC: Pancreatic oval cell; iPS: Induced pluripotent stem cell.

derived from the inner cell mass of blastocysts, are capable of indefinite continuous culture and can generate all cell types in the body. Utilizing liver-specific marker staining and subsequent functional analysis, Jones *et al.*^[30] proved that murine ES cells can differentiate into hepatocytes. Using immunohistochemical assays and reverse transcription-polymerase chain reaction tests for hepatocyte-specific proteins and mRNAs, Kuai *et al.*^[31] confirmed that mouse ES cells can differentiate into functioning hepatocytes in the presence of hepatocyte growth factor and nerve growth factor- β . Similarly, increasing evidence shows that human ES cells can be progressively differentiated into definitive endoderm, LSCs, and hepatocytes/cholangiocytes^[32,33]. Recently, several newly developed techniques have been reported to facilitate the *in vitro* maturation of human ES cell-derived hepatocyte-like cells^[34-36].

BMSCs mainly contain two types of multipotent stem cells: hematopoietic stem cells (HSCs), which give rise to the three classes of mature blood cells; and mesenchymal stem cells (MSCs), which can differentiate into a variety of cell types such as osteoblasts (bone cells), chondrocytes (cartilage cells), myocytes (muscle cells), and adipocytes (fat cells)^[37,38]. Both HSCs^[39] and MSCs^[40,41] have been shown to differentiate/transdifferentiate into oval cells and mature hepatic parenchymal cells, although these phenomena occur weakly and infrequently^[42]. In addition, MSCs can be found in nearly all tissues, and various lines of experimental evidence have shown that non-bone marrow-derived MSCs such as adipose-derived MSCs (AD-MSCs)^[43], umbilical cord-derived MSCs^[44,45], and peripheral blood-derived MSCs^[46] also can give rise to oval cells and mature liver parenchymal cells^[47].

Other cell sources

Strikingly, LSCs also can be transdifferentiated from non-hepatic sources such as pancreatic cells and induced pluripotent stem cells. Rao and Reddy first reported that massive depletion of the acinar cell pool causes a change in the oval and ductular cells that result in transdifferentiation into hepatocytes. Pancreatic hepatocytes exhibit all the morphological and functional properties of liver parenchymal cells. The cells that generate hepatocytes have been thought to be pancreatic oval cells^[48]. The results of the studies by Shen *et al.*^[49] and Marek *et al.*^[50] demonstrated that a rat pancreatic cell line, AR42J-B13, can be transdifferentiated into functional hepatocytes *in vitro*, expressing albumin and functional cytochrome P450s, in response to treatment with dexamethasone.

Induced pluripotent stem cells (also known as iPS cells or iPSCs) are a type of pluripotent stem cell that can be generated directly from adult cells^[51]. Yu *et al.*^[52] reported that liver organogenesis transcription factors (Hnf1 β and Foxa3) are sufficient to reprogram mouse embryonic fibroblasts into induced hepatic stem cells. These reprogrammed cells can be stably expanded *in vitro* and possess the potential for bidirectional differentiation into both hepatocyte and biliary lineages. However, pluripotent stem cells readily form a teratoma when injected into immunodeficient mice, which is considered a major obstacle to their clinical application^[53]. On this basis, Zhu *et al.*^[54] reported the generation of human fibroblast-derived hepatocytes that can proliferate extensively and function similarly to adult hepatocytes by cut short reprogramming to pluripotency to generate an induced multipotent progenitor cell from which hepatocytes can be efficiently differentiated.

THE STEM-CELL ORIGIN OF PLC

Several cell types in the liver, *i.e.*, hepatocytes, cholangiocytes, and LSCs, have the longevity that is needed to be the cellular origin of PLC^[19]. Determining the identity of the founder cells for PLC is more problematic and difficult. Therefore, unveiling the mechanisms by which these cells are activated to proliferate and differentiate during liver regeneration is important for the development of new therapies to treat liver diseases.

It is well known that different tumor cells can show distinct morphological and physiological features, such as cellular morphology, gene expression (including the expression of cell surface markers, growth factors and hormonal receptors), metabolism, proliferation, and immunogenic, angiogenic, and metastatic potential. This heterogeneity occurs both within tumors (intra-tumor heterogeneity) and between tumors (inter-tumor heterogeneity)^[55]. In 1937, Furth *et al.*^[56] first demonstrated that a single malignant white blood cell is capable of producing leukemia. Afterwards, the cancer stem cell (CSC) hypothesis was proposed to explain the tumor heterogeneity phenomenon^[57,58]. This model postulates that most cancer cells have only a limited proliferative potential. However, a small subset of tumor cells has the ability to self-renew and is able to generate diverse tumor cells. These cells are defined as cancer stem cells (CSCs) to reflect their stem cell-like properties: indefinite potential for self-renewal and pluripotency. This theory assumes that only CSCs have the ability to initiate new tumors, both at primary and metastatic sites. Thus, this theory indicates that only elimination of all CSCs is fundamental to eradicate tumors^[57].

Over the past few years, there is a growing realization that many cancers contain a small population of CSCs. However, the cellular origin of PLC is controversial and whether PLC contains cells that possess properties of CSCs requires further exploration. Numerous observations indicate that any proliferative cell in the liver can be susceptible to neoplastic transformation. In the past, it has been considered that HCC is derived from dedifferentiation of hepatocytes and CCC originates from the dedifferentiation of intrahepatic biliary epithelial cells. In contrast, cHCC-CC is thought to be derived from transformed LSCs^[59,60]. More recently, due to the rapid progress of stem cell research, it is widely accepted that cancer is a disease of stem cells, as these are the only cells that persist in the tissue for a sufficient length of time to acquire the requisite number of genetic changes for neoplastic development^[61].

Previous studies reported by Steinberg *et al.*^[62] have shown that transfection of an active Ha-ras proto-oncogene into oval cells can lead to their malignant transformation. By using hepatitis B virus X (HBx) transgenic mice and a drug-induced liver injury model, Wang *et al.*^[63] found that HBx may enable malignant transformation and the acquisition of tumorigenic potential in LSCs, suggesting that liver cancer cells are of LSC origin. The results of Chiba *et al.*^[64,65] implied that disruption of the

self-renewal of LSCs generates a CSC population and highlight the important role of LSCs in hepatocarcinogenesis. A study by You *et al.*^[66] showed that inactivation of the tumor suppressor gene Tg737 results in the malignant transformation of fetal LSCs by promoting cell-cycle progression and differentiation arrest. In a clinical study, Ward *et al.*^[67] concluded that PLC in children often arises from the malignant transformation of LSCs at an early stage. In a similar study, Ishikawa *et al.*^[68] considered that CCC may derive from the oncogenic transformation of normal LSCs. Collectively, extensive animal modeling and clinical studies have demonstrated that PLC is a disease derived from maturation arrest of LSCs^[61].

This theory has been confirmed by the discovery of putative CSCs in the liver. Analysis of the cells in PLC supports the presence of cells with functional properties of somatic CSCs (*e.g.*, immortality, resistance to therapy, and efficient transplantability), which indicates that PLC derives from liver CSCs (LCSCs)^[61]. Suetsugu *et al.*^[69] isolated CD133+ cells from human HCC cell lines and demonstrated that these cells possess cancer stem/progenitor cell-like properties. Ma *et al.*^[70,71] and Yin *et al.*^[72] also identified a CSC population in HCC characterized by a CD133 phenotype, suggesting that CD133 might be one of the markers for HCC cancer stem-like cells. Side population (SP) cells are a sub-population of cells that are distinct from the main population and exhibits distinguishing stem cell-like characteristics. In a study of SP cells in different hepatoma cell lines, Chiba *et al.*^[73] concluded that SP cells in hepatoma cell lines possess extreme tumorigenic potential, which suggests that a minor population of liver cancer cells harbors LCSC-like properties. A variety of recent studies of hepatoma cell lines and clinical samples suggest that epithelial cell adhesion molecule (EpcAM)^[74-76], CD13^[77-80], CD24^[81-83], CD44^[84,85], CD90^[86,87], intercellular adhesion molecule-1 (ICAM-1)^[88], $\alpha 2\delta 1$ subunit of voltage-gated calcium channels^[89], and OV6^[90] may serve as putative LCSC markers. The CSC theory emphasizes the role of LSCs in the hepatocarcinogenesis of PLC. Although the aforementioned proteins and/or molecules have been postulated as putative LCSC markers, no definitive markers have yet been identified directly and widely recognized. Moreover, no LCSCs have been isolated^[61]. Therefore, additional studies are needed to obtain a definitive molecular marker of LCSCs and to isolate LCSCs from PLC cell lines, animal models, and clinical samples.

MOLECULAR MECHANISMS INVOLVED IN THE MALIGNANT TRANSFORMATION OF LSCS

Based on the studies mentioned above, we can scientifically conclude that PLC may derive from neoplastic transformation of LSCs. However, the underlying molecular mechanisms are poorly understood. Studies investigating cancer and CSCs show that several key genes and regulatory signaling pathways are oncogenic, such as

Bmi1, Wnt, Notch, Hedgehog, and transforming growth factor- β (TGF- β), and therefore are potentially involved in the malignant transformation of LSCs^[91]. Here, current knowledge of these pathways is discussed.

Polycomb group gene Bmi1

Polycomb group (PcG) proteins are a family of transcriptional repressors that epigenetically remodel chromatin and participate in the establishment and maintenance of cell fates. These proteins play a central role in hematopoiesis, stem cell self-renewal, cellular proliferation and neoplastic development. To date, four distinct PcG-encoded protein complexes have been purified from different species: Polycomb repressive complex 1 (PRC1), PRC2, Pho repressive complex (PhoRC), and Polycomb repressive deubiquitinase (PR-DUB)^[92].

Bmi1, encoded by the *BMI1* gene (B cell-specific Moloney murine leukemia virus integration site 1), is the most important core subunit of the PRC1 complex, which plays a pivotal role in the self-renewal of both normal stem cells and CSCs. Increasing evidence indicates that Bmi1 protein is elevated in many human malignancies including PLC and has a vital effect on tumorigenesis, cancer progression, and the malignant transformation of stem cells. Therefore, Bmi1 was identified as an important stem cell factor and a proto-oncogene^[93].

In PLC, a number of studies have shown that Bmi1 contributes to the maintenance of tumor-initiating SP cells^[94] and can cooperate with other oncogenic signals to promote hepatic carcinogenesis *in vivo*^[95]. Our empirical results suggest that Bmi1 is highly expressed in patients with PLC and correlates positively with the proliferation and invasiveness of human hepatoma cells^[96,97]. Furthermore, Chiba *et al.*^[64,65] observed that forced expression of Bmi1 promotes the self-renewal of LSCs, and the transplantation of such cells that have been clonally expanded from single LSC produces tumors that exhibit the histologic features of cHCC-CC. The above results indicate that Bmi1 plays a crucial role in the oncogenic transformation of LSCs and therefore drives cancer initiation.

Wnt signaling pathway

The Wnt signaling pathways are ancient and evolutionarily conserved pathways that transmit signals from outside of a cell through cell surface receptors to the inside of the cell and regulate cell-to-cell interactions. Wnt signaling is one of the most well studied molecular pathways during the human life span and involves a large number of proteins that are required for basic developmental processes such as embryonic development, cell fate determination, cell proliferation, cell migration, and cell polarity, in a variety of species and organs^[98].

Three major categories of Wnt signaling pathways are recognized: the canonical Wnt pathway in which the cytoplasmic protein β -catenin is a key mediator, the noncanonical planar cell polarity pathway (β -catenin independent), and the noncanonical Wnt/calcium pathway. Activation of the canonical Wnt/ β -catenin pathway

causes an accumulation of β -catenin in the cytoplasm and its eventual translocation into the nucleus to act as a transcriptional coactivator of transcription factors. Without Wnt signaling, β -catenin would not accumulate in the cytoplasm because it would be degraded by a destruction complex^[99]. Ever since its initial discovery, Wnt signaling has had an association with cancer^[100]. There is substantial evidence to suggest that dysregulation of Wnt signaling is critical for the initiation and progression of PLC^[101,102].

Wnt signaling pathways, particularly the canonical Wnt/ β -catenin pathway, are also involved in the self-renewal and maintenance of embryonic and adult stem cells, and as recent findings demonstrated, in CSCs. Functional characterization of LSCs has revealed that Wnt/ β -catenin pathways were critical for inducing the stem cell properties of hepatoma cells and in promoting self-renewal, tumorigenicity, and chemoresistance^[103]. In the aforementioned HBx-mediated tumorigenic effects, Wang *et al.*^[63] suggest that HBx may enable LSCs with tumorigenic potential *via* activation of the Wnt/ β -catenin signaling pathway. As shown in several *in vivo* and *in vitro* experiments, the Wnt/ β -catenin signaling pathway contributes to the activation of normal and tumorigenic LSCs^[104]. Moreover, Chiba *et al.*^[64] demonstrated that Wnt/ β -catenin signaling activation strongly enhances the self-renewal capability of LSCs and generates a CSC population as an early event, thereby contributing to the initiation of PLC.

Notch signaling pathway

Notch signaling is a complex, highly conserved signal transduction pathway in multicellular organisms. In mammalian cells, the pathway is initiated when Notch ligands (Jagged-1, Jagged-2, and Delta-like 1, 3, and 4) bind to the epidermal growth factor (EGF)-like receptors Notch1-4. Signaling is processed by the enzyme γ -secretase, which results in the subsequent activation of downstream target genes^[105,106]. The Notch signaling pathway functions as a major regulator of cell-fate decisions during embryonic development and adult life, and it is crucial for the regulation of self-renewing tissues. Accordingly, dysregulation of Notch signaling underlies a wide range of human disorders from developmental syndromes to adult-onset diseases and cancer^[105,107].

Like other solid tumors, misregulation of the Notch pathway in PLC has been described as both oncogenic and tumor suppressive, depending on the cellular context^[108]. Qi *et al.*^[109] reported that overexpression of Notch1 inhibits the growth of HCC cells by inducing cell cycle arrest and apoptosis. In 2009, the same authors showed that Notch1 signaling sensitizes tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis in HCC cells^[110]. In addition, Viatour *et al.*^[111] demonstrated that activation of the Notch pathway serves as a negative feedback mechanism to slow HCC growth during tumor progression. At odds with these findings, however, some recent studies have provided strong evidence in favor of the pro-oncogenic activity of

Notch in PLC. For example, Wang *et al.*^[112] showed that aberrantly high expression of Notch1 is significantly associated with metastatic disease parameters in HCC patients, and shRNA-mediated silencing of Notch1 reverses HCC tumor metastasis in a mouse model. In human HCC cell lines, Gao *et al.*^[113] demonstrated that Notch1 activation contributes to tumor cell growth. In accordance, we have shown that Notch1 is overexpressed in human intrahepatic CCC and is associated with its proliferation, invasiveness and sensitivity to 5-fluorouracil *in vitro*^[114]. Taken together, these data highlight the concept that the Notch pathway plays an essential yet controversial role in PLC, presumably depending on the tumor cell type, local inflammatory microenvironment and the status of other signaling pathways^[115,116].

The aforementioned hypothesis was further supported by recent studies examining Notch signaling in the regulation of stem cell and in the development of LSC-driven PLC^[117,118]. Utilizing a genetically engineered mouse model and comparative functional genomics, Strazzabosco *et al.*^[115], Villanueva *et al.*^[119] and Razumilava *et al.*^[120] observed that liver-specific Notch activation in mice recapitulates different stages of human hepatocarcinogenesis and results in HCC, including histological features associated with stem cell expansion. They also confirmed that Notch1 is a bona fide oncogene in experimental liver cancer. Using a transgenic mouse model, Zender *et al.*^[116] proved that stable overexpression of Notch 1 in bipotential LSCs causes the formation of intrahepatic CCCs. Dill *et al.*^[121] and Cardinale *et al.*^[122] also reported that liver-specific expression of the intracellular domain of Notch2 (N2ICD) in mice is sufficient to induce HCC formation, while DEN^{N2ICD} (diethylnitrosamine-induced HCCs with constitutive Notch2 signaling) mice develop large hepatic cysts, dysplasia of the biliary epithelium, and eventually CCC. These studies also suggested that the LSC compartment is the most likely candidate for oncogenic events^[115,116,119-122].

Nevertheless, these newly published studies raise one question: how can one pathway, Notch signaling, contribute to two different subtypes of PLC: HCC and CCC? Of note, the balance between Notch/Wnt signaling has been proposed to be crucial for the determination of the LSC cell fate in liver disease. Activation of Notch signaling in LSCs leads to biliary specification; in contrast, Wnt signaling activation inhibits default-activated Notch signaling *via* Numb (a target of canonical Wnt signaling), allowing LSCs to escape the biliary cell fate and acquire a hepatocellular specification^[123-125]. Therefore, based on previous studies and to the best of our knowledge^[123-126], we propose that the balance between Notch/Wnt signaling pathways determines the oncogenic transformation of LSCs into HCC, CCC, or cHCC-CC phenotype. The predominance of Notch over the Wnt signaling in LSCs leads to the CCC phenotype, and activation of Wnt signaling likely prevents activation of the Notch pathway and thus leads to the HCC phenotype. When the comparison is balanced between the two signaling pathways,

the cell has a higher probability of entering the cHCC-CC phenotype. In summary, the role of such a pleiotropic pathway in liver regeneration and liver diseases seems to be highly context dependent. Additional research is required to clearly establish the effects of the Notch signaling pathway during hepatocarcinogenesis.

Hedgehog signaling pathway

The Hedgehog signaling pathway is one of the key regulators of embryonic development. Mammals have three Hedgehog homologues, Sonic (SHH), Indian (IHH), and Desert (DHH), of which Sonic is the best studied. Like the Wnt and Notch pathways, the Hedgehog signaling pathway also plays significant roles in stem cell self-renewal^[127] and cancer cell proliferation^[128,129].

Sicklick *et al.*^[130] showed that Hedgehog signaling is conserved in hepatic progenitors from fetal development through adulthood and is essential for the maintenance of LSC survival. In a study reported by Jeng *et al.*^[131], the SHH pathway is activated in CD133+ mouse liver cancer cells that harbor stem cell features. In human CCC tissues and cell lines, El Khatib and colleagues^[132] demonstrated that inhibition of Hedgehog signaling attenuates carcinogenesis *in vitro* and increases necrosis in CCC. Chen *et al.*^[133] showed that enhanced Hedgehog signaling activity may be responsible for the invasion and chemoresistance of hepatoma subpopulations. In a fibrosis-associated hepatocarcinogenesis model, Philips *et al.*^[134] further established that Hedgehog signaling pathway activation promotes hepatocarcinogenesis while inhibiting Hedgehog signaling safely reverses this process even in advanced HCC.

TGF- β signaling pathway

The TGF- β signaling pathway is involved in various cellular functions in both the developing embryo and the adult organism including cell growth, cell differentiation, apoptosis, and cellular homeostasis. The pathway is activated upon binding of TGF- β to its receptors, TGF- β receptor I (TGFB1) and TGFB2, resulting in the translocation of Smad proteins to the nucleus where they act as transcription factors and participate in the regulation of target gene expression^[135,136].

The role of TGF- β in tumors is rather complicated. In healthy tissue, it acts as a tumor suppressor controlling the cell cycle and inducing apoptosis. During carcinogenesis, TGF- β acts as a potent inducer of cell motility, invasion and metastasis. In liver cancer, TGF- β has been shown to have both tumor-promoting and tumor-suppressing effects, and its expression is decreased in early but increased in later stages of carcinogenesis. Although the underlying molecular mechanisms remain largely undefined, it had been speculated that the dual role of TGF- β signaling in liver cancer results from its effect on the tumor microenvironment^[135,136].

It has long been known that TGF- β signaling is vitally involved in stem cell renewal and lineage specification, including in LSCs^[137]. Recently, TGF- β signaling has also

been linked to the malignant transformation of LSCs in hepatocarcinogenesis. Nishimura *et al.*^[138] reported that TGF- β treatment increases the percentage of SP cells in a hepatoma cell line. Yuan *et al.*^[139] reported that HCC cells with aberrantly high expression of TGF- β signaling that are positive for Oct4 (octamer-binding transcription factor 4) are likely cancer progenitor cells with the potential to give rise to HCC. Using several experimental approaches, Wu *et al.*^[140] confirmed that long-term treatment of oval cells with TGF- β impaired their LSC potential but granted them tumor-initiating cell (TIC) properties including the expression of TIC markers, increased self-renewal capacity, stronger chemoresistance, and tumorigenicity in nude mice. In opposition to these findings, however, Tang *et al.*^[141,142] showed that activation of the interleukin-6 (IL-6) signaling pathway induces neoplastic transformation of LSCs along with inactivation of the TGF- β signaling pathway. Lin *et al.*^[143] suggested that disruption of TGF- β signaling is an important molecular event in the transformation of normal LSCs to cancer progenitor/stem cells. These data suggest an important but contradictory role for TGF- β signaling in LSC-driven hepatocarcinogenesis, potentially due to the interaction with other signaling pathways.

A NEW CONCEPT UNDERLYING THE LCSC LINEAGE: VASCULAR ENDOTHELIAL TRANSDIFFERENTIATION

Interestingly, CSCs can potentially transdifferentiate into cell types other than the original type from which the tumor arose. Several recent studies have shown that CSCs also can transdifferentiate into functional vascular endothelial cells that line the tumor vasculature, mediating tumor growth and metastasis^[144-146]. In 2010, Wang *et al.*^[147] and Ricci-Vitiani *et al.*^[148] provided strong evidence that a proportion of the endothelial cells that contribute to blood vessels in glioblastoma originate from the tumor itself, having differentiated from tumor stem-like cells. Wang *et al.*^[147] also demonstrated that blocking VEGF (vascular endothelial growth factor) or silencing VEGFR2 (VEGF receptor 2) inhibits the maturation of tumor endothelial progenitors into endothelium but not the transdifferentiation of tumor stem-like cells into endothelial progenitors, whereas γ -secretase inhibition or Notch1 silencing blocks the transition into endothelial progenitors. Subsequently, multiple studies have confirmed the presence of tumor-derived endothelial cells in several other malignancies, such as renal^[149,150], ovarian^[151], and breast cancers^[152,153], which suggests that this is a general phenomenon in CSCs.

Similarly, Marfels *et al.*^[154] found that chemoresistant hepatoma cells show increased pluripotent capacities and the ability to transdifferentiate into functional endothelial like cells both *in vitro* and *in vivo*. These tumor-derived endothelial cells possess increased angiogenesis and drug resistance capability (including chemotherapeutics and

angiogenesis inhibitors) compared with normal endothelial cells^[155,156]. Taken together, these data may provide new perspectives on the biology of CSCs and reveal new insights into the mechanisms of resistance to anti-angiogenesis therapy.

CONCLUSION

Our review of the literature shows that identification of the cellular origin and the signaling pathways involved is challenging issues in PLC with pivotal implications in the therapeutic perspectives. Although dedifferentiation of mature hepatocytes/cholangiocytes in hepatocarcinogenesis cannot be excluded, neoplastic transformation of a stem cell subpopulation more easily explains hepatocarcinogenesis. Elimination of LCSCs in PLC could result in the degeneration of downstream cells, making them potential targets for liver cancer therapies. Therefore, LSCs could represent a new target for therapeutic approaches to PLC in the near future. However, though LSCs have a bright future, their efficient therapeutic applications will demand further scientific advances.

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