Genome Medicine

REVIEW

Role of cancer stem cells in hepatocarcinogenesis

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Abstract

There has been considerable interest in cancer stem cells (CSCs) among cancer biologists and clinicians, most likely because of their role in the heterogeneity of cancer and their potential application in cancer therapeutics. Recent studies suggest that CSCs play a key role in liver carcinogenesis. A small subpopulation of cancer cells with CSC properties has been identified and characterized from hepatocellular carcinoma (HCC) cell lines, animal models and human primary HCCs. Considering the high mortality and ineffectiveness of current therapies for HCC, understanding the characteristics and function of CSCs is likely to lead to development of new therapies resulting in improvement of patient survival. This review summarizes recent progress in liver cancer stem cell research with regard to the identification, cell origin, regulation of self-renewal capacity, and therapeutic implications of liver CSCs.

Background

Tumors are characterized by uncontrolled proliferation of abnormal cells that frequently display morphological and functional heterogeneity [1]. Two models have been proposed to explain the heterogeneity of tumors, namely the stochastic [2] and hierarchical models [3]. The stochastic model suggests that all cells within a tumor are biologically homogenous and, therefore, have equal capacity to regenerate the tumor. The hierarchical model, also known as the cancer stem cell model, proposes that only a small subset of tumor cells, designated cancer stem cells (CSCs) or tumor-initiating cells, within a tumor exhibit the capacity to initiate and sustain tumor growth. CSCs possess two important properties similar to those of normal stem cells, namely self-renewal and differentiation capacity [4]. Although CSCs were first identified 26 years ago in acute myelogenous leukemia with the cell surface marker $CD34^+CD38^-$ [5], the isolation of these cells from solid tumors was accomplished only in 2003 [6]. This study showed that $CD44^+CD24^-$ cells from breast cancer specimens are able to generate xenografts that phenotypically resemble the initial tumor. Furthermore, the xenografts could be serially passed to generate similar tumors, indicating the self-renewal ability of these cells. Following this seminal study, CSCs were isolated from various solid tumors such as brain, colon, pancreas, lung and liver. The isolation of CSCs was based on a series of cell surface markers, including CD133 [7,8], CD44 [6,9], CD90 [10], CD24 [11] and EpCAM [12,13], as well as functional markers such as ALDH1 [14] and ABCB5 [15], and side population (SP) cells [16].

Hepatocellular carcinoma (HCC) is a deadly disease with no promising therapeutic options. It is the fifth most prevalent cancer in the world, and the third leading cause of cancer-related death, with an annual death rate exceeding 500,000. The incidence of HCC among the population between the ages of 45 and 60 years is rising dramatically, particularly in the western world, due to an increase in hepatitis C virus infection, and alcoholic and non-alcoholic fatty liver disease. While surgical resection/ablation and liver transplantation are potentially curative, the high mortality is due to diagnosis at an advanced stage [17,18]. Although the multikinase inhibitor sorafenib has recently been approved for treatment of HCC, poor response of the late-stage cancer to this and almost all other available chemotherapeutic agents continues to be a major obstacle to successful HCC therapy. The 5-year survival rate for this cancer is only 5%, and the death rate is expected to rise in the next 20 years. Primary HCC, the most common malignant tumor, accounts for >90% of all primary liver cancer. Its development is a complex, multistep process. Elucidation of the molecular mechanisms of liver carcinogenesis is critical to determine the specific pathways involved in the initiation and progression of HCC that will eventually lead to identification of novel molecular targets for therapy. As observed in other solid tumors, recent studies suggested that CSCs may be involved in the development of liver cancer [10,13,19-21]. This review will summarize recent progress in the potential role of CSCs in hepatocarcinogenesis.

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CSCs in hepatocellular carcinoma

In an early attempt to isolate liver-cancer-specific CSCs, SP cells with CSC characteristics were separated from a total population of HCC cells [19]. SP sorting was initially used to detect hematopoietic stem cells exploiting the ability of these cells to efflux Hoechst 33342 through an ATP-binding cassette membrane transporter. This property contributes to multidrug resistance of these cells, and this is a key feature of CSCs. Only a very small proportion of HCC cells (0.25% in Huh7 and 0.8% in PLC/RPL5 cells) possess a SP phenotype; these show higher proliferative and tumorigenic potential compared with non-SP cells. Indeed, 1×10^3 SP cells were sufficient for tumor formation in xenograft transplantation while at least 1×10^6 unsorted HCC cells were required to form a tumor, indicating enrichment of CSCs in SP cells. Despite these key observations, it remains uncertain whether SP cells are authentic CSCs, since Hoechst 33342 is cytotoxic, and non-SP cells are unable to grow in the presence of this dye. Consequently, differential resistance to Hoechst 33342 rather than the intrinsic difference in the stem cell properties between SP and non-SP cells could contribute to the difference in tumor formation between these cell types.

In the ensuing several years, significant efforts were made to identify CSCs in HCC cell lines, mouse models and primary HCC (Table 1). CD133, also known as prominin-1 (Prom1), encoding a pentaspan transmembrane glycoprotein [22,23], has been used as a marker for both somatic stem cells [24-26] and CSCs in different tissues, including brain [7], colon [8], pancreas [27] and skin [28]. Recently, several studies have shown that $CD133^+$ cells isolated from various liver cancer cell lines [20,21,29] exhibit the properties of CSCs and only a small fraction of $CD133^+$ cells produce hepatocellular carcinoma in severe combined immunodeficiency mice. Although CD133 has been used to identify CSCs from HCC cell lines and mouse models, some studies have suggested that $CD133^-$ cells can form tumors in immunodeficient mice at higher cell number [21], indicating that CD133 may not be an effective CSC marker in HCC. To identify CSCs in human primary HCC specimens, Yang *et al.* [10] used CD90 cell surface markers and found that, unlike $CD90^-$ cells, $CD90^+$ cells from HCC specimens as well as patient blood samples exhibited tumorigenic potential. This study suggested that CD90 is a potent marker of liver CSCs and that circulating CSCs probably contributed to tumor metastasis.

Since CSCs share key features with normal stem cells, including self-renewal and differentiation, it is conceivable that these cells may possess similar cell surface markers. Indeed, some markers, such as EpCAM [13] and OV6 [30], that were used to isolate hepatic stem cells have also been shown to function as specific markers for CSCs in HCC.

Table 1. Cell surface markers of liver cancer stem cells

Marker	Cell line/primary tumor	Reference
Side population	Huh7 and PLC/PRF/5 cell lines	Chiba *et al.* (2006) [19]
$CD133^+$	SMMC-7721 cell line, human primary HCC	Yin *et al.* (2007) [20]
$CD133^+$	Huh7, PLC8024, Hep3B cell lines, human primary HCC	Ma *et al.* (2007) [21]
$CD133^+$ $CD44^+$	SMMC-7721, MHCC-LM3 and MHCC-97L cell lines	Zhu *et al.* (2010) [29]
CD90	HepG2, Hep3B, PLC, Huh7, MHCC97L, and MHCC97H cell lines, human primary HCC	Yang *et al.* (2008) [10]
EpCAM	HuH1, HuH7, and Hep3B cell lines, human primary HCC	Yamashita *et al.* (2009) [13]
OV6	Huh7, PLC, SMMC7721, Hep3B, and HepG2 cell lines	Yang *et al.* (2008) [30]

HCC, hepatocellular carcinoma.

Cellular origin of liver CSCs

It has been proposed that CSCs may arise through mutations accrued in normal tissue stem cells or more differentiated tissue progenitor cells [31]. Recently, crypt cells expressing Lgr5 in small intestine and colon tissues have been identified as long-lived stem cells [32]. Apc deletion in these cells was necessary and sufficient to produce tumors *in vivo* [32]. Another study showed that CD133 marks the intestinal stem cells, and overproduction of β-catenin can transform $CD133^+$ cells into CSCs and generate tumors [33]. Those studies suggested that normal stem cells may be the origin of CSCs. In contrast, introduction of MLL-AF9 into committed granulocyte macrophage progenitors produced leukemia stem cells [34], suggesting that CSCs could also be derived from committed progenitors.

The existence of stem cells in adult liver has been debated for many years. Recent studies have suggested that hepatic progenitor cells (HPCs) or oval cells, a subset of small epithelial cells with an oval nucleus located in the bile ductules and canals of Hering in adult liver [35], may function as stem cells in adult liver. HPCs can be activated to proliferate and differentiate into hepatocytes and biliary lineage in response to severe liver damage [36-41], such as that induced by treatment of rats with 2-acetylaminofluorene after partial hepatectomy (AAF/PH model) [42]. Under this condition, hepatocytes were unable to proliferate to replace the lost tissues. HPCs are, therefore, considered to be the bipotent liver stem cells.

HPCs play an important role in carcinogenesis in at least some subtypes of HCC. The number of HPCs in human chronic liver diseases, such as hepatitis B and C infection and alcoholic liver disease, are directly related to disease severity, suggesting that oval cell proliferation is associated with increased risk of development of HCC

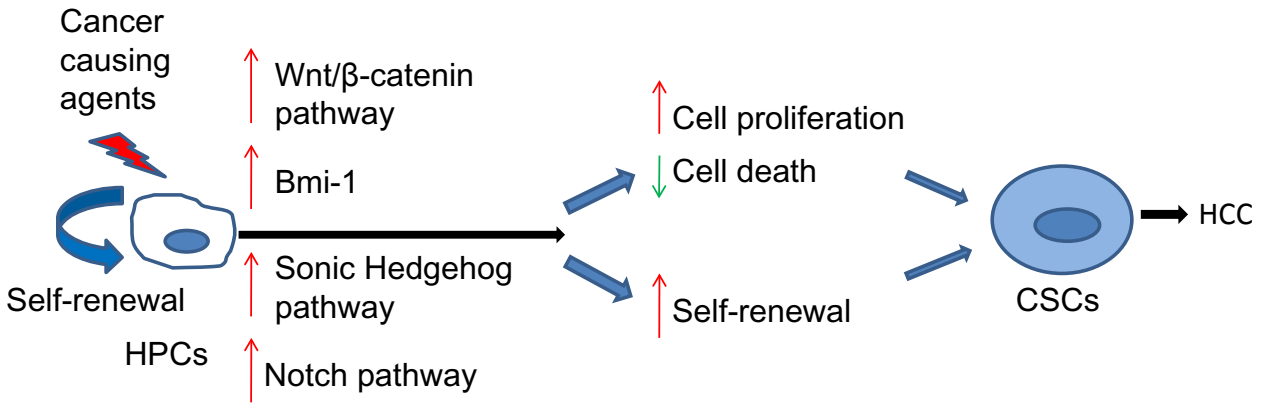

Figure 1. Proposed model for the cell origin and transformation of liver cancer stem cells. Dysregulation of signaling pathways involved in self-renewal and proliferation of normal stem cells (hepatic progenitor cells, HPCs) is likely to cause uncontrolled proliferation and increased self-renewal capacity of HPCs, resulting in the transformation of HPCs into cancer stem cells (CSCs). HCC, hepatocellular carcinoma.

in chronic liver disease [41]. A recent study has shown that certain subtypes of primary human HCCs with poor prognosis may be derived from HPCs [43], based on the identification of human HCCs that exhibited gene expression profiles identical to that of hepatoblasts. Further, this subtype of HCC can be distinguished from other types by the markers of HPCs. HPCs are also activated in several mouse models of hepatocarcinogenesis, suggesting their potential role in the initiation and progression of liver cancer [44-48]. Fang *et al.* [44] found that oval cell proliferation was prominent from the initial liver injury stage to the occurrence and development of HCC in a HCC model fed 3,3'-diaminobenzidine. More importantly, blocking the expansion of oval cells by targeting c-Kit with imatinib mesylate significantly reduced liver tumor formation in mice fed a choline-deficient, ethionine-supplemented diet [49]. Similarly, TNF was upregulated during oval cell proliferation induced by a choline-deficient, ethionine-supplemented diet, and was produced by oval cells [50]. Oval cell proliferation and tumorigenesis were substantially impaired in TNF receptor type 1 knockout mice. These two studies suggested that oval cells may be directly involved in the initiation and progression of liver cancer in this mouse model. More direct evidence for the involvement of oval cells in hepatocarcinogenesis was revealed in a study that demonstrated tumor formation in *nude* mice upon subcutaneous injection of p53 *null* oval cells [46]. Further studies showed that $CD133^{+}CD45^{-}$ oval cells isolated from Mat1a (methionine adenosyltransferase 1a) [51] and Pten (phosphatase and tensin homolog deleted on chromosome 10) [52] knockout mice exhibited greater tumorigenic capacity. Furthermore, these oval cells exhibited high levels of resistance to chemotherapeutic agents, consistent with cancer stem cell characteristics. These findings, together with the notion that CSCs may arise from adult stem cells or progenitor cells, suggest that liver CSCs are probably derived from HPCs.

However, it should be noted that not all HCCs arise from HPCs, since oval cell proliferation is very rare in diethylnitrosamine-induced HCC. It is widely accepted that the HCC induced by this carcinogen develops from hepatocytes [53]. Based on these observations, the exact origin of CSCs in the liver appears to depend on the etiology of the cancer.

Signaling pathways involved in maintaining the characteristics of liver CSCs

The maintenance of self-renewal and tumorigenic capacity of CSCs involves several cancer-related signaling pathways, including Bmi-1 [54], Wnt/β-catenin [55], Notch [56], and Sonic Hedgehog [55,57] (Figure 1). *Bmi-1*, a polycomb group (PcG) gene, has previously been shown to regulate the self-renewal of hematopoietic stem cells [58]. Overexpression of *Bmi-1* enhanced self-renewal of both hematopoietic stem cells and neural stem cells, indicating that Bmi-1 may function as a universal determinant of stem cell self-renewal. Overexpression of *Bmi-1* in HPCs dramatically promoted the self-renewal of these cells, and transplantation of *Bmi-1*-transduced HPCs produced tumors *in vivo*, indicating the important role of Bmi-1 in regulating the self-renewal of normal or cancer stem cells in the liver [54]. Notably, upregulation of *Bmi-1* has also frequently been observed in human primary HCC [59].

The Wnt/β-catenin signaling pathway can play a critical role in stem cell proliferation and potency maintenance [60], as well as differentiation of stem cells in a variety of tissues [61]. The activation of Wnt/β-catenin has been shown to be associated with hepatocyte proliferation in liver development, regeneration and tumor growth [62]. As observed in Bmi-1, mutation in β-catenin and

consistent activation of this signaling pathway significantly enhanced the self-renewal of HPCs and promoted cancer initiation [54].

Although the Notch signaling pathway plays an important role in regulating stem cell self-renewal and differentiation [3], it may function as a tumor suppressor or oncogene in different tissues depending on other signaling pathways [63]. In breast tissue, the Notch signaling pathway was shown to be upregulated, and inhibition of the Notch pathway reduced the formation of mammosphere, an indicator of stem-like cells [56]. Similarly, inhibition of the Notch pathway selectively depleted glioblastoma CSCs [64]. The role of the Notch signaling pathway in HCC is controversial. One study found that activation of Notch1 signaling inhibits growth of human HCC [65], suggesting that it may function as a tumor suppressor. In contrast, another study showed that the Notch1 signaling pathway is constitutively activated in HCC [66], indicating a potential oncogenic role of this pathway in HCC.

A variety of tumors exhibit activating mutations or upregulation of Sonic Hedgehog (HH) pathway components, such as PTCH1, SMO and GLI1[67]. Recent studies showed that the HH pathway also regulates the self-renewal ability of CSCs in breast cancer [68], glioma [69] and multiple myeloma [70]. Although the role of the HH pathway in liver CSCs has not been well characterized, this pathway is known to be activated in HCC [71]. It would be of interest to investigate its potential role in regulating the self-renewal ability of liver CSCs.

Therapeutic implications of liver CSCs

Recent studies have shown that CSCs are more resistant to chemotherapy [11,72] and radiotherapy [73] compared with non-CSCs within the same tumor, indicating that CSCs may be responsible for the recurrence and eventual metastasis of these tumors. It is conceivable that targeting liver CSCs would provide new alternatives to treat HCC, since HCC is more resistant to traditional therapies. Drugs targeting signaling pathways involved in regulating the self-renewal ability of CSCs may prove to be effective in HCC therapy. For example, cyclopamine, which targets the HH pathway, significantly reduces the self-renewal of glioma [74], whereas gamma secretase inhibitor, which targets the Notch pathway, decreases self-renewal of breast CSCs [75]. It is worth investigating whether such drugs can also effectively eliminate liver CSCs. Another strategy to target CSCs is to induce differentiation of liver CSCs. In this context, Yin *et al.* [76] have shown that the forced expression of HNF4α, a central regulator of the differentiated hepatocyte phenotype, in CSCs can induce the differentiation of CSCs into mature hepatocytes and abolish their tumorigenesis in mice, implicating a novel strategy for HCC therapy.

Conclusions

Although significant progress has been made in liver cancer stem cell research in HCC, many issues remain to be resolved, such as the identity and origin of liver CSCs. Several markers are currently used to isolate CSCs. Although CSCs thus identified are known to form tumors in a xenograft model, it is not known whether these markers coexist or whether they are present in different cells. Additionally, the origin of liver CSCs needs to be studied by a lineage tracing study. The development of single cell sorting and *in vivo* imaging technology, as well as the use of mouse HCC models and syngeneic transplantation, will facilitate the identification of unique and specific liver CSC markers. It is critical to elucidate the detailed mechanisms underlying the transformation of liver CSCs. Comparing the gene expression profiles between normal stem cells (HPCs) and CSCs by microarray analysis or next-generation sequencing may prove to be useful to identify the dysregulated oncogenes or tumor suppressors and signaling pathways that are involved in the transformation of liver CSCs. Finally, these findings are likely to lead to the development of new diagnostic markers and therapeutic targets for HCC.

Abbreviations

CSC, cancer stem cell; HCC, hepatocellular carcinoma; HH, Sonic Hedgehog; HPC, hepatic progenitor cell; SP, side population; TNF, tumor necrosis factor.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

BW extensively reviewed the literature and prepared the first draft after discussion with SJ. SJ reviewed the draft, edited and helped to prepare the final draft.

Acknowledgements

We thank Dr Kalpana Ghoshal for critical review of the article and useful suggestions. The work done in the authors' laboratory was supported, in part, by NIH grants CA101956 and CA086978 (STJ).

Published: 22 February 2011

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doi:10.1186/gm225
Cite this article as: Wang B, Jacob ST: **Role of cancer stem cells in hepatocarcinogenesis.** *Genome Medicine* 2011, **3:**11.