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Mini-review

# Characteristics of liver cancer stem cells and clinical correlations

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ABSTRACT

Liver cancer is an aggressive malignant disease with a poor prognosis. Patients with liver cancer are usually diagnosed at an advanced stage and thus miss the opportunity for surgical resection. Chemotherapy and radiofrequency ablation, which target tumor bulk, have exhibited limited therapeutic efficacy to date. Liver cancer stem cells (CSCs) are a small subset of undifferentiated cells existed in liver cancer, which are considered to be responsible for liver cancer initiation, metastasis, relapse and chemoresistance. Elucidating liver CSC characteristics and disclosing their regulatory mechanism might not only deepen our understanding of the pathogenesis of liver cancer but also facilitate the development of diagnostic, prognostic and therapeutic approaches to improve the clinical management of liver cancer. In this review, we will summarize the recent advances in liver CSC research in terms of the origin, identification, regulation and clinical correlation.



## Introduction

Liver cancer is the sixth most common malignancy and is second only to lung cancer in mortality [1]. Hepatocellular carcinoma (HCC) is the major pathological type and accounts for approximately 80% of cases of liver cancer. Despite the enormous advances in the diagnosis and treatment of liver cancer during the past several decades, the therapeutic effect of this deadly disease remains disappointing. The first-line curative approaches for liver cancer mainly involve partial liver resection and liver transplantation. Unfortunately, most patients miss the opportunity for resection due to a late diagnosis [2–5]. Even after surgical resection, the long-term prognosis remains very poor due to frequent recurrence. Chemotherapy via either systemic treatment or transarterial chemoembolization is the second-line treatment, but the overall response rate is rather low due to the high chemoresistance of liver cancer [6]. Emerging evidence has shown that cancer stem cells (CSCs), also termed tumor-initiating cells (T-ICs), exist in solid tumors and are responsible for cancer relapse, metastasis and chemoresistance [7]. The existence of liver CSCs has been reported in numerous studies [8], and these cells are considered to account for the heterogeneous and hierarchical organizations of HCCs [9,10]. In this review, we present a brief and up-to date overview of the characteristics of liver CSCs and their clinical correlations.

## Origin of liver cancer stem cells

Although the existence of liver CSCs has been widely accepted, the origin of these cells remains controversial. One possible origin of liver CSCs is the transformed liver stem/progenitor cells [11]. Hepatoblasts are the stem cells of the liver that retain the ability to self-renew and proliferate to provide liver progenitor cells (LPCs), which can differentiate into hepatocytes and cholangiocytes. Malignant transformation of hepatoblasts could be the most direct way to generate liver CSCs. LPCs, a subpopulation of small and oval-shaped cells, usually reside quiescently in the canals of Hering and bile canaliculi. It was reported that LPCs could be derived from hematopoietic stem cells or mesenchymal stem cells [12,13]. Most recently, Sahin et al. demonstrated that hepatocytes were the origin of LPCs [14]. LPCs are known to differentiate into hepatocytes or cholangiocytes and engage in liver regeneration when the replication of liver parenchymal cells is restricted [15]. Nevertheless, chronic liver disease alone with long-lasting inflammation and hepatocyte regeneration might facilitate the transformation of LPCs into liver CSCs [16,17]. Our previous study demonstrated that long-term TGF-β exposure drove the transformation of LPCs into liver CSCs, contributing to cirrhosis-elicited hepatocarcinogenesis [18]. Another possible origin of liver CSCs is transformed adult hepatocytes/cholangiocytes through mutation and dedifferentiation. Distinct from other types of mature parenchymal cells, adult hepatocytes possess the ability to regenerate during liver damage and can differentiate into both hepatocyte and biliary lineages exhibiting stem cell properties [15]. Acquisition of stemness during or after the transformation of hepatocytes might give rise to liver CSC generation (Fig. 1). Recently, Thorgeirsson et al. stably transduced oncogenic H-Ras into

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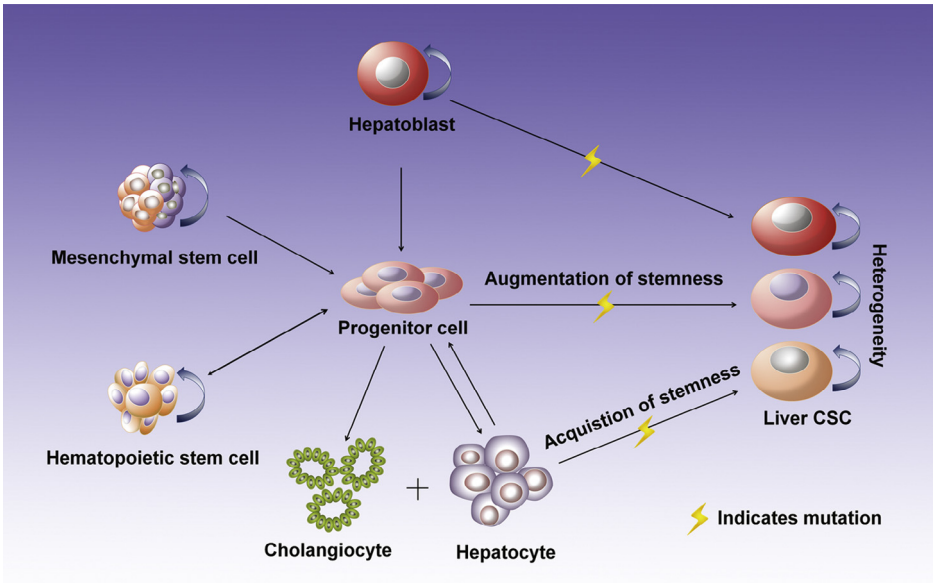


**Fig. 1.** Potential origin and generation of liver CSC.

murine hepatoblasts, LPCs and adult hepatocytes and found that all of these transformed cells acquired stem cell properties and converted into CSCs after the genetic/epigenetic alterations [19]. Additionally, phenotypic flexibility characterizes a subset of cancer cells possessing the capability of interconverting between differentiated and stem-like status via a continuum of cell fate specifications [20]. Recent studies have suggested that non-stem breast cancer cells dedifferentiated into breast CSCs following a typical EMT (epithelial–mesenchymal transition) process [21,22]. These findings implied that the plasticity of liver CSCs might be responsible for the heterogeneous organization of liver cancers and the distinct survival of patients.

## Identification of liver cancer stem cells

In general, liver CSCs can be isolated and enriched based on their immunogenic or functional characteristics. The antigenic approach targets certain cell surface markers and has been utilized to achieve distinct liver CSC fractions from human HCCs. The functional isolation depends on the particular characteristics of liver CSCs, such as side population (SP), high aldehyde dehydrogenase (ALDH) activity and autofluorescence. Considering the plasticity and heterogeneity of the liver CSC origin, it is impossible to define liver CSCs by a single marker or one functional property alone. Therefore, a combination of antigenic and functional approaches could be more appropriate to identify liver CSCs.

### *Surface markers of liver CSCs*

Distinct cell surface proteins have been reported as liver CSC markers, including CD133, EpCAM, CD90, CD44, OV-6, CD13, CD24, DLK1, α2δ1, ICAM-1 and CD47 (Fig. 2). Moreover, some of these markers have been elucidated to possess a regulatory role in liver CSC, which include CD24, CD133, CD47, CD13, CD44 and ICAM-1 [23]. CD133, also known as prominin 1, is a transmembrane glycoprotein expressed in the adult stem cells. Ma et al. reported, for the first time, that CD133 was a liver CSC marker and was required for maintenance of liver CSCs through activation of neurotensin/IL-8/CXCL1 signaling [24,25]. Yamashita et al. proposed the utility of EpCAM for HCC classification and observed that EpCAM$^+$ HCC cells possessed CSC phenotypes, including the capacity to self-renew, differentiate and initiate tumors [26]. Yang et al. isolated CD90$^+$ liver CSCs from MHCC97H and PLC/PRF/5 cell lines and unraveled their CSC properties, including self-renewal, tumor formation and metastatic capacity [27]. Unlike other known liver CSC markers, CD44 serves as a liver CSC marker only in combination with other CSC markers, such as CD133 [28] or CD90 [29]. In addition, a CD44 variant has been unveiled to regulate the redox status by stabilizing xCT and protecting CSCs from oxidative stress [30]. Our lab clarified that the CD133$^+$ cell population was significantly enriched in OV6$^+$ HCC cells and that OV6$^+$ HCC cells possess greater tumorigenicity and chemoresistance compared with OV6$^-$ cells, indicating that OV6 could be another liver CSC marker [31]. Haraguchi et al. identified that CD13$^+$ HCC cells were liver CSCs and were enriched in SP cells isolated from Huh-7, PLC/PRF/5 or Hep3B cells respectively. In further study, they showed that the CD13$^+$ population existed predominantly in the G1/G0 phase and that CD13 expression reduced the cell damage triggered by genotoxic reagents, which was consistent with the quiescent characteristic of

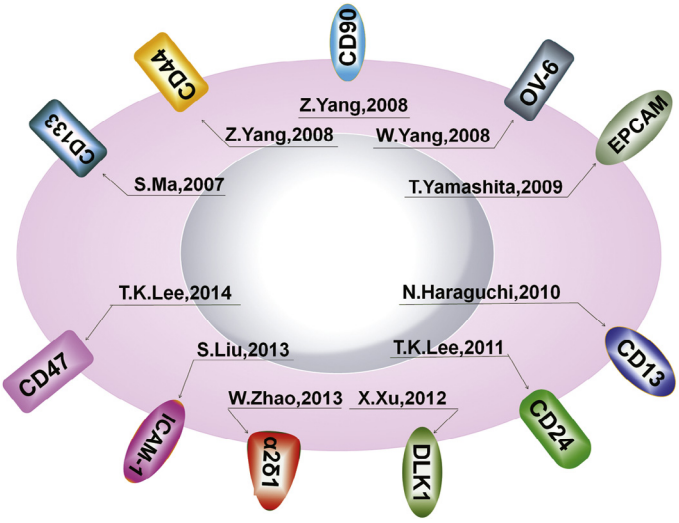


**Fig. 2.** Liver CSC surface markers identified to date.