



## Liver cancer stem cell markers: Progression and therapeutic implications

Jing-Hui Sun, Qing Luo, Ling-Ling Liu, Guan-Bin Song

Jing-Hui Sun, Qing Luo, Ling-Ling Liu, Guan-Bin Song, Key Laboratory of Biorheological Science and Technology, Ministry of Education, College of Bioengineering, Chongqing University, Chongqing 400044, China

**Author contributions:** Sun JH designed the main concepts and wrote, revised the manuscript; Song GB supervised the writing, drafting and critical revision and final approval of the final version; Luo Q and Liu LL provided scientific and technical knowledge.

**Supported by** The Natural National Science Foundation of China, No. 11272365.

**Conflict-of-interest statement:** We declare that the authors have no conflict of interest.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Guan-Bin Song, PhD, Professor of Biomedical Engineering, Key Laboratory of Biorheological Science and Technology, Ministry of Education, College of Bioengineering, Chongqing University, No.174, Shapingba Street, Shapingba District, Chongqing 400044, China. [song@cqu.edu.cn](mailto:song@cqu.edu.cn)  
Telephone: +86-23-65102507  
Fax: +86-23-65102507

Received: December 21, 2015  
Peer-review started: December 22, 2015  
First decision: January 28, 2016  
Revised: February 12, 2016  
Accepted: March 2, 2016  
Article in press: March 2, 2016  
Published online: April 7, 2016

### Abstract

Cancer stem cells (CSCs) are a small subpopulation in cancer, have been proposed to be cancer-initiating cells, and have been shown to be responsible for chemotherapy resistance and cancer recurrence. The identification of CSC subpopulations inside a tumor presents a new understanding of cancer development because it implies that tumors can only be eradicated by targeting CSCs. Although advances in liver cancer detection and treatment have increased the possibility of curing the disease at early stages, unfortunately, most patients will relapse and succumb to their disease. Strategies aimed at efficiently targeting liver CSCs are becoming important for monitoring the progress of liver cancer therapy and for evaluating new therapeutic approaches. Herein, we provide a critical discussion of biological markers described in the literature regarding liver cancer stem cells and the potential of these markers to serve as therapeutic targets.

**Key words:** Liver cancer; Cancer recurrence; Liver cancer stem cells; Cancer stem cell markers; Targeted therapy

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Liver cancer is the fifth most common cancer and the third leading cause of cancer-related mortality worldwide despite remarkable progress in understanding hepatocarcinogenesis and new therapeutic approaches. Recently, the presence of highly resistant cancer stem cells (CSCs) in liver cancer has been proposed to be responsible for tumor growth, invasion, metastasis and recurrence. CSC involvement in liver cancer pathogenesis also highlights them as preferential targets for therapy. This review specifically focuses on the markers used to define human liver

cancer stem cells, the therapeutic implications of the expression of these markers in patient's primary tumors, and the potential of the markers to serve as therapeutic targets.

Sun JH, Luo Q, Liu LL, Song GB. Liver cancer stem cell markers: Progression and therapeutic implications. *World J Gastroenterol* 2016; 22(13): 3547-3557 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v22/i13/3547.htm> DOI: <http://dx.doi.org/10.3748/wjg.v22.i13.3547>

## INTRODUCTION

Hepatocellular carcinoma (HCC), the most common liver cancer, is the third leading cause of cancer-related mortality worldwide<sup>[1]</sup>, mainly due to its high rate of recurrence, which can be as high as 70% following conventional treatments, such as chemotherapy, arterial embolization, surgical resection, and radiofrequency ablation<sup>[2]</sup>. Some research studies have demonstrated that liver cancers are derived from liver stem cells that are present in adult liver tissue with endogenous or exogenous liver origin, where the former are oogonia located in the smallest terminal of the intrahepatic bile duct. However, the cellular origin of HCC recurrence remains poorly understood, and no specific treatment strategy has been developed that focuses on HCC recurrence. Although the cytological pathogenesis of HCC remains unclear, it has been proposed that HCCs are not created equally and display a great deal of heterogeneity<sup>[3,4]</sup>, as there are abundant distinct tumor cell populations expressing different markers. Only a rare subset of cancer cells with stem cell properties, often referred to as liver cancer stem cells (LCSCs), are considered to be responsible for tumor growth, metastasis and recurrence of HCC as well as for the failure of chemotherapy and radiotherapy<sup>[5]</sup>. These findings indicate that liver cancer therapies, although killing a majority of tumor cells, may ultimately fail because they do not eliminate LCSCs, which survive to regenerate new tumors (Figure 1). Therefore, the cancer stem cell theory offers novel insight into tumor diagnosis, treatment and prevention.

Recent rapid progress in CSC research has encountered increasing challenges in which the identification of CSC-specific marker sets and targeted therapeutic destruction are the most frequently debated topics. CSC markers must be clearly defined for each tissue, and clarifying cellular and signaling functions of CSCs is key to conducting better identification and diagnosis based on CSC biomarkers for targeting CSCs, which will undoubtedly improve prevention and treatment for many types of CSCs. To achieve better understanding and treatment of LCSCs, we must better understand the markers of stemness and cell fractions associated with prognosis, metastasis, and resistance. These markers are necessary to isolate CSCs and analyze

their biological characteristics to target them efficiently for therapeutic purposes. Therefore, we summarize here current knowledge on putative markers that define LCSCs, potential functional implications, and therapeutic targets of these markers and provide insights into new therapeutic approaches for more specific targeting and eradication of liver CSCs.

## CELL SURFACE MARKERS OF LIVER CSCS

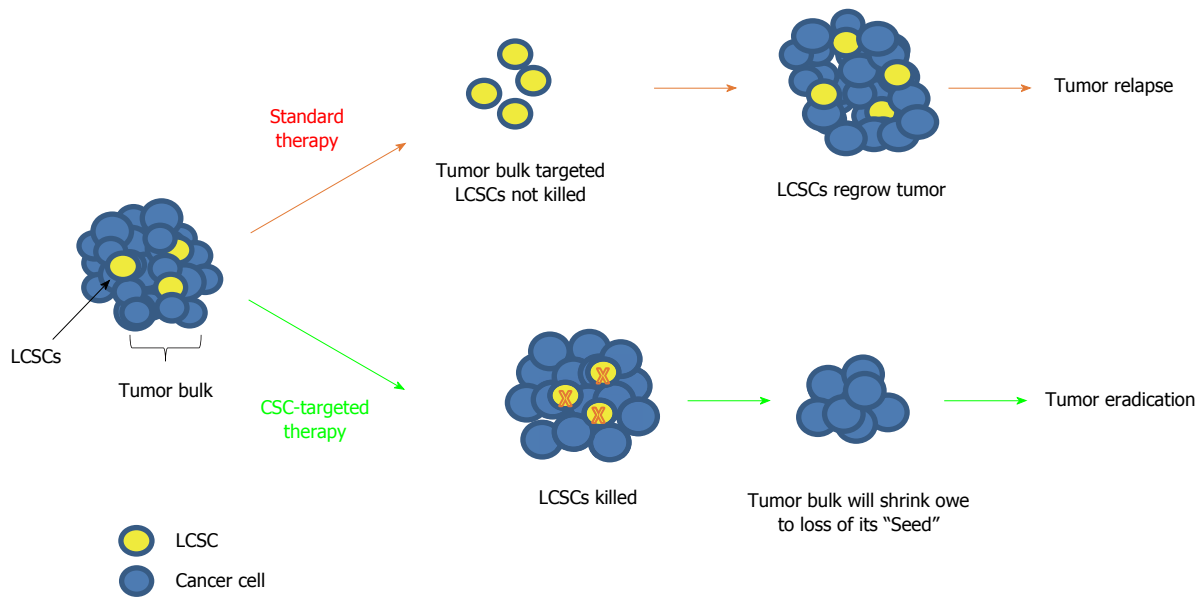
Current surface markers or a particular phenotype are used to identify CSCs. Several markers proposed in the literature to identify CSCs in liver cancer using cell surface antigens are enriched in LCSCs (isolated by FACS or Ab-conjugated magnetic beads). Additionally, cytokeratin 7 and 19 may also serve as relatively specific markers of LCSCs, playing significant roles in hepatocellular carcinoma<sup>[6,7]</sup>.

### CD133

One of the most commonly described surface markers in LCSCs is CD133. CD133, also known as Prominin-1, is a membrane glycoprotein encoded by the CD133/Prom-1 gene<sup>[8]</sup>. It was first detected as a marker of hematopoietic stem cells and has since been shown to be a marker of CSCs in the prostate, colon, and ovaries<sup>[9-11]</sup>. CD133<sup>+</sup> HCC cells were first identified as a potential CSC subpopulation by Suetsugu *et al.*<sup>[12]</sup>. They found that the isolated CD133<sup>+</sup> HCC cells from Huh7 HCC cell lines exhibited higher proliferative and tumorigenic potential and expressed lower levels of mature hepatocyte markers than those of the CD133<sup>-</sup> counterparts. Subsequent reports from Yin *et al.*<sup>[13]</sup> demonstrated a similar result, where the CD133<sup>+</sup> population of HCC SMMC-7721 cells exhibits higher *in vivo* clonogenicity and *in vitro* tumorigenicity than those of the CD133<sup>-</sup> counterparts. We have also researched the enrichment and characterization of LCSCs through a sphere culture system and found that CD133 was significantly enriched in liver CSCs compared with that in MHCC97H cells. Additionally, liver CSCs proliferated significantly faster and induced more tumor colonies than those of MHCC97H cells<sup>[14]</sup>. Enhanced CD133 expression is also found to be an independent prognostic indicator for survival and tumor recurrence in HCC patients<sup>[15]</sup>. Furthermore, CD133-positive cells seemed to be increased with the loss of differentiation of the tumor<sup>[16]</sup>.

### Aldehyde dehydrogenase

Aldehyde dehydrogenase (ALDH) is a detoxifying enzyme responsible for the oxidation of intracellular aldehydes, which is engaged in early differentiation of stem cells by retinol oxidation to retinoic acid<sup>[17]</sup>. ALDH activity has been found to be upregulated in murine and neural stem and human hematopoietic and progenitor cells<sup>[18]</sup>. ALDH is also widely used as a CSC marker in



**Figure 1 Targeting liver cancer stem cells is necessary to prevent tumor recurrence.** LCSCs are resistant to the current standard of care: surgical resection, radiotherapy and chemotherapy. Therapeutic approaches and strategies to target LCSCs in addition to the differentiated tumor cells are necessary to effectively treat the entire cancer and prevent tumor recurrence. LCSCs: Liver cancer stem cells.

many types of cancer, including colon<sup>[19]</sup>, breast<sup>[20]</sup>, ovary<sup>[21]</sup>, bladder<sup>[22]</sup> and prostate<sup>[23]</sup>. In liver cancer, Yin *et al.*<sup>[13]</sup> suggested that ALDH is expressed in LCSCs and is positively correlated with CD133 expression. The combination of these markers can define LCSCs more accurately; dual-color FACS analysis found that the majority of ALDH<sup>+</sup>HCC cells were CD133<sup>+</sup>, yet not all CD133<sup>+</sup>HCC cells were ALDH<sup>+</sup>. A hierarchical organization of cells that differentially express CD133 and ALDH exhibit descending tumorigenic potential in the order of CD133<sup>+</sup>ALDH<sup>+</sup> > CD133<sup>+</sup>ALDH<sup>-</sup> > CD133<sup>-</sup>ALDH<sup>-</sup><sup>[13]</sup>, which implies that ALDH expression along CD133 can be used to characterize the tumorigenic liver CSC population more specifically.

### CD90

CD90 is a 25-37 kDa heavily N-glycosylated, glyco-phosphatidylinositol (GPI)-anchored protein expressed in many cells, such as thymocytes, T-cells, neurons, endothelial cells and fibroblasts<sup>[24]</sup>. CD90 operates as an important regulator of cell-cell and cell-matrix interactions, apoptosis, adhesion, migration, cancer and fibrosis<sup>[25]</sup>. CD90 is also expressed in bone marrow-derived stem cells<sup>[26]</sup> and hepatic stem/progenitor cells from adult or fetal livers but not in adult hepatocytes<sup>[27-29]</sup>. It has been identified to be one potential marker in CSCs, including in HCC. Yamashita *et al.*<sup>[30]</sup> investigated the expression patterns of three CSC makers (CD 133, EpCAM, CD90) in 15 primary HCCs with high viability, where EpCAM, CD90 and CD133 are positive in 3, 7 and 15 cell strains, respectively. Although strong correlation of CD90<sup>+</sup> proportions in tumor cells and liver cancer distant metastasis was suggested, the intrinsic mechanics still need to be determined. Additionally, the feasibility of

eradicating cancer cells committed to mesenchymal endothelial lineages by imatinib mesylate, in which CD90<sup>+</sup> cells are believed to be chemosensitive, is proposed. Yang *et al.*<sup>[31]</sup> found that the number of CD90<sup>+</sup> cells increased with the tumorigenicity and metastatic potential in a panel of HCC cell lines. Moreover, CD45<sup>-</sup>CD90<sup>+</sup> cells were detected in all of blood samples from HCC patients, but none in normal subjects or patients with cirrhosis. The CD45<sup>-</sup>CD90<sup>+</sup> subpopulation has the capacity to initiate and maintain tumor formation in SCID/Beige mice, whereas the CD90<sup>-</sup> and CD45<sup>-</sup>CD90<sup>-</sup> cells do not. In conclusion, these results provide evidence of the tumorigenicity and stem cell-like properties of CD45<sup>-</sup>CD90<sup>+</sup> and CD90<sup>+</sup> populations from HCC patients.

### CD44

CD44 is a ubiquitous multi-structural and multi-functional cell surface glycoprotein involved in adhesive cell-cell and cell-matrix interactions, cell migration, cell homing, cell proliferation and angiogenesis<sup>[32]</sup>. All of these biological properties are essential to normal cell physiology, but under certain conditions they are associated with pathological activities, in particular, those of cancer cells<sup>[33]</sup>. Moreover, CD44 is the receptor for hyaluronic acid and has been identified as a CSC marker for several human cancers, including breast<sup>[34]</sup>, gastric, colon, prostate<sup>[35]</sup>, colorectal<sup>[36]</sup>, pancreatic<sup>[37]</sup>, and head and neck squamous cell carcinomas<sup>[38]</sup>. In human liver cancer, CD44 is also an important marker. CD44 and other markers were reported to more accurately define the surface phenotype of liver CSCs. The CD90<sup>+</sup>CD44<sup>+</sup> cells showed a more aggressive phenotype than the CD90<sup>+</sup>CD44<sup>-</sup> counterpart and formed metastatic lesions in immunodeficient

mice. CD44 blockade prevented the formation of local and metastatic tumor nodules, which showed that concomitantly expressed CD44 modulates the biological activity of the CD90<sup>+</sup> CSCs<sup>[39]</sup>. Another study demonstrated that CD44 was preferentially expressed in aCD133<sup>+</sup> population in four HCC cell lines, including Huh7, SMMC7721, MHCCLM3 and MHCC97L. Compared with CD133<sup>+</sup>CD44<sup>-</sup> cells, CD133<sup>+</sup>CD44<sup>+</sup> HCC cells showed more stem cell properties, including extensive proliferation, self-renewal, and differentiation into the bulk of cancer cells. Furthermore, cells double positive for CD133 and CD44 exhibited preferential expression of some stem cell-related genes and were more resistant to chemotherapeutic agents<sup>[40]</sup>.

### CD13

CD13 antigen, a membrane-bound zinc-dependent type II exopeptidase, is widely distributed in many tissues of mammals, such as the intestine, kidney, and liver as well as the central nervous system<sup>[41-43]</sup>. CD13 participates in the final hydrolysis of nutrients and the degradation of bioactive molecules, such as enkephalin and endorphin. In addition, CD13 is highly expressed in many tumor cells and has been considered as a tumor marker that plays a crucial role in tumor cell growth, invasion, metastasis and angiogenesis. Importantly, CD13 is involved in angiogenesis-generating and -modulating signals and in the process of capillary tube formation and is a marker of angiogenic vessels<sup>[43-51]</sup>.

Recently, Haraguchi *et al.*<sup>[52]</sup> identified CD13 as a candidate liver cancer stem cell marker by a surface marker screen based on microarray analysis. CD13<sup>+</sup> liver CSCs were enriched in side population (SP) cells isolated from Huh-7, PLC/PRF/5 and Hep3B cells, which is known as the multi-drug resistant cell fraction with ATP-binding cassette (ABC) transporter expression, and also localized predominantly in G1/G0 phase. These results suggested that CD13<sup>+</sup> cells represent the dormant or slow-growing population that is believed to account for the chemoresistant capacity in HCC. *In vivo* chemosensitivity assays indicated the high multi-drug resistant property of CD13<sup>+</sup> cells. Treatment of liver cancer cells with a CD13 inhibitor or CD13 neutralizing antibody efficiently induced cellular apoptosis *in vitro*, suggesting that CD13 is a liver CSC target.

In mouse xenograft models, the combination of a CD13 inhibitor and the genotoxic chemotherapeutic fluorouracil (5-FU) more efficiently reduced tumor volume compared with either agent alone. CD13 inhibition suppressed the self-renewing and tumor-initiating abilities of LCSCs. In other respects, reactive oxygen species (ROS) have been found to be negatively correlated with surface marker CD13 in cells, where CD13<sup>+</sup> cells have relatively higher ROS than in their CD13<sup>-</sup> counterparts. Furthermore, CD13 inhibition is also able to increase ROS expression. These results suggest that chemo-resistant properties

of CD13<sup>+</sup> cells are regulated by ROS, indicating a positive prospect of treating liver cancer with a CD13 inhibitor and ROS-inducing chemo/radiation therapy<sup>[52]</sup>.

### EpCAM

Epithelial cell adhesion/activating molecule (EpCAM) is encoded by the TACSTD1 gene, one of the first tumor-associated antigens identified<sup>[64]</sup>. EpCAM is highly expressed in a large variety of human adenocarcinomas and squamous cell carcinomas<sup>[53]</sup>. Yamashita *et al.*<sup>[54]</sup> demonstrated that EpCAM<sup>+</sup> HCC showed a distinct molecular signature with features of hepatic progenitor cells, including the presence of known stem/progenitor markers, such as cytokeratin 19, EpCAM, c-Kit, and activated Wnt/ $\beta$ -catenin signaling, whereas EpCAM<sup>-</sup> HCC expressed genes related to mature hepatocytes. Because its expression is highly elevated in premalignant hepatic tissues and in a subset of HCC, EpCAM may serve as an early biomarker of HCC<sup>[55]</sup>. Similar results were observed by other researchers<sup>[56-58]</sup>, reiterating the significance of EpCAM in HCC development. The EpCAM<sup>+</sup>/AFP<sup>+</sup> subtype of HCC was significantly correlated with a poor prognosis for HCC patients. Functional analysis showed that EpCAM<sup>+</sup> HCC cells possessed CSC phenotypes, including the ability to self-renew, differentiate and initiate tumors<sup>[54]</sup>. Moreover, these cells demonstrated EpCAM enrichment by activation of Wnt/ $\beta$ -catenin signaling using the GSK-3 $\beta$  inhibitor BIO, suggesting that EpCAM is a downstream effect or of the Wnt/ $\beta$ -catenin signaling pathway. These data support therapeutic strategies targeting EpCAM<sup>+</sup> liver CSCs through the suppression of Wnt/ $\beta$ -catenin signaling. EpCAM knockdown by RNA interference (RNAi) was shown to cause significant inhibition of cell invasion, sphere formation, and tumorigenicity of HCC cells<sup>[55]</sup>. In addition, knockdown of EpCAM suppressed colony-forming ability in sorted EpCAM<sup>+</sup> HCC cells by EpCAM shRNA (shEpCAM) *in vitro*. EpCAM<sup>+</sup> liver cancer cells highly express the chromatin-remodeling enzyme CHD4, whose knockdown and overexpression separately increased chemosensitivity and chemoresistance to epirubicin *in vitro*<sup>[59]</sup>. Histone deacetylase and poly (ADP-ribose) polymerase are regulators of CHD4. The inhibitory effects of their inhibitors suberohydroxamic acid and AG-014699 were assessed by Nio *et al.*<sup>[60]</sup>, who proposed that either inhibitor alone reduced the number of EpCAM<sup>+</sup> liver cancer stem cells *in vitro* and that the combination of the two inhibitors successfully inhibited tumor growth in a mouse xenograft model.

### OV-6

Hepatic oval cells are an important origin of liver stem cells. OV-6 has been found to be a useful marker for rat oval cells and is thought to be a hepatic stem cell marker<sup>[61-64]</sup>. Oval cells arise in the intraportal area of the liver after treatment with hepatocarcinogens

Table 1 Cell surface markers of liver cancer stem cells

Marker	Cell line/primary tumor	Characteristics of marker-positive CSCs	Inhibitors	Ref.
CD133	PLC8024, Huh7, Hep3B, primary HCC	Self-renewal, tumorigenicity, chemoresistance, invasiveness	Lupeol, Anti-CD133 antibody, antisense oligonucleotides	[10-14]
ALDH	Huh7, HPLC8024, Hep3B	Chemoresistance tumorigenicity	Diethylaminobenzaldehyde	[10,11]
CD90	HepG2, Hep3B, PLC, Huh7, MHCC97L, MHCC97H, Primary HCC	Tumorigenicity, metastasis, circulation	Anti-CD44 antibody	[28,29]
CD44	PLC/PRL/5	Tumorigenicity, invasiveness, chemoresistance, metastasis	RNAi interference, Anti-CD44 antibody, antisense oligonucleotides	[38]
CD13	PLC/PRL/5, Huh7, Hep3B	Tumor formation, cell cycle arrest, chemoresistance, self-renewal	Anti-CD13 antibody, CD13 inhibitor ubenimex	[51]
EpCAM	Huh1, Huh7, primary HCC	Invasiveness, Self-renewal, tumor formation, chemoresistance	RNAi interference, GSK-3 $\beta$ inhibitor BIO, Bispecific antibody EpCAMxCD3	[53,54,60]
OV6	Huh7, SMMC7721, primary HCC	Tumorigenicity, chemoresistance, invasiveness, metastasis	RNAi interference, targeting $\beta$ -catenin	[70]
1B50-1	Huh7, HepG2Hep-12, SMMC7721	Tumorigenic, invasiveness self-renewal	RNAi interference ERK1/2 inhibitor U0126	[71]
SALL4	PLC/PRF/5, Huh7	Proliferation, chemoresistance, tumorigenic	ERK1/2 inhibitor U0126 RNAi interference	[76]
ICAM-1	Hep3B, Huh7	Tumorigenic, metastasis	RNAi interference	[78,79]

CSCs: Cancer stem cells.

or hepatotoxins in rats, these cells and their progeny have the ability to proliferate and differentiate into either biliary cells or hepatocytes<sup>[62,65-69]</sup>. Yang *et al.*<sup>[70]</sup> showed that OV6<sup>+</sup> cells possessed a greater ability to form tumors *in vivo* and that these cells showed a substantial resistance to standard chemotherapy when compared with OV6<sup>-</sup> tumor cells. The OV6<sup>+</sup> population was enriched after Wnt pathway activation, whereas inhibition of  $\beta$ -catenin signaling led to a decrease in the OV6<sup>+</sup> population. OV6<sup>+</sup> HCC cells were more chemoresistant than the OV6<sup>-</sup> counterparts, but this characteristic was reversed upon lentivirus-delivered stable expression of a microRNA targeting  $\beta$ -catenin. This result suggested the importance of the Wnt/ $\beta$ -catenin pathway in the activation and expansion of OV6<sup>+</sup> populations within tumors. Therefore, therapies targeting Wnt/ $\beta$ -catenin signaling may be a promising approach to reverse the chemoresistant nature of OV6<sup>+</sup> liver CSCs<sup>[70]</sup>.

### 1B50-1

In human HCC cells, mAb 1B50-1, which binds to  $\alpha 2\delta 1^+$  isoform 5, selectively targeted LCSCs. Recent studies show that 1B50-1<sup>+</sup> cells could initiate tumors. 1B50-1 binds to a subpopulation of HCC cells, hereafter termed  $\alpha 2\delta 1^+$  cells, that display stem cell-like properties, such as the expression of stem cell-associated genes (OCT4, SOX2, NANOG, and BMI1), increased self-renewal, increased invasiveness and the ability to give rise to both  $\alpha 2\delta 1^+$  and  $\alpha 2\delta 1^-$  cells<sup>[71]</sup>. Interestingly, 1B50-1<sup>+</sup> cells overlapped with CD133<sup>+</sup>, EpCAM<sup>+</sup>, CD13<sup>+</sup>, and ALDH<sup>+</sup> populations of Hep-12 cells. Although the majority of 1B50-1<sup>+</sup> cells were also positive for CD133, EpCAM, CD13, and ALDH in Huh7 cells, only a small fraction of CD133<sup>+</sup>, EpCAM<sup>+</sup>, CD13<sup>+</sup>, or ALDH<sup>+</sup> cells were 1B50-1<sup>+</sup>. A similar correlation

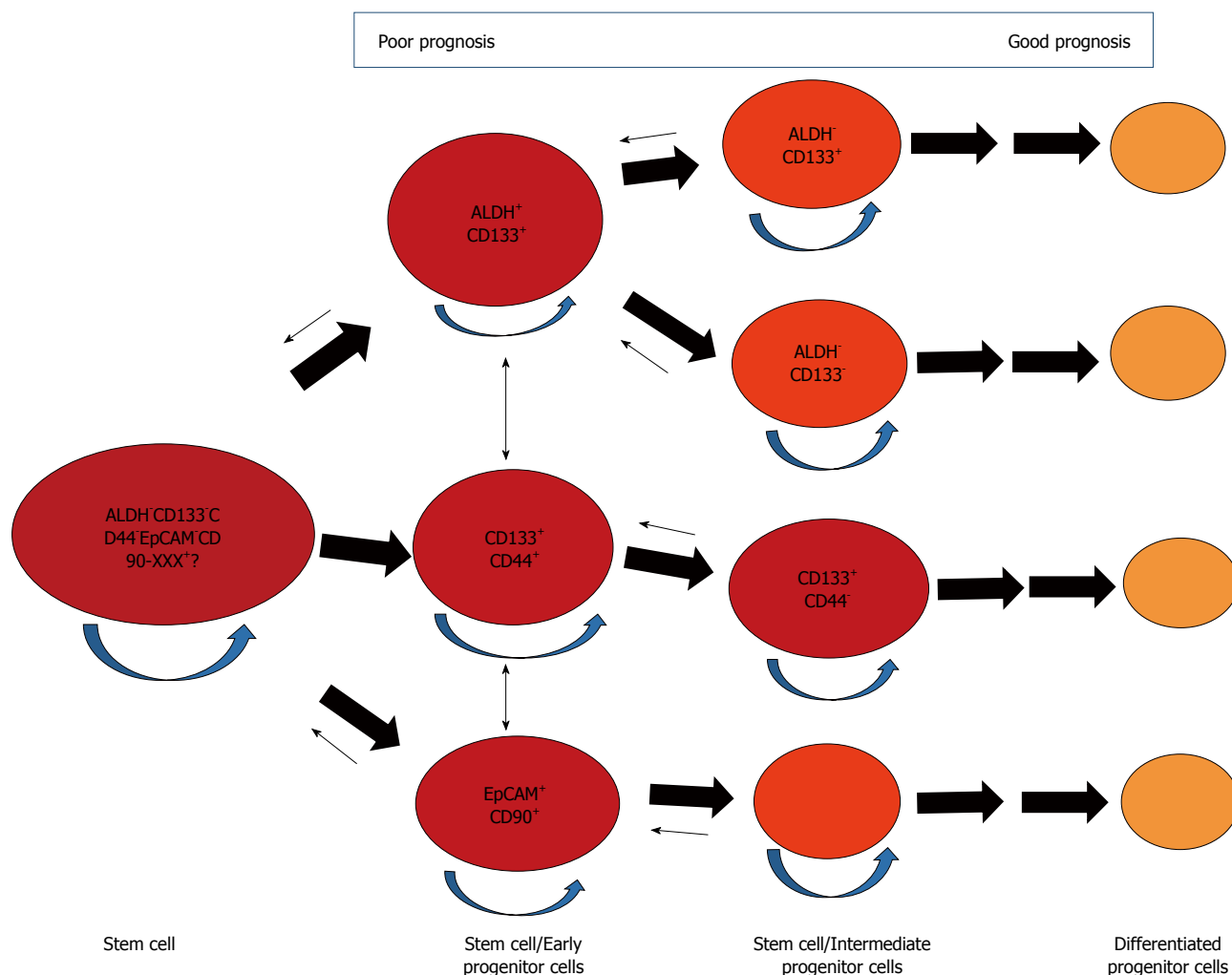
between 1B50-1 and these reported liver CSC markers was also found in other HCC cell lines and patient-derived cells (Table 1). Thus, 1B50-1<sup>+</sup> cells represent fractions of CD133<sup>+</sup>, EpCAM<sup>+</sup>, and CD13<sup>+</sup> populations but not vice versa<sup>[71]</sup>.

### SALL4

Sal-like protein 4 (SALL4) is a member of a family of zinc finger transcription factors that regulates embryogenesis, organogenesis, and pluripotency. SALL4 is able to elicit reprogramming of somatic cells and is a marker of stem cells. Some research studies have shown that SALL4 is constitutively expressed in hematopoietic stem cells and is a potent regulator of their expansion<sup>[72,73]</sup>. SALL4 has also been identified as a novel molecule in reprogramming of somatic cells to become iPSCs<sup>[74,75]</sup>. Recent research shows that SALL4 is a novel therapeutic target for liver cancers. Bioinformatics analyses showed that elevated expression of SALL4 in tumors is closely related to poor survival of HCC patients. *In vitro*, overexpression of SALL4 promotes cell proliferation and elevates the expression of EpCAM, cytokeratin 19 (CK19), and adenosine triphosphate (ATP)-binding cassette-G2 (ABCG2)<sup>[76]</sup>. In summary, SALL4 may be a prognostic marker of liver cancer and an indicator of stem cells, playing roles in 5-FU resistance and growth of cells, and tumors with suppressed SALL4 results in differentiation and delayed tumor growth.

### ICAM-1

Intercellular adhesion molecule 1 (ICAM-1), a 90-kD cell surface glycoprotein of the immunoglobulin superfamily, is believed to be responsible for HCC metastasis<sup>[77]</sup>. Previous studies have shown that hepatocytes are negative for ICAM-1 in cancerous



**Figure 2 Proposed hierarchy of liver cancer stem cells according to the current literature.** Liver cancer stem cell markers have been defined that can give rise to ALDH<sup>+</sup>CD133<sup>+</sup>, EpCAM<sup>+</sup>CD90<sup>+</sup>, CD133<sup>+</sup>CD44<sup>+</sup> and other early progenitor cells, each of which can subsequently divide into more differentiated progenitor cells. Rounded arrows show cells with self-renewal capacity that have the potential to serve as CSCs. Small arrows show the unproven potential for "de-differentiation". CSCs: Cancer stem cells.

areas but not on hepatocytes in noncancerous areas. The expression of ICAM-1 has been reported to mediate adhesion-dependent cell-cell interactions and facilitate the movement of cells through the extracellular matrix, and it has been shown to be positively correlated with tumor size and poor prognosis in HCC<sup>[78]</sup>. Liu *et al.*<sup>[79]</sup> indicated that ICAM-1 could be used as a potential CSC marker and may therefore be helpful in developing an effective treatment against cancer. ICAM-1<sup>+</sup> HCC have also been shown to be highly sphere-forming, have high tumorigenic capability and have increased expression of stemness-related genes in comparison with their ICAM-1<sup>-</sup> counterparts.

### ESTABLISHMENT OF A HIERARCHY IN LIVER CANCER THROUGH LIVER CANCER STEM CELL MARKERS

Some research studies have indicated that different

biomolecules are used as markers to identify and isolate cell populations with liver cancer stem cell properties, including the ability to generate tumors through the stem cell processes of self-renewal, ability to differentiate into multiple cell types, ability to undergo asymmetric division, and increased resistance to radio-/chemotherapy. One important task will be to confirm the relationship among these markers, as different markers of liver CSCs may be organized as a hierarchy of liver cancer cells (Figure 2). The establishment of such a hierarchy allows for the identification of which markers regulate CSC self-renewal, proliferation and differentiation. These abilities of LCSCs represent important therapeutic targets.

In the basic research field, Ma *et al.*<sup>[80]</sup> found that ALDH and CD133 as CSC markers could be used either alone or in combination to identify different chemoresistant liver CSC populations and define a simple hierarchy. ALDH<sup>+</sup> cells with CD133<sup>+</sup> or CD133<sup>-</sup> phenotype could initiate tumors in mice. The study also showed that the majority of ALDH<sup>+</sup> cells were

CD133<sup>+</sup>, yet not all CD133<sup>+</sup> HCC cells were ALDH<sup>+</sup>. A hierarchical organization of cells that differentially express CD133 and ALDH exhibit an ascending tumorigenic potential in the order of CD133<sup>+</sup>ALDH<sup>+</sup>, CD133<sup>+</sup>ALDH<sup>-</sup>, and CD133<sup>-</sup>ALDH<sup>-</sup>. Similarly, Zhu *et al.*<sup>[40]</sup> observed that CD44 was consistently preferentially expressed in CD133<sup>+</sup> cells at the mRNA level compared to the corresponding CD133<sup>-</sup> cells from HCC cell lines. Multimarker analyses by flow cytometry revealed similar preferential expression of CD44 in the CD133<sup>+</sup> cell population. Specifically, the majority of CD133<sup>+</sup> cells from the SMMC-7721, MHCC-LM3 and MHCC-97L cell lines also expressed CD44. For Huh7, although the percentage of CD133<sup>+</sup> cells was more than 60%, only 1.88% of cells co-expressed CD133 and CD44, more likely representing a minority of the CSC subset. CD133<sup>+</sup>CD44<sup>+</sup> HCC cells showed stem cell properties, including extensive proliferation, self-renewal, and differentiation into the bulk of cancer cells. *In vivo* xenograft experiments revealed that, actually, the highly tumorigenic capacity of CD133<sup>+</sup> cells as previously described was primarily attributed to the CD133<sup>+</sup>CD44<sup>+</sup> cell subpopulation instead of their CD133<sup>+</sup>CD44<sup>-</sup> counterparts. Moreover, cells double-positive for CD133 and CD44 exhibited preferential expression of some stem cell-associated genes and were more resistant to chemotherapeutic agents due to the upregulation of ATP-binding cassette (ABC) superfamily transporters, including ABCB1, ABCC1, and ABCG2, further supporting that these cells are of HCC cell origin. These findings suggest that CD133<sup>+</sup>CD44<sup>+</sup> cells might represent true cancer stem/progenitor cells in HCC, which could allow for a better understanding of HCC initiation and progression as well as establish a precise target for the development of more effective therapies<sup>[40]</sup>. Captivatingly, in the clinical research field, Yamashita *et al.*<sup>[54]</sup> found that EpCAM<sup>+</sup> and EpCAM<sup>-</sup> HuH1 cells equally expressed CD133, but only EpCAM<sup>+</sup> cells developed large hypervascular tumors. In addition, these results suggested that EpCAM may be a better marker than CD133 for enriching HCC tumor-initiating cells from AFP<sup>+</sup> tumors. They also found that CD90 expression was limited to HCC cell lines that are EpCAM<sup>-</sup>AFP<sup>-</sup>, and Wnt/ $\beta$ -catenin signaling had little effect on CD90<sup>+</sup> cell enrichment. These results identified that the expression patterns of various stem cell markers in tumor-initiating cells with stem/progenitor cell features may be different in each HCC subtype, possibly due to the heterogeneity of activated signaling pathways in normal stem/progenitor cells where these tumor-initiating cells may originate. Consequently, it would be useful to comprehensively research the expression patterns of stem cell markers to characterize the population of CSCs that may correlate with the activation of their distinct molecular pathways.

From all of the observations described above, asymmetric division of liver CSCs gives rise to CD133<sup>+</sup>CD44<sup>+</sup> or CD133<sup>+</sup>ALDH<sup>+</sup> early progenitor CSCs,

and additional tumor-forming progenitors with more differentiated histology could be produced by further asymmetric division of these early progenitor cells.

## LIVER CSC MARKERS AS THERAPEUTIC TARGETS

CSCs are defined by several markers that could represent potentially important therapeutic targets. In addition, these markers may be functionally important for CSCs, making them even more attractive as therapeutic targets. Despite reports that some markers are useful in the isolation and study of liver CSCs, other tissues may share these markers with hepatocellular carcinoma because of histological variation. The CSC phenotype might not necessarily be universal in all cancer subtypes. It thus appears relevant to identify specific CSC biomarkers, including cell surface markers, to improve prognosis and ultimately patient survival. New treatment strategies involve the development of antibodies that can target these markers. Antibody therapies against tumor cell surface antigens have improved clinical prognosis through inhibition of specific signaling pathways or enhanced activation of direct immune effectors. In some cases, these antibodies are conjugated to a bioactive drug that enables selective targeting of chemotherapeutic agents. Additionally, they block a signaling pathway in which the marker may be involved. Antibodies may also act by an antibody-dependent cytotoxicity (ADCC)/complement-dependent cytotoxicity (CDC) mechanism, thereby enhancing the immune response against CSCs<sup>[81]</sup>. CD133-expressing cells have been suggested to be critical tumorigenic progenitors in HCC, conferring chemoresistance by preferential activation of AKT/PKB and Bcl-2 cell survival response<sup>[82]</sup>. The treatment of CD133<sup>+</sup> HCC cells with an AKT1 inhibitor, which is specific to the Akt/PKB pathway, significantly reduced the expression of survival proteins. In addition, suppression of CD133 by a murine antibody to human CD133 conjugated to a potent cytotoxic drug reduced the proliferation rate of Hep3B cells *in vitro* and delayed tumor growth in a SCID mouse model<sup>[83]</sup>. Potential drug-resistant cell subpopulations can hopefully be eliminated in many cancers, such as liver cancer, retinoblastoma, ovarian cancer, prostatic adenocarcinoma, pancreatic cancer, or colorectal cancer, through the development of CD133 targeting antibodies. Multimarker methods have been applied in the characterization of CSCs in breast<sup>[34]</sup> and pancreatic cancers<sup>[37]</sup>. In liver cancer, CD133<sup>+</sup>/CD44<sup>+</sup> HCC cells were more tumorigenic than those of CD133<sup>+</sup>/CD44<sup>-</sup> cells *in vivo*. A recent study suggested that the CSC phenotype could be precisely defined by co-expression of CD133 and CD44 cell surface markers. CD133<sup>+</sup>/CD44<sup>+</sup> HCC cells showed stem cell properties, including extensive proliferation, self-renewal and differentiation into the bulk of cancer cells. Additionally, recent studies

also revealed that blocking CD44 signaling using an anti-CD44 antibody might be a potential strategy to eradicate liver CSCs and consequently cure those patients<sup>[40]</sup>. A previous study demonstrated that CD90<sup>+</sup>CD44<sup>+</sup> HCC cells possess a high capacity for tumorigenicity. Researchers who have characterized this subpopulation of cells have also examined the potential benefits of targeting CD44 *via* a neutralizing antibody approach. The systemic administration of anti-human CD44 antibodies in immunodeficient mice, formed by the intrahepatic inoculation of CD90<sup>+</sup> liver CSCs, suppressed tumor nodule formation of liver tissue and metastatic lesions in lung tissue. Furthermore, the administration of CD44 antibodies was also shown to induce apoptosis in both CD90<sup>+</sup> and CD90<sup>-</sup> cells *in vitro*<sup>[39]</sup>. In research on CD13<sup>+</sup> liver CSCs, Haraguchi *et al.*<sup>[52]</sup> have also indicated that the combination of a CD13 inhibitor and 5-FU dramatically reduced tumor volume compared with that of either agent alone. 5-FU inhibited proliferating CD13<sup>+</sup> semi-quiescent CSCs, and the self-renewing and tumor-initiating abilities of liver CSCs were suppressed by CD13 inhibition. These studies demonstrated a novel treatment strategy of liver cancer by combining a CD13 inhibitor with reactive oxygen species (ROS) -inducing chemo/radiation therapy. Currently, several EpCAM-targeting antibodies are in clinical development, which include Catumaxomab and Adecatumumab. Clinical trials have been conducted in various cancers, including breast, prostate and colon cancers<sup>[84,85]</sup>. In liver cells, RNAi targeting of EpCAM significantly decreased the CSC pool and reduced both tumorigenicity and invasive capacity of CSCs<sup>[52,57]</sup>. Because EpCAM expression is a downstream target of Wnt/ $\beta$ -catenin, these results may have implications for the development of novel target therapies.

In addition to antibody-targeted therapy, a recent discovery by Lee *et al.*<sup>[86]</sup> showed that lupeol, a phytochemical present in fruits and vegetables, could target CD133<sup>+</sup> liver CSCs by inhibiting their self-renewal and tumorigenic capacity. In addition, lupeol was able to sensitize HCC cells to chemotherapeutic agents (doxorubicin and cisplatin) *via* the PTEN-AKT-ABCG2 signaling pathway. The combination of lupeol, doxorubicin and cisplatin was found to exert a synergistic effect on tumor suppression, allowing the use of a lower dosage of conventional chemotherapeutic drugs, which may substantially reduce the cytotoxic side effects.

Other approaches have also been applied to target liver CSCs utilizing mechanisms that are not dependent on CSC-specific markers. Research studies targeting stem cell-related signaling pathways have shown some efficiency, and these therapeutic studies have been reviewed elsewhere<sup>[87,88]</sup>.

quantity of work researching markers that identify liver CSCs, and these discoveries have contributed to one of the most important developments in cancer treatment. Nevertheless, some important issues still need to be resolved. For example, some of the pivotal markers that are significant to CSCs are also shared by normal stem cells; thus, drugs targeting these markers could have a negative effect on normal stem cells. To specifically target CSCs without unnecessarily affecting normal stem cells, molecular differences between them need to be delineated. In addition, in the coming years, one of the major challenges will be to determine how these different liver CSC markers relate to one another. There is growing concern that a single marker cannot isolate a LCSC population. Increasing evidence has demonstrated that combinations of multiple markers can specifically label CSC populations.

In summary, in the future, more effective liver CSC markers are required to identify and design more specific anti-CSC marker therapies. The apparent advantages of specifically targeting CSCs in improving the potency of existing therapies are revealed in current knowledge, leading to long-term clinical benefits by providing an important framework for developing a novel therapeutic regimen.

## REFERENCES

- 1 **Forner A**, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet* 2012; **379**: 1245-1255 [PMID: 22353262 DOI: 10.1016/S0140-6736(11)61347-0]
- 2 **Bruix J**, Colombo M. Hepatocellular carcinoma: current state of the art in diagnosis and treatment. *Best Pract Res Clin Gastroenterol* 2014; **28**: 751 [PMID: 25260305 DOI: 10.1016/j.bpg.2014.08.010]
- 3 **Nishi M**, Sakai Y, Akutsu H, Nagashima Y, Quinn G, Masui S, Kimura H, Perrem K, Umezawa A, Yamamoto N, Lee SW, Ryo A. Induction of cells with cancer stem cell properties from nontumorigenic human mammary epithelial cells by defined reprogramming factors. *Oncogene* 2014; **33**: 643-652 [PMID: 23318426 DOI: 10.1038/onc.2012.614]
- 4 **Hamburger AW**, Salmon SE. Primary bioassay of human tumor stem cells. *Science* 1977; **197**: 461-463 [PMID: 560061 DOI: 10.1126/science.560061]
- 5 **Xu XL**, Xing BC, Han HB, Zhao W, Hu MH, Xu ZL, Li JY, Xie Y, Gu J, Wang Y, Zhang ZQ. The properties of tumor-initiating cells from a hepatocellular carcinoma patient's primary and recurrent tumor. *Carcinogenesis* 2010; **31**: 167-174 [PMID: 19897602 DOI: 10.1093/carcin/bgp232]
- 6 **Durnez A**, Verslype C, Nevens F, Fevery J, Aerts R, Pirenne J, Lesaffre E, Libbrecht L, Desmet V, Roskams T. The clinicopathological and prognostic relevance of cytokeratin 7 and 19 expression in hepatocellular carcinoma. A possible progenitor cell origin. *Histopathology* 2006; **49**: 138-151 [PMID: 16879391]
- 7 **Kim H**, Choi GH, Na DC, Ahn EY, Kim GI, Lee JE, Cho JY, Yoo JE, Choi JS, Park YN. Human hepatocellular carcinomas with "Stemness"-related marker expression: keratin 19 expression and a poor prognosis. *Hepatology* 2011; **54**: 1707-1717 [PMID: 22045674 DOI: 10.1002/hep.24559]
- 8 **Grosse-Gehling P**, Fargeas CA, Dittfeld C, Garbe Y, Alison MR, Corbeil D, Kunz-Schughart LA. CD133 as a biomarker for putative cancer stem cells in solid tumours: limitations, problems and challenges. *J Pathol* 2013; **229**: 355-378 [PMID: 22899341 DOI: 10.1002/path.4086]
- 9 **Vander Griend DJ**, Karthaus WL, Dalrymple S, Meeker A,

## CONCLUSION

During the past few years there has been a great



- DeMarzo AM, Isaacs JT. The role of CD133 in normal human prostate stem cells and malignant cancer-initiating cells. *Cancer Res* 2008; **68**: 9703-9711 [PMID: 19047148 DOI: 10.1158/0008-5472.CAN-08-3084]
- 10 **Shmelkov SV**, Butler JM, Hooper AT, Hormigo A, Kushner J, Milde T, St Clair R, Baljevic M, White I, Jin DK, Chadburn A, Murphy AJ, Valenzuela DM, Gale NW, Thurston G, Yancopoulos GD, D'Angelica M, Kemeny N, Lyden D, Rafii S. CD133 expression is not restricted to stem cells, and both CD133+ and CD133- metastatic colon cancer cells initiate tumors. *J Clin Invest* 2008; **118**: 2111-2120 [PMID: 18497886 DOI: 10.1172/JCI34401]
  - 11 **Qin Q**, Sun Y, Fei M, Zhang J, Jia Y, Gu M, Xia R, Chen S, Deng A. Expression of putative stem marker nestin and CD133 in advanced serous ovarian cancer. *Neoplasma* 2012; **59**: 310-315 [PMID: 22296500 DOI: 10.4149/neo\_2012\_040]
  - 12 **Suetsugu A**, Nagaki M, Aoki H, Motohashi T, Kunisada T, Moriwaki H. Characterization of CD133+ hepatocellular carcinoma cells as cancer stem/progenitor cells. *Biochem Biophys Res Commun* 2006; **351**: 820-824 [PMID: 17097610]
  - 13 **Yin S**, Li J, Hu C, Chen X, Yao M, Yan M, Jiang G, Ge C, Xie H, Wan D, Yang S, Zheng S, Gu J. CD133 positive hepatocellular carcinoma cells possess high capacity for tumorigenicity. *Int J Cancer* 2007; **120**: 1444-1450 [PMID: 17205516]
  - 14 **Sun J**, Luo Q, Liu L, Zhang B, Shi Y, Ju Y, Song G. Biomechanical profile of cancer stem-like cells derived from MHCC97H cell lines. *J Biomech* 2016; **49**: 45-52 [PMID: 26627368 DOI: 10.1016/j.jbiomech.2015.11.007]
  - 15 **Galizia G**, Gemei M, Del Vecchio L, Zamboli A, Di Noto R, Mirabelli P, Salvatore F, Castellano P, Orditura M, De Vita F, Pinto M, Pignatelli C, Lieto E. Combined CD133/CD44 expression as a prognostic indicator of disease-free survival in patients with colorectal cancer. *Arch Surg* 2012; **147**: 18-24 [PMID: 22250106 DOI: 10.1001/archsurg.2011.795]
  - 16 **Liu H**, Zhang W, Jia Y, Yu Q, Grau GE, Peng L, Ran Y, Yang Z, Deng H, Lou J. Single-cell clones of liver cancer stem cells have the potential of differentiating into different types of tumor cells. *Cell Death Dis* 2013; **4**: e857 [PMID: 24136221 DOI: 10.1038/cddis.2013.340]
  - 17 **Koppaka V**, Thompson DC, Chen Y, Ellermann M, Nicolaou KC, Juvonen RO, Petersen D, Deitrich RA, Hurley TD, Vasilioiu V. Aldehyde dehydrogenase inhibitors: a comprehensive review of the pharmacology, mechanism of action, substrate specificity, and clinical application. *Pharmacol Rev* 2012; **64**: 520-539 [PMID: 22544865 DOI: 10.1124/pr.111.005538]
  - 18 **Zhang L**, Wang L, Liu X, Zheng D, Liu S, Liu C. ALDH expression characterizes G1-phase proliferating beta cells during pregnancy. *PLoS One* 2014; **9**: e96204 [PMID: 24787690 DOI: 10.1371/journal.pone.0096204]
  - 19 **Huang EH**, Hynes MJ, Zhang T, Ginestier C, Dontu G, Appelman H, Fields JZ, Wicha MS, Boman BM. Aldehyde dehydrogenase 1 is a marker for normal and malignant human colonic stem cells (SC) and tracks SC overpopulation during colon tumorigenesis. *Cancer Res* 2009; **69**: 3382-3389 [PMID: 19336570 DOI: 10.1158/0008-5472.can-08-4418]
  - 20 **Ginestier C**, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, Jacquemier J, Viens P, Kleer CG, Liu S, Schott A, Hayes D, Birnbaum D, Wicha MS, Dontu G. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. *Cell Stem Cell* 2007; **1**: 555-567 [PMID: 18371393 DOI: 10.1016/j.stem.2007.08.014]
  - 21 **Landen CN**, Goodman B, Katre AA, Steg AD, Nick AM, Stone RL, Miller LD, Mejia PV, Jennings NB, Gershenson DM, Bast RC, Coleman RL, Lopez-Berestein G, Sood AK. Targeting aldehyde dehydrogenase cancer stem cells in ovarian cancer. *Mol Cancer Ther* 2010; **9**: 3186-3199 [PMID: 20889728 DOI: 10.1158/1535-7163.MCT-10-0563]
  - 22 **Su Y**, Qiu Q, Zhang X, Jiang Z, Leng Q, Liu Z, Stass SA, Jiang F. Aldehyde dehydrogenase 1 A1-positive cell population is enriched in tumor-initiating cells and associated with progression of bladder cancer. *Cancer Epidemiol Biomarkers Prev* 2010; **19**: 327-337 [PMID: 20142235 DOI: 10.1158/1055-9965.EPI-09-0865]
  - 23 **van den Hoogen C**, van der Horst G, Cheung H, Buijs JT, Lippitt JM, Guzmán-Ramírez N, Hamdy FC, Eaton CL, Thalmann GN, Cecchini MG, Pelger RC, van der Pluijm G. High aldehyde dehydrogenase activity identifies tumor-initiating and metastasis-initiating cells in human prostate cancer. *Cancer Res* 2010; **70**: 5163-5173 [PMID: 20516116 DOI: 10.1158/0008-5472.CAN-09-3806]
  - 24 **Sukowati CH**, Anfuso B, Torre G, Francalanci P, Crocè LS, Tiribelli C. The expression of CD90/Thy-1 in hepatocellular carcinoma: an in vivo and in vitro study. *PLoS One* 2013; **8**: e76830 [PMID: 24116172 DOI: 10.1371/journal.pone.0076830]
  - 25 **Rege TA**, Hagood JS. Thy-1 as a regulator of cell-cell and cell-matrix interactions in axon regeneration, apoptosis, adhesion, migration, cancer, and fibrosis. *FASEB J* 2006; **20**: 1045-1054 [PMID: 16770003 DOI: 10.1096/fj.05-5460rev]
  - 26 **Davies OG**, Cooper PR, Shelton RM, Smith AJ, Scheven BA. Isolation of adipose and bone marrow mesenchymal stem cells using CD29 and CD90 modifies their capacity for osteogenic and adipogenic differentiation. *J Tissue Eng* 2015; **6**: 2041731415592356 [PMID: 26380065 DOI: 10.1177/2041731415592356]
  - 27 **Herrera MB**, Bruno S, Buttiglieri S, Tetta C, Gatti S, Deregibus MC, Bussolati B, Camussi G. Isolation and characterization of a stem cell population from adult human liver. *Stem Cells* 2006; **24**: 2840-2850 [PMID: 16945998 DOI: 10.1634/stemcells.2006-0114]
  - 28 **Dan YY**, Riehle KJ, Lazaro C, Teoh N, Haque J, Campbell JS, Fausto N. Isolation of multipotent progenitor cells from human fetal liver capable of differentiating into liver and mesenchymal lineages. *Proc Natl Acad Sci USA* 2006; **103**: 9912-9917 [PMID: 16782807 DOI: 10.1073/pnas.0603824103]
  - 29 **Lázaro CA**, Croager EJ, Mitchell C, Campbell JS, Yu C, Foraker J, Rhim JA, Yeoh GC, Fausto N. Establishment, characterization, and long-term maintenance of cultures of human fetal hepatocytes. *Hepatology* 2003; **38**: 1095-1106 [PMID: 14578848]
  - 30 **Yamashita T**, Honda M, Nakamoto Y, Baba M, Nio K, Hara Y, Zeng SS, Hayashi T, Kondo M, Takatori H, Yamashita T, Mizukoshi E, Ikeda H, Zen Y, Takamura H, Wang XW, Kaneko S. Discrete nature of EpCAM+ and CD90+ cancer stem cells in human hepatocellular carcinoma. *Hepatology* 2013; **57**: 1484-1497 [PMID: 23174907 DOI: 10.1002/hep.26168]
  - 31 **Yang ZF**, Ngai P, Ho DW, Yu WC, Ng MN, Lau CK, Li ML, Tam KH, Lam CT, Poon RT, Fan ST. Identification of local and circulating cancer stem cells in human liver cancer. *Hepatology* 2008; **47**: 919-928 [PMID: 18275073 DOI: 10.1002/hep.22082]
  - 32 **van der Windt GJ**, Schouten M, Zeerleder S, Florquin S, van der Poll T. CD44 is protective during hyperoxia-induced lung injury. *Am J Respir Cell Mol Biol* 2011; **44**: 377-383 [PMID: 20463290 DOI: 10.1165/rcmb.2010-0158OC]
  - 33 **Naor D**, Nedvetski S, Golan I, Melnik L, Faitelson Y. CD44 in cancer. *Crit Rev Clin Lab Sci* 2002; **39**: 527-579 [PMID: 12484499]
  - 34 **Al-Hajj M**, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA* 2003; **100**: 3983-3988 [PMID: 12629218 DOI: 10.1073/pnas.0530291100]
  - 35 **Collins AT**, Berry PA, Hyde C, Stower MJ, Maitland NJ. Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res* 2005; **65**: 10946-10951 [PMID: 16322242 DOI: 10.1158/0008-5472.CAN-05-2018]
  - 36 **Dalerba P**, Dylla SJ, Park IK, Liu R, Wang X, Cho RW, Hoey T, Gurney A, Huang EH, Simeone DM, Shelton AA, Parmiani G, Castelli C, Clarke MF. Phenotypic characterization of human colorectal cancer stem cells. *Proc Natl Acad Sci USA* 2007; **104**: 10158-10163 [PMID: 17548814 DOI: 10.1073/pnas.0703478104]
  - 37 **Li C**, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, Wicha M, Clarke MF, Simeone DM. Identification of pancreatic cancer stem cells. *Cancer Res* 2007; **67**: 1030-1037 [PMID: 17283135 DOI: 10.1158/0008-5472.CAN-06-2030]
  - 38 **Prince ME**, Sivanandan R, Kaczorowski A, Wolf GT, Kaplan MJ,

- Dalerba P, Weissman IL, Clarke MF, Ailles LE. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. *Proc Natl Acad Sci USA* 2007; **104**: 973-978 [PMID: 17210912 DOI: 10.1073/pnas.0610117104]
- 39 **Yang ZF**, Ho DW, Ng MN, Lau CK, Yu WC, Ngai P, Chu PW, Lam CT, Poon RT, Fan ST. Significance of CD90+ cancer stem cells in human liver cancer. *Cancer Cell* 2008; **13**: 153-166 [PMID: 18242515 DOI: 10.1016/j.ccr.2008.01.013]
- 40 **Zhu Z**, Hao X, Yan M, Yao M, Ge C, Gu J, Li J. Cancer stem/progenitor cells are highly enriched in CD133+CD44+ population in hepatocellular carcinoma. *Int J Cancer* 2010; **126**: 2067-2078 [PMID: 19711346 DOI: 10.1002/ijc.24868]
- 41 **Chen H**, Kinzer CA, Paul WE. p161, a murine membrane protein expressed on mast cells and some macrophages, is mouse CD13/aminopeptidase N. *J Immunol* 1996; **157**: 2593-2600 [PMID: 8805662]
- 42 **Riemann D**, Kehlen A, Langner J. CD13--not just a marker in leukemia typing. *Immunol Today* 1999; **20**: 83-88 [PMID: 10098327 DOI: 10.1016/S0167-5699(98)01398-X]
- 43 **Mina-Osorio P**. The moonlighting enzyme CD13: old and new functions to target. *Trends Mol Med* 2008; **14**: 361-371 [PMID: 18603472 DOI: 10.1016/j.molmed.2008.06.003]
- 44 **Pasqualini R**, Koivunen E, Kain R, Lahdenranta J, Sakamoto M, Stryhn A, Ashmun RA, Shapiro LH, Arap W, Ruoslahti E. Aminopeptidase N is a receptor for tumor-homing peptides and a target for inhibiting angiogenesis. *Cancer Res* 2000; **60**: 722-727 [PMID: 10676659]
- 45 **Bhagwat SV**, Lahdenranta J, Giordano R, Arap W, Pasqualini R, Shapiro LH. CD13/APN is activated by angiogenic signals and is essential for capillary tube formation. *Blood* 2001; **97**: 652-659 [PMID: 11157481 DOI: 10.1182/blood.V97.3.652]
- 46 **Bhagwat SV**, Petrovic N, Okamoto Y, Shapiro LH. The angiogenic regulator CD13/APN is a transcriptional target of Ras signaling pathways in endothelial morphogenesis. *Blood* 2003; **101**: 1818-1826 [PMID: 12406907 DOI: 10.1182/blood-2002-05-1422]
- 47 **Bauvois B**. Transmembrane proteases in cell growth and invasion: new contributors to angiogenesis? *Oncogene* 2004; **23**: 317-329 [PMID: 14724562 DOI: 10.1038/sj.onc.1207124]
- 48 **Bauvois B**, Dauzonne D. Aminopeptidase-N/CD13 (EC 3.4.11.2) inhibitors: chemistry, biological evaluations, and therapeutic prospects. *Med Res Rev* 2006; **26**: 88-130 [PMID: 16216010 DOI: 10.1002/med.20044]
- 49 **Fukasawa K**, Fujii H, Saitoh Y, Koizumi K, Aozuka Y, Sekine K, Yamada M, Saiki I, Nishikawa K. Aminopeptidase N (APN/CD13) is selectively expressed in vascular endothelial cells and plays multiple roles in angiogenesis. *Cancer Lett* 2006; **243**: 135-143 [PMID: 16466852 DOI: 10.1016/j.canlet.2005.11.051]
- 50 **Mahoney KM**, Petrovic N, Schacke W, Shapiro LH. CD13/APN transcription is regulated by the proto-oncogene c-Maf via an atypical response element. *Gene* 2007; **403**: 178-187 [PMID: 17897790 DOI: 10.1016/j.gene.2007.08.010]
- 51 **Yang E**, Shim JS, Woo HJ, Kim KW, Kwon HJ. Aminopeptidase N/CD13 induces angiogenesis through interaction with a pro-angiogenic protein, galectin-3. *Biochem Biophys Res Commun* 2007; **363**: 336-341 [PMID: 17888402 DOI: 10.1016/j.bbrc.2007.08.179]
- 52 **Haraguchi N**, Ishii H, Mimori K, Tanaka F, Ohkuma M, Kim HM, Akita H, Takiuchi D, Hatano H, Nagano H, Barnard GF, Doki Y, Mori M. CD13 is a therapeutic target in human liver cancer stem cells. *J Clin Invest* 2010; **120**: 3326-3339 [PMID: 20697159 DOI: 10.1172/JCI42550]
- 53 **Herlyn M**, Steplewski Z, Herlyn D, Koprowski H. Colorectal carcinoma-specific antigen: detection by means of monoclonal antibodies. *Proc Natl Acad Sci USA* 1979; **76**: 1438-1442 [PMID: 286328]
- 54 **Yamashita T**, Forgues M, Wang W, Kim JW, Ye Q, Jia H, Budhu A, Zanetti KA, Chen Y, Qin LX, Tang ZY, Wang XW. EpCAM and alpha-fetoprotein expression defines novel prognostic subtypes of hepatocellular carcinoma. *Cancer Res* 2008; **68**: 1451-1461 [PMID: 18316609 DOI: 10.1158/0008-5472.CAN-07-6013]
- 55 **Kim JW**, Ye Q, Forgues M, Chen Y, Budhu A, Sime J, Hofseth LJ, Kaul R, Wang XW. Cancer-associated molecular signature in the tissue samples of patients with cirrhosis. *Hepatology* 2004; **39**: 518-527 [PMID: 14768006 DOI: 10.1002/hep.20053]
- 56 **de Boer CJ**, van Krieken JH, Janssen-van Rhijn CM, Litvinov SV. Expression of Ep-CAM in normal, regenerating, metaplastic, and neoplastic liver. *J Pathol* 1999; **188**: 201-206 [PMID: 10398165]
- 57 **Ruck P**, Wichert G, Handgretinger R, Kaiserling E. Ep-CAM in malignant liver tumours. *J Pathol* 2000; **191**: 102-103 [PMID: 10767726]
- 58 **Breuhahn K**, Baeuerle PA, Peters M, Prang N, Töx U, Köhne-Volland R, Dries V, Schirmacher P, Leo E. Expression of epithelial cellular adhesion molecule (Ep-CAM) in chronic (necro-)inflammatory liver diseases and hepatocellular carcinoma. *Hepatol Res* 2006; **34**: 50-56 [PMID: 16364680 DOI: 10.1016/j.hepres.2005.10.006]
- 59 **Kimura O**, Takahashi T, Ishii N, Inoue Y, Ueno Y, Kogure T, Fukushima K, Shiina M, Yamagiwa Y, Kondo Y, Inoue J, Kakazu E, Iwasaki T, Kawagishi N, Shimosegawa T, Sugamura K. Characterization of the epithelial cell adhesion molecule (EpCAM)+ cell population in hepatocellular carcinoma cell lines. *Cancer Sci* 2010; **101**: 2145-2155 [PMID: 20707805 DOI: 10.1111/j.1349-7006.2010.01661.x]
- 60 **Nio K**, Yamashita T, Okada H, Kondo M, Hayashi T, Hara Y, Nomura Y, Zeng SS, Yoshida M, Hayashi T, Sunagozaka H, Oishi N, Honda M, Kaneko S. Defeating EpCAM(+) liver cancer stem cells by targeting chromatin remodeling enzyme CHD4 in human hepatocellular carcinoma. *J Hepatol* 2015; **63**: 1164-1172 [PMID: 26095183 DOI: 10.1016/j.jhep.2015.06.009]
- 61 **Dunsford HA**, Sell S. Production of monoclonal antibodies to preneoplastic liver cell populations induced by chemical carcinogens in rats and to transplantable Morris hepatomas. *Cancer Res* 1989; **49**: 4887-4893 [PMID: 2474376]
- 62 **Dunsford HA**, Karnasuta C, Hunt JM, Sell S. Different lineages of chemically induced hepatocellular carcinoma in rats defined by monoclonal antibodies. *Cancer Res* 1989; **49**: 4894-4900 [PMID: 2474377]
- 63 **Sell S**, Dunsford HA. Evidence for the stem cell origin of hepatocellular carcinoma and cholangiocarcinoma. *Am J Pathol* 1989; **134**: 1347-1363 [PMID: 2474256]
- 64 **Biggaard HC**, Parmelee DC, Dunsford HA, Sechi S, Thorgeirsson SS. 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. *Mol Carcinog* 1993; **7**: 60-66 [PMID: 7679578]
- 65 **Dunsford HA**, Maset R, Salman J, Sell S. Connection of ductlike structures induced by a chemical hepatocarcinogen to portal bile ducts in the rat liver detected by injection of bile ducts with a pigmented barium gelatin medium. *Am J Pathol* 1985; **118**: 218-224 [PMID: 2578739]
- 66 **Evarts RP**, Nagy P, Nakatsukasa H, Marsden E, Thorgeirsson SS. In vivo differentiation of rat liver oval cells into hepatocytes. *Cancer Res* 1989; **49**: 1541-1547 [PMID: 2466557]
- 67 **Elmore LW**, Sirica AE. Phenotypic characterization of metaplastic intestinal glands and ductular hepatocytes in cholangiofibrotic lesions rapidly induced in the caudate liver lobe of rats treated with furan. *Cancer Res* 1991; **51**: 5752-5759 [PMID: 1655260]
- 68 **Novikoff PM**, Yam A, Oikawa I. Blast-like cell compartment in carcinogen-induced proliferating bile ductules. *Am J Pathol* 1996; **148**: 1473-1492 [PMID: 8623918]
- 69 **Golding M**, Sarraf CE, Lalani EN, Anilkumar TV, Edwards RJ, Nagy P, Thorgeirsson SS, Alison MR. Oval cell differentiation into hepatocytes in the acetylaminofluorene-treated regenerating rat liver. *Hepatology* 1995; **22**: 1243-1253 [PMID: 7557877]
- 70 **Yang W**, Yan HX, Chen L, Liu Q, He YQ, Yu LX, Zhang SH, Huang DD, Tang L, Kong XN, Chen C, Liu SQ, Wu MC, Wang HY. Wnt/beta-catenin signaling contributes to activation of normal and tumorigenic liver progenitor cells. *Cancer Res* 2008; **68**: 4287-4295 [PMID: 18519688 DOI: 10.1158/0008-5472.CAN-07-6691]

- 71 **Zhao W**, Wang L, Han H, Jin K, Lin N, Guo T, Chen Y, Cheng H, Lu F, Fang W, Wang Y, Xing B, Zhang Z. 1B50-1, a mAb raised against recurrent tumor cells, targets liver tumor-initiating cells by binding to the calcium channel  $\alpha 2\delta 1$  subunit. *Cancer Cell* 2013; **23**: 541-556 [PMID: 23597567 DOI: 10.1016/j.ccr.2013.02.025]
- 72 **Aguila JR**, Liao W, Yang J, Avila C, Hagag N, Senzel L, Ma Y. SALL4 is a robust stimulator for the expansion of hematopoietic stem cells. *Blood* 2011; **118**: 576-585 [PMID: 21602528]
- 73 **Ma Y**, Cui W, Yang J, Qu J, Di C, Amin HM, Lai R, Ritz J, Krause DS, Chai L. SALL4, a novel oncogene, is constitutively expressed in human acute myeloid leukemia (AML) and induces AML in transgenic mice. *Blood* 2006; **108**: 2726-2735 [PMID: 16763212]
- 74 **Wong CC**, Gaspar-Maia A, Ramalho-Santos M, Reijo Pera RA. High-efficiency stem cell fusion-mediated assay reveals Sall4 as an enhancer of reprogramming. *PLoS One* 2008; **3**: e1955 [PMID: 18414659]
- 75 **Tsubooka N**, Ichisaka T, Okita K, Takahashi K, Nakagawa M, Yamanaka S. Roles of Sall4 in the generation of pluripotent stem cells from blastocysts and fibroblasts. *Genes Cells* 2009; **14**: 683-694 [PMID: 19476507]
- 76 **Oikawa T**, Kamiya A, Zeniya M, Chikada H, Hyuck AD, Yamazaki Y, Wauthier E, Tajiri H, Miller LD, Wang XW, Reid LM, Nakauchi H. Sal-like protein 4 (SALL4), a stem cell biomarker in liver cancers. *Hepatology* 2013; **57**: 1469-1483 [PMID: 23175232 DOI: 10.1002/hep.26159]
- 77 **van de Stolpe A**, van der Saag PT. Intercellular adhesion molecule-1. *J Mol Med (Berl)* 1996; **74**: 13-33 [PMID: 8834767]
- 78 **Zhu PP**, Yuan SG, Liao Y, Qin LL, Liao WJ. High level of intercellular adhesion molecule-1 affects prognosis of patients with hepatocellular carcinoma. *World J Gastroenterol* 2015; **21**: 7254-7263 [PMID: 26109813 DOI: 10.3748/wjg.v21.i23.7254]
- 79 **Liu S**, Li N, Yu X, Xiao X, Cheng K, Hu J, Wang J, Zhang D, Cheng S, Liu S. Expression of intercellular adhesion molecule 1 by hepatocellular carcinoma stem cells and circulating tumor cells. *Gastroenterology* 2013; **144**: 1031-1041.e10 [PMID: 23376424 DOI: 10.1053/j.gastro.2013.01.046]
- 80 **Ma S**, Chan KW, Lee TK, Tang KH, Wo JY, Zheng BJ, Guan XY. Aldehyde dehydrogenase discriminates the CD133 liver cancer stem cell populations. *Mol Cancer Res* 2008; **6**: 1146-1153 [PMID: 18644979 DOI: 10.1158/1541-7786.MCR-08-0035]
- 81 **Okamoto OK**, Perez JF. Targeting cancer stem cells with monoclonal antibodies: a new perspective in cancer therapy and diagnosis. *Expert Rev Mol Diagn* 2008; **8**: 387-393 [PMID: 18598221 DOI: 10.1586/14737159.8.4.387]
- 82 **Ma S**, Lee TK, Zheng BJ, Chan KW, Guan XY. CD133+ HCC cancer stem cells confer chemoresistance by preferential expression of the Akt/PKB survival pathway. *Oncogene* 2008; **27**: 1749-1758 [PMID: 17891174 DOI: 10.1038/sj.onc.1210811]
- 83 **Smith LM**, Nesterova A, Ryan MC, Duniho S, Jonas M, Anderson M, Zabinski RF, Sutherland MK, Gerber HP, Van Orden KL, Moore PA, Ruben SM, Carter PJ. CD133/prominin-1 is a potential therapeutic target for antibody-drug conjugates in hepatocellular and gastric cancers. *Br J Cancer* 2008; **99**: 100-109 [PMID: 18542072 DOI: 10.1038/sj.bjc.6604437]
- 84 **Kurtz JE**, Dufour P. Adecatumumab: an anti-EpCAM monoclonal antibody, from the bench to the bedside. *Expert Opin Biol Ther* 2010; **10**: 951-958 [PMID: 20426706 DOI: 10.1517/14712598.2010.0482098]
- 85 **Gires O**, Bauerle PA. EpCAM as a target in cancer therapy. *J Clin Oncol* 2010; **28**: e239-e40; author reply e239-e40; [PMID: 20385979 DOI: 10.1200/JCO.2009.26.8540]
- 86 **Lee TK**, Castilho A, Cheung VC, Tang KH, Ma S, Ng IO. Lupeol targets liver tumor-initiating cells through phosphatase and tensin homolog modulation. *Hepatology* 2011; **53**: 160-170 [PMID: 20979057 DOI: 10.1002/hep.24000]
- 87 **Gedaly R**, Galuppo R, Daily MF, Shah M, Maynard E, Chen C, Zhang X, Esser KA, Cohen DA, Evers BM, Jiang J, Spear BT. Targeting the Wnt/ $\beta$ -catenin signaling pathway in liver cancer stem cells and hepatocellular carcinoma cell lines with FH535. *PLoS One* 2014; **9**: e99272 [PMID: 24940873 DOI: 10.1371/journal.pone.0099272]
- 88 **Morell CM**, Strazzabosco M. Notch signaling and new therapeutic options in liver disease. *J Hepatol* 2014; **60**: 885-890 [PMID: 24308992 DOI: 10.1016/j.jhep.2013.11.028]

**P- Reviewer:** Hubscher SG, Mizuguchi T **S- Editor:** Ma YJ

**L- Editor:** A **E- Editor:** Zhang DN



