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Review

Review

Liver cancer stem cells as a hierarchical society: yes or no?

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Abstract

Cancer stem cells (CSCs) are cells possessing abilities of self-renewal, differentiation, and tumorigenicity in NOD/SCID mice. Based on this definition, multiple cell surface markers (such as CD24, CD133, CD90, and EpCAM) as well as chemical methods are discovered to enrich liver CSCs in the recent decade. Accumulated studies have revealed molecular signatures and signaling pathways involved in regulating different liver CSCs. Among liver CSCs positive for different markers, some molecular features and regulatory pathways are commonly shared, while some are only unique in certain CSC populations. These studies imply that liver CSCs exhibit diverse heterogeneity, while a functional relationship also exists. The aim of this review is to revisit the society of liver CSCs and summarize the common or unique molecular features of known liver CSCs. We hope to call for attention of researchers on the relationship of the liver CSC subgroups and to provide clues on the hierarchical structure of the liver CSC society.

Key words: cancer stem cell, liver cancer, heterogeneity, EpCAM, CD24

Introduction

About 30 years ago, Dick and his colleagues studied functional heterogeneity within leukemia. They reported the isolated $CD34^+CD38^-$ human leukemic cells initiating acute leukemia after transplantation into immunodeficient mice and predicting relapse in high-risk B-lineage acute lymphoblastic leukemia [1,2]. The isolated $CD34^+CD38^-$ cells were then named leukemic cancer stem cells (CSCs). From then on, CSCs were gradually identified in many solid tumors including breast cancer [3], melanoma [4], pancreas [5,6], prostate [7], ovarian [8,9], lung [10,11], colorectal cancer [12–14], liver cancer [15,16], and brain cancer [17,18]. Now, it is well known that CSCs are the special cancer cells that possess the characteristics associated with normal stem cells, i.e. self-renewal and differentiation, and exhibit an ability of giving rise to cancer cells inheriting all parental features as well as resistance to chemo-/radio-therapy [19].

Up to date, CSC populations expressing different biomarkers have been identified in many cancers. Functional characterization of these CSC subpopulations further reveals the common or unique deregulated molecular signaling pathways in maintaining their CSC features. In this review, we focused on liver CSCs and summarized the current methods to enrich different liver CSCs as well as the common or unique molecular pathways in regulating their stemness features. According to these, we aimed to provide an integrative view whether these known liver CSC populations were potentially hierarchically related to each other.

Primary Liver Cancer and Liver CSCs

Primary liver cancer is the second most deadly human malignancy in men world widely [20,21]. It consists of hepatocellular carcinoma (HCC; 75%–85%), intrahepatic cholangiocarcinoma (iCCA; 10%–15%) as well as other rare types [20]. The main HCC risk factors are well defined such as hepatitis B virus, hepatitis C virus, alcohol, aflatoxin B1, and non-alcoholic fatty liver disease in patients with metabolic syndrome and diabetes [22]. Risk factors of iCCA development include biliary diseases (primitive sclerosing cholangitis, cholelithiasis, biliary cirrhosis), type II diabetes, and the risk factors

contributing to HCC [23,24]. HCC and iCCA are independent tumors derived from distinct cell populations. However, recent studies also indicated that some HCC and iCCA tumors shared similar molecular signatures and even driver genes [25,26].

HCC exhibits strong heterogeneity on its risk factors, clinical parameters as well as molecular profiles. Consistently, multiple cell surface markers, i.e. epithelial cell adhesion molecule (EpCAM), CD133, CD90, CD47, CD44, and CD24 etc., have been identified to enrich corresponding CSCs in HCC. They all present similar 'stemness' features, i.e. the abilities to self-renew and differentiate, chemoresistance, and tumorigenicity in NOD/SCID mice even after serial transplantation. The existence of these different hepatic CSCs partially explains HCC heterogeneity. Researchers have also explored the expressions of hepatic CSC biomarkers in iCCA cell lines and primary iCCA tissues [27]. Higher expression levels of these hepatic CSC markers are related to poor prognosis of iCCA patients and these biomarkers are CD133 [28], EpCAM [29], CD44 [30], CD13 [31], as well as CD90 [32]. However, these hepatic CSC biomarkers have not been used to enrich iCCA CSCs, and there are no reported iCCA CSC biomarkers yet that could meet the current definition of CSCs, i.e. cells possessing abilities of self-renewal, differentiation, chemo/radio-resistance and tumorigenesis. In this review, we thus mainly focused on hepatic CSCs.

Identification of Hepatic CSCs

Until now, there are two main approaches to enrich hepatic CSCs, i.e. the antigenic methods targeting cell surface markers via cell sorting and the functional isolation methods. The functional isolation methods rely on CSC features. With these approaches, hepatic CSCs are a subset of cells isolated from either HCC cell lines or primary HCC tissues and possess CSC features.

Identification of hepatic CSCs by cell surface markers

Distinct surface markers have been identified to characterize liver CSC subpopulations such as EpCAM, CD133 (Prominin 1), CD90 (Thy-1), CD44, CD24, CD47, ICAM1, α2δ1, and oval cell marker OV6, *etc.* [33–36] (**Table 1**). The methods of fluorescence-activated cell sorting (FACS) and magnetic-activated cell sorting with the corresponding antibodies are used to isolate CSC populations from HCC cell lines or the tumor bulk of HCC patients. These enriched subpopulations exhibit the CSC abilities including self-renewal by spheroid formation assay, differentiation as shown by asymmetric division or loss of the expression of CSC biomarkers, tumorigenesis and chemo/radio-therapeutic resistance. For example, isolated $EpCAM^+$ subpopulation from AFP^+ HCC cell lines and AFP^+ primary HCC specimens exhibit such features. This subpopulation could form more and larger spheroids than $EpCAM^-$ cells, differentiate into a mixture of $EpCAM^+$ and $EpCAM^-$ populations, and are highly chemoresistant under the exposure to 5-fluorouracil (5-FU) and doxorubicin treatment. $EpCAM^+$ CSCs from HCC cell lines as well as primary HCC tissues initiate highly invasive tumors in NOD/SCID mice, even after serial transplantation. However, $EpCAM^-$ cells do not possess these properties [16]. **Table 1** summarizes the tumorigenic capabilities of various enriched hepatic CSC subpopulations from HCC cell lines and primary HCC tissues. Given the long-term *in vitro* culture of cancer cell lines, each cancer cell line is relatively homogenous and carries much more genetic and epigenetic variations than its parental cells originally derived from patients. On the other hand, cancer cells from HCC primary specimens of different patients are freshly isolated and more representative. Thus, the results from HCC cell lines are valuable, especially when consistent data are obtained from multiple cell lines. Nevertheless, data from fresh primary HCC tissues are much representative, present the solid evidence of the tumor initiating ability of hepatic CSCs and possess high translational values.

Individual hepatic CSC markers have also been combined to characterize CSC subpopulations, such as the combination of CD44 and CD133 as well as the combination of CD44 and CD90 [37,38]. The results revealed that double positive cells present more aggressive features than cells with the corresponding single CSC biomarker alone. $CD90^+CD44^+$ HCC cells are more tumorigenic than $CD90^+CD44^-$ cells in mice and form lung metastasis lesions while $CD90^+CD44^-$ cells do not. $CD133^+CD44^+$ cells express higher levels of stem cell-associated genes, form more colonies, and are more tumorigenic in immuno-deficient mice as well as more chemoresistant than $CD133^+CD44^-$ cells.

In addition, some groups also constructed lentiviral green fluorescent protein (GFP) vectors with human Nanog or Sox9 promoter, and then employed GFP to monitor and isolate CSCs [39,40]. For example, Shan *et al.* [40] employed a Nanog promoter-driven GFP gene in HCC cells. GFP^+ and GFP^- HCC cells were then sorted via FACS and their CSC features were compared. The sorted GFP^+ cells expressed higher levels of Nanog, OCT4, and Sox2 and exhibited enhanced abilities of self-renewal, differentiation, chemoresistance, as well as tumor initiation than sorted GFP^- cells [40]. A similar assay was also designed with a Sox9 promoter-driven enhanced GFP and comparable results were noticed [39].

Identification of hepatic CSCs through functional assays

Researchers have also enriched hepatic CSCs based on functional features such as the capacity of sphere formation [41], the resistance to chemicals, screening cells with high aldehyde dehydrogenase (ALDH) activity, and staining side population (SP) cells by Hoechst dye 33342 [42]. ALDH is responsible for the oxidation of intracellular aldehydes and ALDH activity; thus ALDH is considered to be a stem cell marker [43], though its role in isolating liver CSCs needs to be further clarified. In different HCC cell lines (Hep3B, Huh7, PLC8024, and HepG2), ALDH activity shows at 89%, 49%, 46%, and 8% of cells, respectively. The isolated $ALDH^+$ HCC cells present chemoresistance. Dual-color flow cytometry analysis showed the majority of $ALDH^+$ HCC cells to be $CD133^+$. The HCC tumorigenicity ability varies with the following order: $CD133^+ALDH^+ > CD133^+ALDH^- > CD133^-ALDH^-$ [44]. However, in primary HCC tissues, Tanaka *et al.* [45] reported that ALDH1A1-overexpressing cells did not show the positive immunohistochemistry staining of many other hepatic CSC markers including EpCAM, BMI1, CD13, CD24, CD90, and CD133. In this vein, assays need to be designed to carefully characterize the CSC features of cells with high ALDH ability, although ALDH activity is likely a potential specific marker for $CD133^+$ CSCs in HCC.

SP cells are cells with low Hoechst staining. They are thought to be CSCs due to the high expression level of adenosine triphosphate-binding cassette transporters, which efflux Hoechst 33342 dye. In many HCC cell lines, SP cells possess higher stemness gene expression and tumorigenesis ability compared with non-SP population [42]. In NOD/SCID mice, 1000 sorted SP cells are able to form tumors and such a tumorigenicity is maintained after serial transplantation. As a control, an injection of 1 million non-SP cells does not initiate tumors

Table 1. Cell surface biomarkers or methods to enrich hepatic CSCs

Markers	Identification source		Tumorigenicity (minimal number of cells)		Ref
	Cell line	Primary tissue	Cell line	Primary tissue	
EpCAM	HuH1, Huh7	Yes	2×10^2	1×10^3	[16,47]
CD133	Huh7, PLC8024, SMMC7721	Yes	1×10^2	2×10^4	[15,48–50]
CD90	HepG2, Hep3B, PLC, Huh7, MHCC97	Yes	5×10^2	5×10^2	[38,47,51]
CD24	PLC/PRF/5, HLE	Yes	5×10^2	4×10^3	[52]
CD44	HepG2	Yes	1×10^2	1×10^3	[53]
ICAM1	Huh7, Hep3B	Yes	1×10^3	2.5×10^3	[34]
CD47	Huh7, PLC/PRF/5	Yes	1×10^3	5×10^2	[35]
α2δ1	Huh7, Hep-11, Hep-12, HepG2, SMMC7721	Yes	1×10^2	1×10^2	[36]
$CD44^+CD133^+$	SMMC7721, MHCC97L, MHCC-LM3	/	1×10^2	/	[37]
$CD44^+CD90^+$	PLC, MHCC97L	/	5×10^2	/	[38]
CD13	Huh7, PLC/PRF/5	/	1×10^2	/	[31]
OV6	Huh7, PLC, SMMC7721, Hep3B, HepG2	/	5×10^3	/	[54]
DLK1	Hep3B, HuH7	/	1×10^4	/	[55]
SP cells	Huh7, PLC/PRF/5	/	1×10^3	/	[42]
KRT19	Huh7, PLC/PRF/5, Hep3B	/	1×10^4	/	[56]
Lgr5	PLC, Hep3B	/	1×10^2	/	[57]
KIAA1114	Huh7, SK-Hep1	/	1×10^3	/	[58]
CD34	PLC/PRF/5	/	1×10^2	/	[59]
ALDH	/	Yes	/	1×10^3	[60]
ALDH, $CD133^+$	PLC8024	/	5×10^2	/	[44]

[42]. Toh *et al.* [46] also reported that flow-sorted SP and $CD44^+$ cells from AKT/β-catenin-driven mouse tumor spheres were more tumorigenic than non-SP and $CD44^-$ cells in immunodeficient mice.

Co-expressed hepatic CSC biomarkers

The establishment of various methods for isolating hepatic CSCs has assisted researchers to explore the heterogeneity feature of hepatic CSCs and sheds light on clarifying potential relationships among heterogenous CSCs. Yamashita *et al.* [16] have revealed that isolated $EPCAM^+$ HCC cells express a high level of CD133 via FACS assay. Furthermore, cells co-expressing CD90 and CD44 are isolated, and they could differentiate to a mixed population of $CD90^+CD44^+$, $CD90^+CD44^-$, and $CD90^-CD44^-$. $CD90^+CD44^+$ CSCs have an earlier tumor onset time and larger tumors in tumorigenicity assay than the $CD90^+CD44^-$ counterpart and also form lung metastasis nodules, while $CD90^+$ cells do not have such a feature [38]. Similar results were also obtained in CSCs co-expressed with CD133 and CD44 [37], CD133 and ALDH [44], as well as CD44 and SP population [46]. Cells with co-expressed hepatic CSC markers exhibited a more tumorigenic ability than their corresponding controls positive with single hepatic CSC biomarker. These results not only show the co-expression of these biomarkers but also indicate their potential hierarchical relationship in hepatic CSC society.

In Table 2, we summarized the known hepatic CSC biomarkers that are co-expressed in different hepatic CSC subpopulations. In literature, CD133, CD90, and EpCAM are early identified hepatic CSC biomarkers and also the most frequently reported markers co-expressed in various hepatic CSCs. CD133 is positive in most of hepatic CSC subpopulations including $EpCAM^+$ cells, $CD24^+$ cells, $CD13^+$ cells, OV6 enriched cells, as well as SP cells. CD90 is positive for $EpCAM^+$ cells, $CD44^+$ cells, $CD13^+$ cells, and OV6 enriched cells. EpCAM is positive in $CD133^+$ cells, $CD24^+$ cells, and OV6 enriched cells. Such a co-expression might indicate their possible key node role in the hepatic CSC hierarchical relationship. However, we need to pay attention to these three biomarkers reported earlier than many other hepatic CSC markers, which likely leads to a frequent detection of their expression in various hepatic CSCs.

Meanwhile, the co-expression of some CSC biomarkers varies among different HCC cell lines. For example, the percentages of $SP/CD44^+$ cell populations are investigated via flow cytometry among six human HCC cell lines including Hep3B, Huh7, LM3, SNU387, SNU398, and SNU449. About 32% and 6% of $SP/CD44^+$ cells are shown in LM3 and SNU449, respectively. Whereas, no $SP/CD44^+$ cells exist in the rest of four cell lines. On the other hand, there is also no solid evidence yet that any hepatic CSC biomarkers might be mutually exclusive. However, from the literature (Table 2), we do notice that CD44 is rarely co-expressed with EpCAM. Thus, in the hierarchal structure of hepatic CSCs, if there is any, the $EpCAM^+$ CSC branch would locate away from $CD44^+$ branch but next to the CD133 branch. In this vein, more broad and in-depth studies are necessary to achieve a panorama on the co-expression of hepatic CSC biomarkers, which will be helpful on untangling their potential linear relationship.

The Origins of Hepatic CSCs

Although the cellular origin of hepatic CSCs remains inconclusive, cells contributing to regenerate hepatocytes under an injured liver condition are thought to be potential resources of hepatic CSCs. Bone marrow stem cells including hematopoietic stem cells and mesenchymal stem cells have high plasticity and could differentiate into mature hepatocytes via forming pluripotent stem cells or by fusion with hepatocytes in normal liver or injured liver [47–49]. Replication of hepatocytes is the major resource of liver regeneration after partial hepatectomy. While replicative ability of hepatocytes is damaged,

Table 2. Co-expressed hepatic CSC biomarkers

CSCs	Other markers	Method	Cell line or primary HCC	Ref
EpCAM	CD133	FACS	Huh7, Hep3B	[16]
	CD90	FACS	primary HCC	[47]
CD133	CD44	FACS	Huh7, PLC8024, SMMC-7721, MHCC-LM3, MHCC97L	[15,37]
	CD24, CD44	FACS	Huh7, Hep3B	[51]
	CD44, EpCAM	FACS	primary HCC, Huh7, PLC8024, Hep3B	[61]
CD24	CD44	FACS	MHCC97L, MHCC97H	[51]
	EpCAM, CD133	FACS	Huh7	[52]
CD44	CD90, CD24	FACS	primary HCC	[53]
CD90	CD44	FACS	MHCC97L, PLC, primary HCC	[38]
OV6	CD133	FACS	Huh7, SMMC7721	[54]
	EpCAM, CD90, CD133	FACS	WB-TβLT	[62]
CD13	CD133	FACS	Huh7	[31]
	CD90	FACS	PLC/PRF/5, primary HCC	[62]
SP cells	BMI1	IHC	Huh7, PLC/PRF/5	[63]
	CD44	FACS	LM3, SNU449	[46]
	CD133, KRT14	IHC	Huh7	[64]

IHC indicates immuno-histochemistry.

liver progenitor cells could also repopulate the damaged liver and restore most hepatocytes [50–52]. Due to their active proliferation and their longevity, stem cells, progenitor cells as well as hepatocytes in liver are favored as targets of oncogenic transformation especially when chronic damage exits. Current researches related to hepatic CSC origin are mainly on these three populations (Fig. 1).

The liver is the hematopoietic organ of the fetus. Hematopoietic stem cells likely reside in adult liver and contribute to HCC development. The existence of hematopoietic stem cells in the liver has been shown by significant frequencies of donor bone marrow-derived cells in clinical patients who received gender-mismatched liver transplant [53]. Zeng *et al.* [54] have reported that $CD34^+$ liver CSCs are positive for OV6, CD43, CD31, and CD45, which are markers of liver stem cells and myelomonocytic cells. They proposed that this $CD34^+$ liver CSC population is formed via a fusion of liver progenitor cells with $CD34^+$ hematopoietic precursor-derived myeloid intermediates. However, in the choline-deficient, ethionine-supplemented diet rat model, observation has also been reported that bone marrow-derived cells are not involved in hepatic tumorigenesis but could fuse with hepatic oval cells [55]. Meanwhile, CD133 is a well-established cell surface marker of hematopoietic stem cells and hepatic progenitor cells [56,57]. In HCC, the expression of CD133 is related to the poor prognosis of HCC patients and enriched $CD133^+$ cells possess hepatic CSC abilities [15,58–61]. These findings indicated that hematopoietic stem cells might be one of the potential resources of hepatic CSCs, whereas more in-depth and direct evidence is still needed to reach a solid conclusion.

Liver progenitor cells and hepatocytes are the resource of liver tumor. At earlier time, it was reported that liver progenitor cells isolated from p53 null mice could initiate tumor and the traced hepatocytes via β-galactosidase expression existed in formed tumor of diethylnitrosamine-induced HCC mouse model [62,63]. Holczbauer *et al.* [64] also co-transduced murine hepatic progenitor cells, hepatoblasts, and hepatocytes with oncogenic H-Ras and SV40LT and investigated the ability of these hepatic lineage-related cells to acquire CSC properties. Strikingly, all these transduced hepatic lineage cells are capable to reprogram into CSCs with increased SP populations, CD133 expression and self-renewing spheroid formation. In the context of chronic hepatocellular injury and inflammation, lineage-tracing studies also revealed that hepatic CSCs may be derived from hepatocytes undergoing dedifferentiation [65]. Moreover, Karin *et al.* [66] described that differentiated hepatocytes were transformed into HCC progenitor cells in the early stage of tumor development. CHD1L is reported to promote the dedifferentiation of HCC cells as a chromatin remodeling factor. It maintains an open chromatin structure for key transcriptional factors related to pluripotency such as TCF4, which allows HCC cells to gain the CSC abilities [67].

It might also be taken into consideration that hepatic CSCs with different markers potentially represent different cellular origins. As mentioned above, CD34 and CD133 are hematopoietic stem cell markers. CD90 is shared by both normal hepatic stem cells and bone marrow-derived mesenchymal stem cells, implying its possible mesenchymal stem cell origin [68]. EpCAM and CK19 are liver progenitor markers, which might indicate the liver progenitor cellular origin [69]. Unfortunately, for many other hepatic CSC subpopulations such as $CD24^+$ and $DLK1^+$ CSCs, it remains unknown about their potential cellular origins. *In vivo* systematically lineage-tracing investigations on different biomarkers may assist to decode the relationship between various hepatic CSC subpopulations.

Molecular Signaling Pathways in Regulating Hepatic CSCs

Data continue to mount on the molecular pathways in regulating the differential expressions of various hepatic CSC biomarkers and the stemness maintenance of CSCs. Here, we summarized the major signaling pathways in hepatic CSC populations with different biomarkers.

Wnt signaling pathway, mainly in $EpCAM^+$ and $CD133^+$ hepatic CSCs

The Wnt signaling pathway is crucial in embryogenesis, cell growth, and tumor formation. Activation of the Wnt/β-catenin pathway starts when Wnt binds to its receptor Frizzled (FZD) proteins. The cytoplasmic protein disheveled is then recruited and phosphorylated, which binds to Axin and disassociates the β-catenin destruction complex (GSK3β/β-catenin/APC/axin). β-catenin thus accumulates

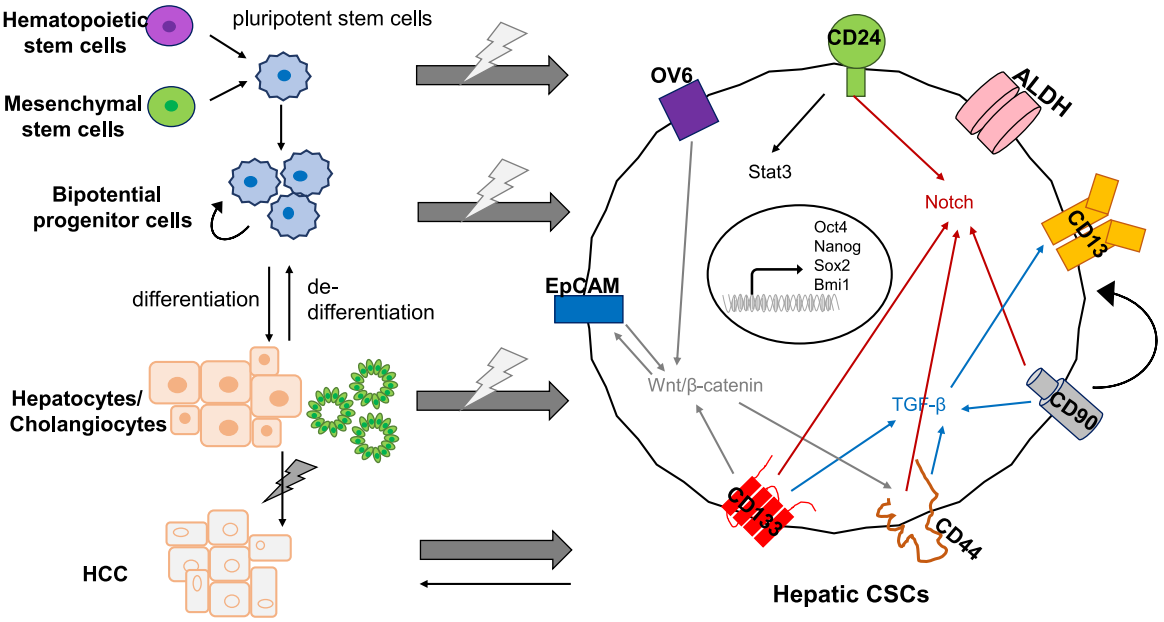

Figure 1. Potential origins and characterization of liver CSCs Pluripotent stem cells, bipotential progenitor cells, hepatocytes or cholangiocytes, and liver cancer cells are potential cellular origins of hepatic CSCs via malignant transformation. Currently, many hepatic CSC biomarkers have been identified such as EpCAM, CD24, CD44, CD90, and CD133. For each CSC population, its associated signaling pathways have also been explored.

and translocates to nucleus where it activates the expression of TCF/LEF target genes such as cyclin D1, c-Myc, and EpCAM [70–74]. Collected evidence suggests that Wnt pathway plays an important role in hepatic CSCs. In many hepatic CSC populations such as CD133$^+$, EpCAM$^+$, Lgr5$^+$, and OV6$^+$ hepatic CSCs, activation of this pathway has been documented [15,16,75,76] (Fig. 2). Among the currently identified hepatic CSC markers, EpCAM is found to be a direct transcriptional target of β-catenin [77].

In liver cancer, it has been frequently observed of mutations in β-catenin, APC and Axin, over-expression of the FZD receptor, and inactivation of GSK-3. These all contribute to aberrant activation of Wnt/β-catenin signaling in HCC [78,79]. Knockdown of YB-1 (a transcriptional factor) suppresses the expression of Wnt ligands (Axin1/2) and β-catenin, impairs the Wnt/β-catenin signaling pathway, and reduces the numbers of hepatic CSCs, such as EpCAM$^+$ cells [80]. βII-Spectrin (SPTBN1), an adapter protein for Smad3/Smad4 complex, maintains the expression of kallistatin, which in turn suppresses Wnt pathway. Consistently, in the SPTBN1-knockout mice, Wnt pathway is activated and EpCAM$^+$ hepatic CSC cells are largely enriched [81]. In Lgr5$^+$ hepatic CSCs, it was reported that LSD1 inhibits the expression of several suppressors of β-catenin signaling such as Prickle1 and APC [75]. HBx enhances the stemness properties of OV6$^+$ HCC cells through activating the MDM2/β-catenin signaling axis. HBx promotes the nucleus translocation of MDM2 and in turn enhances the transcriptional expression of CXCL12 and CXCR4 [82].

Various noncoding RNAs also modulate the stemness features of hepatic CSCs via targeting Wnt pathway. MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression by directing their mRNA targets for translational repression or sometimes degradation. miR-181 family is significantly upregulated in EpCAM$^+$ HCCs and functionally important in maintaining the stemness of EpCAM$^+$ hepatic CSCs. miR-181 inhibits cell differentiation via targeting the GATA-binding protein 6 and CDX2 as well as promotes HCC stemness via target the Wnt signaling inhibitor NLK [83]. Meanwhile, Wnt/β-catenin pathway could also induce the expression of miR-181 family members via directly binding to the promoter region of this family, which leads to a Wnt/miR-181 positive feedback [84]. miR-214 could inhibit the β-catenin pathway directly via targeting CTNNB1 (encoding β-catenin) or the enhancer of Zeste homolog 2 (EZH2), leading to the reduction of EpCAM$^+$ hepatic CSCs [85]. miR-1246 activates the Wnt/β-catenin pathway in CD133$^+$ liver CSCs via targeting Axin2 and GSK3β, two key members of the β-catenin destruction complex [86]. Let-7b reduces the proportion of CD24$^+$CD133$^+$ hepatic CSCs by inhibiting Wnt signaling via downregulating Frizzled4 [87]. Liu *et al.* [88] demonstrate that miR-200a is markedly downregulated in isolated SP cells of F344 rat HCC. miR-200a targets CTNNB1 and knockdown of miR-200a partially activates Wnt pathway, leading to an enhanced spheroid-formation and the high expression of liver CSC markers (EpCAM, CD133, and ABCG2). miR-452 increases the population of liver CSCs by inhibiting SOX7, which directly interacts with β-catenin and TCF4 and blocks the activation of the Wnt/β-catenin pathway [89].

Long non-coding RNAs (LncRNAs) are non-coding RNAs longer than 200 nt and involved in regulating gene transcription, mRNA processing and gene post-transcriptional control [90]. They are also functionally important in regulating Wnt pathway and contribute to the stemness features of liver CSCs, especially CD133$^+$ subpopulations. LncTCF7 is highly expressed in CD133$^+$CD13$^+$ liver CSCs. It recruits the SWI/SNF complex to the TCF7 promoter region, which induces TCF7 expression and then activates Wnt signaling [91]. Lnc-β-Catm is important for the self-renewal of CD133$^+$CD13$^+$ hepatic CSCs via activating Wnt pathway [92]. Through recruiting EZH2, a histone methyltransferase, Lnc-β-Catm induces β-catenin methylation, which abrogates the phosphorylation and degradation of β-catenin. LncTIC1 interacts with the N-terminal of β-catenin and inhibits β-catenin phosphorylation, which helps maintain the stability of β-catenin and consequently activates Wnt signaling. Through these, LncTIC1 participates in CD133$^+$ liver CSC self-renewal [93]. LncAPC activates Wnt/β-catenin signaling and promotes the

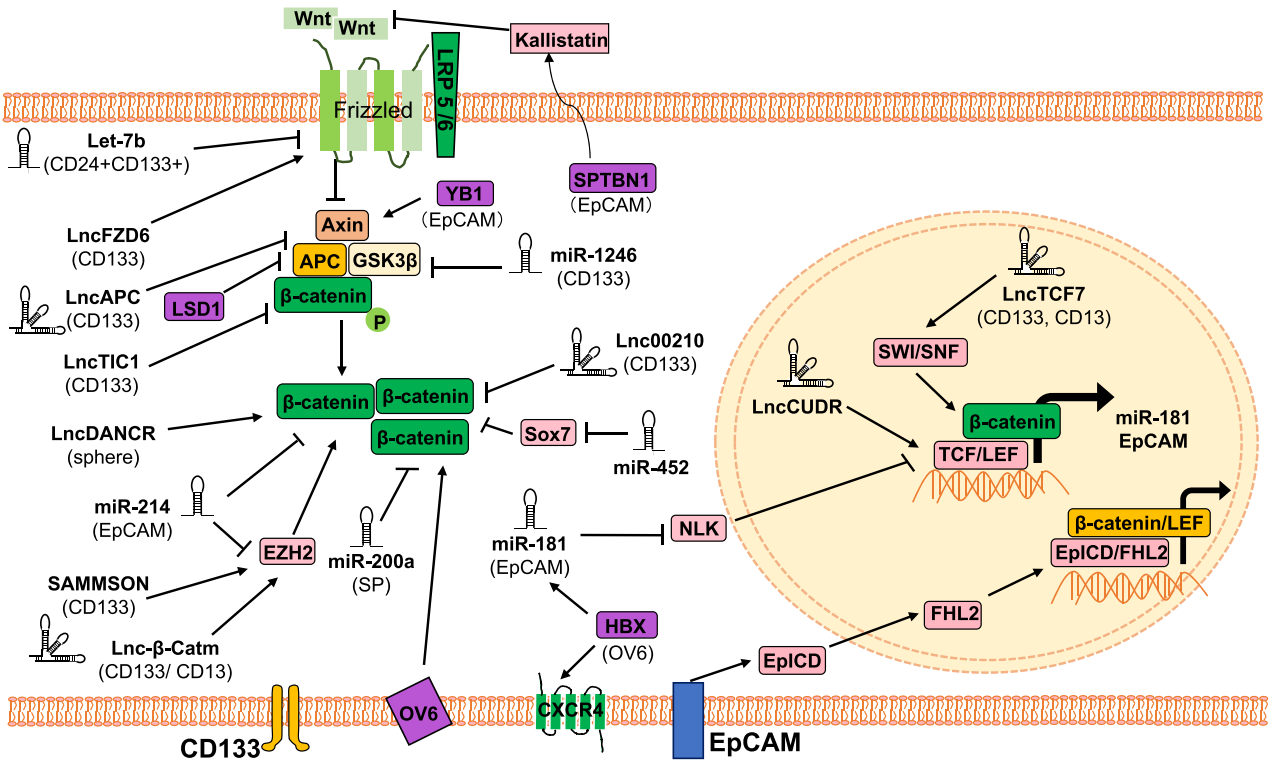

Figure 2. WNT signaling pathway in hepatic CSCs Activation of Wnt signaling pathway has been documented mainly in CD133$^+$ and EpCAM$^+$ hepatic CSCs. Many abnormally expressed genes and non-coding RNAs in HCC have been shown to directly or indirectly regulate Wnt pathway, contributing to the alteration of certain haptic CSC populations. A hepatic CSC marker filled in a bracket in this figure refers to that this hepatic CSC population is altered by the gene typed right above this CSC biomarker.

self-renewal of CD133$^+$ liver CSCs. It locates near the APC locus and recruits EZH2 to APC promoter, which impairs the EZH2-dependent transcription of APC [94]. CTNNBIP1 is a negative regulator of Wnt activation via blocking the interaction of β-catenin with TCF/LEF. Lnc00210 interacts with CTNNBIP1 and blocks the interaction of β-catenin and CTNNBIP1, which consequently drives the activation of Wnt/β-catenin signaling to promote self-renewal and tumor initiating capability of liver CD133$^+$ cells [95]. LncFZD6 promotes Wnt/β-catenin activation and liver CD133$^+$ CSC self-renewal through activating FZD6 expression [96]. In HCC, LncRNA SAMMSON drives self-renewal of CD133$^+$ cells through EZH2-dependent Wnt/β-catenin activation. SAMMSON interacts with EZH2 and then binds to the CTNNBIP1 promoter, contributing to the suppressed expression of CTNNBIP1 in EZH2 dependent manner [97]. In addition, both LncDANCR and LncCUDR are functionally important in liver CSCs via enhancing β-catenin. LncCUDR promotes β-catenin promoter-enhancer chromatin DNA looping formation mediated by CUDR-CTCF complex [98]. LncDANCR reduces the posttranscriptional suppression of β-catenin by miRNA via occupying several miRNA binding sites in the 3′UTR region of CTNNB1 mRNA. These miRNAs are miR-214, miR-320a, and miR-199a [99].

TGF-β signaling pathway, mainly in CD133$^+$ and EpCAM$^+$ hepatic CSCs

The TGF-β signaling pathway is initiated when TGF-β binds to the type II receptor (TGF-βRII). Such a binding leads to the recruitment of the type I receptor (TGF-βRI), and the phosphorylation of receptor-regulated Smads (Smad2/3). The complexes of phosphorylated Smad2/3 and Smad4 are then formed and move into the nucleus, which regulates gene transcription together with other transcription factors. Recently, accumulated results suggest that the TGF-β pathway can induce CD133 expression and is involved in the stemness maintenance of several marker-positive hepatic CSC populations (Fig. 3).

You *et al.* [100] reported that TGF-β1 induces CD133 expression in a time- and dose-dependent manner, via demethylating CD133 promoter region by inhibiting the expression of DNA methyltransferase DNMT1 and DNMT3β. BMP4 could also induce Erk1/2 phosphorylation in a dose-dependent manner, which in turn increases CD133$^+$ hepatic CSCs [101]. Tumor-associated macrophages directly secrete TGF-β1, which promotes epithelial mesenchymal transition (EMT) and CSC-like features as well as increases EpCAM$^+$ hepatic CSC populations [102]. Meanwhile, Cyclin D1 could increase the CD90$^+$ and EpCAM$^+$ hepatic CSC populations, increasing stemness gene expression and increasing chemo-resistance via directly activating Smad2/3 and Smad4 [103]. TGF-β pathway activation by FAM83D could also promote CD44 expression [104].

Many non-coding RNAs are also reported to be involved in TGF-β pathway by which contribute to the stemness regulation of hepatic CSCs. miR-155 significantly increases the EpCAM$^+$ hepatic CSCs [105,106], while TGF-β1 could further induce miR-155 expression [105]. In TGF-β1-induced HCC EMT model, the expression of miR-125b is drastically reduced. Over-expressed miR-125b directly targets Smad2 and Smad4, by which it attenuates EMT phenotype and CSC features of HCC cells such as chemo-resistance,

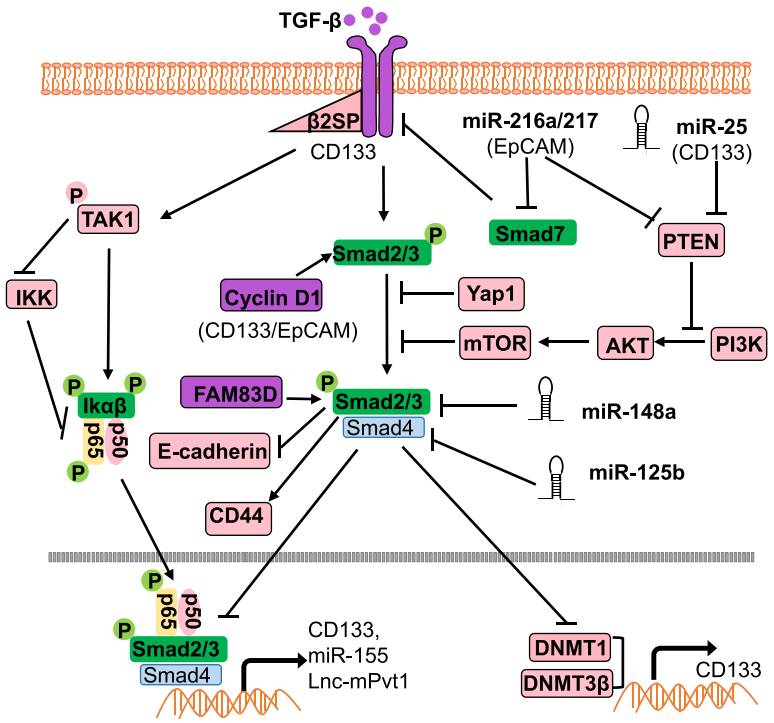

Figure 3. TGF-β signaling pathway in hepatic CSCs TGF-β signaling pathway is involved in the stemness maintenance of several marker-positive hepatic CSC populations, mainly in CD133$^+$ and EpCAM$^+$ hepatic CSCs. Molecules functionally involved in regulating hepatic CSCs via TGF-β signaling pathway are included in this figure. A hepatic CSC marker filled in a bracket in this figure refers to that this hepatic CSC population is altered by the gene typed right above this CSC biomarker.

tumor incidence as well as metastasis in mice model [107]. miR-216a/217 cluster could activate TGF-β and PI3K/AKT pathways by targeting Smad7 and PTEN, contributing to tumor recurrence, sorafenib resistance, and stem-like properties of HCC cells with increased EpCAM$^+$ HCC cells [108]. miR-148a suppresses TGF-β1 signal pathways by repressing Smad2 and ACVR1, a key member of BMP type I receptors, by which miR-148a suppresses cell proliferation, invasion, and subcutaneous tumor growth of HCC cells [109,110].

IL-6/STAT3 signaling pathway, mainly in CD44$^+$ and CD133$^+$ hepatic CSCs

IL-6/STAT3 signaling activation plays an important role in the survival and self-renewal of stem cells (Fig. 4). Wan *et al.* [111] demonstrated that the number of CD44$^+$ cells correlates with the number of tumor-associated macrophages in primary HCC tissues. Furthermore, IL-6 is produced by these tumor-associated macrophages, which activates STAT3 signaling pathway in HCC cells and significantly promotes the sphere formation of CD44$^+$ cells in culture and facilitates tumor growth in xenograft mouse model [111]. TM4SF5 could interact with CD44 through their extracellular domains and such an interaction also activates the c-Src/STAT3/Twist1/Bmi1 signaling, which in turn promotes more spheroid formation [112].

OSM is a pleiotropic cytokine that belongs to the IL-6 family. It shares the gp130 receptor subunit and activates STAT3 pathway. OSM treatment leads to cell division and differentiation of dormant EpCAM$^+$ liver CSCs. Together with 5-FU, OSM could efficiently target both differentiated liver cells and liver CSCs [113]. AQP3 promotes the stimulation and nuclear translocation of STAT3, which further enhances CD133$^+$ hepatic CSC populations [114]. Through cooperation with NF-κB and HIF-1α, IL-6/STAT3 signaling also induces the expression of CD133 during liver carcinogenesis [115]. STAT3 also cooperates with TLR4 signaling via Nanog to activate EMT master regulator Twist1 and consequently promotes the formation of CD133$^+$ liver CSCs in mice [116]. Lee *et al.* [117] also demonstrated that STAT3 activation enhances the self-renewal of CD24$^+$ liver CSCs by upregulating Nanog expression.

Via targeting SOCS2/5 and PTPN1/11, two inhibitors of IL-6 pathway, miR-589-5p enhances spheroid formation, fraction of CD133$^+$ HCC cells and SP cells of HCC cells, as well as tumorigenicity in HCC mouse model [118]. LncDILC is significantly downregulated in HCC spheroids. Over-expressed LncDILC in HCC cells binds to IL-6 promoter and blocks NF-κB-induced IL-6 expression, which contributes to a reduced interaction of hepatic inflammation with CD24$^+$ and EpCAM$^+$ hepatic CSCs [119]. LncSOX4 could bind to STAT3 and recruit STAT3 to the SOX4 promoter region. Consequently, LncSOX4 enhances STAT3-induced SOX4 expression and promotes the self-renewal and tumorigenesis of CD133$^+$ liver CSCs [120].

Other signaling pathways

Phosphatase and tensin homolog deleted on chromosome 10 (PTEN)

PTEN is a phosphatase of phosphoprotein and phospholipid that often acts as a tumor suppressor by antagonizing the phosphatidylinositol 3-kinase/AKT pathway. Mice with liver-specific PTEN deletion form liver cancer spontaneously within a year and present

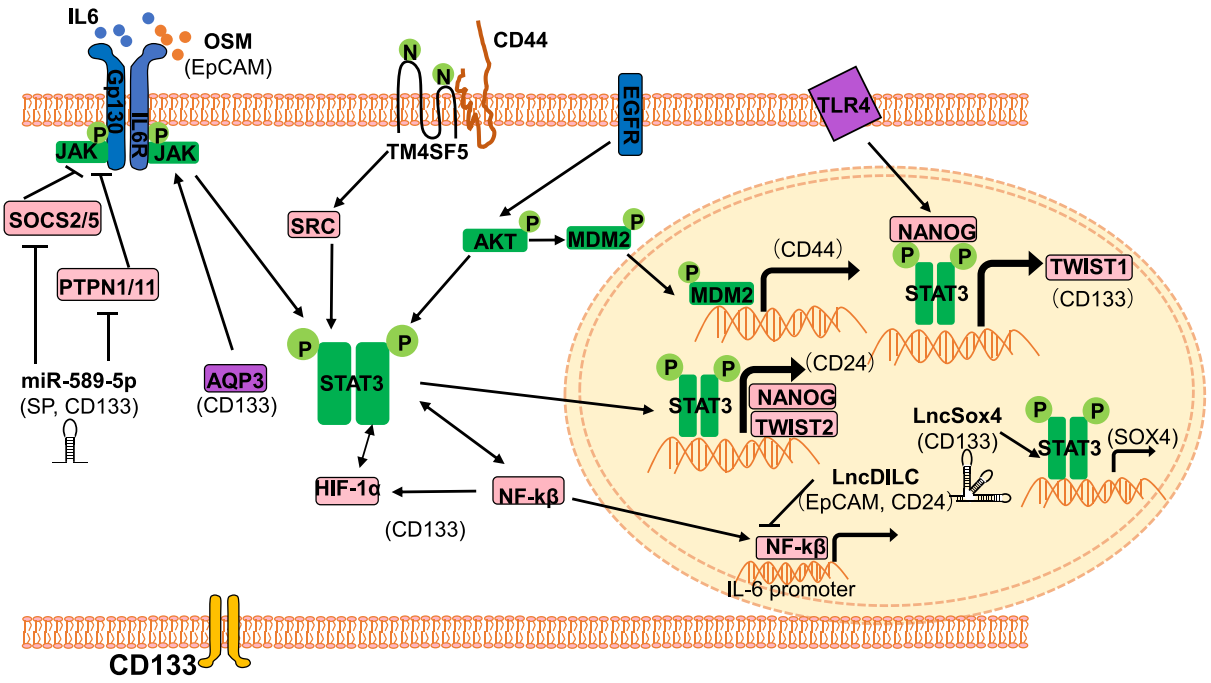

Figure 4. STAT3 signaling pathway in hepatic CSCs IL-6/STAT3 signaling activation plays an important role in the stemness features of hepatic CSCs, mainly in CD44$^+$ and CD133$^+$ populations. Genes and non-coding RNAs in this figure have been reported to regulate the activation of IL-6/STAT3 pathway, leading to the alteration of stemness features in HCC. A hepatic CSC marker filled in a bracket in this figure refers to that this hepatic CSC population is altered by the gene typed right above this CSC biomarker.

the expansion of CD133$^+$ populations [58,121]. Dr Galicia further demonstrated that PTEN loss upregulates AKT2, contributing to the expansion of progenitor cells [122]. Moreover, overexpression of PTEN by adenovirus gene delivery or by celecoxib treatment significantly reduced the populations of CD44$^+$ and CD133$^+$ hepatic CSCs as well as HCC tumor formation in mice [123]. Lineage-tracing experiments showed that PTEN haplodeficiency in miR-122a-null mice accelerates liver carcinogenesis potentially via promoting the expansion of periportal tumor-initiating cells [124]. In addition, miR-25 has been found to enhance the resistance of liver CSCs to TRAIL-induced apoptosis by directly targeting PTEN [125]. LncCUDR overexpression cooperates with PTEN loss to accelerate the proliferation of liver CSCs both *in vitro* and *in vivo* [126].

The Notch signaling pathway

The Notch signaling pathway is crucial in cell development, proliferation, and tissue homoeostasis. It is activated upon a direct interaction of Notch ligands including 'Delta' and 'Jagged' and Notch receptor. Notch ligand-receptor interaction results in the release of the Notch intracellular domain that translocates to the nucleus and induces the transcriptional activation of Notch target genes [127,128]. Notch activation has been observed in CSCs from different cancers such as glioblastoma [129] and colon [130,131] and breast cancers [132]. However, there are only limited findings in the abnormal activation of Notch pathway in liver CSCs. In CD133$^+$ HCC cells, the expressions of Notch receptor and its ligand Jagged are highly elevated [15]. RUNX3 reduces EpCAM$^+$ CSCs by suppressing JAG1-mediated Notch signaling [133]. Zhu *et al.* [134] reported that C8orf4 suppresses the self-renewal of CD13$^+$CD133$^+$ hepatic CSCs via targeting Notch2 signaling. Furthermore, via activating the Notch1 signaling, Musashi2 promotes the population of CD44$^+$ hepatic CSCs [135].

Bmi1

Bmi1, a polycomb-group protein, functions as an oncogene and in the maintenance of the self-renewal of stem cells. The expression of BMI1 is increased in isolated populations of CD133$^+$ hepatic CSCs [15]. It also functions in the maintenance of tumor-initiating SP cells [136].

The Hedgehog signaling pathway

The Hedgehog (Hh) signaling pathway is evolutionally conserved and essential for embryonic development [137]. In HCC, this pathway is also activated and associated with invasion of poorly differentiated HCC [138,139]. Hh activation is essential for chemoresistance and invasion of CD133$^+$ and EpCAM$^+$ cells, which could be blocked by cyclopamine, an Hh pathway inhibitor [140]. Moreover, NanoHHI is a novel nanoparticle-encapsulated inhibitor of the Hh transcription factor Gli1 and could also dramatically reduce the population of CD133$^+$ HCC cells [141].

The above accumulated data show that several signaling pathways such as Wnt and TGF-β are activated in both EpCAM$^+$ CSCs and CD133$^+$ CSCs in HCC. As summarized above, EpCAM and CD133 are also co-expressed in various hepatic CSC populations. These observations further imply a close relationship of EpCAM$^+$ CSCs and CD133$^+$ CSCs in the hepatic CSC society. Interestingly, IL-6 pathway activation is shared by CD44$^+$ and CD133$^+$ hepatic CSCs, while CD44 and CD133 are also co-expressed in hepatic CSCs, which indicates a close relationship between CD44$^+$ and CD133$^+$ cells too. However, in literature there is no evidence to show the co-expression of CD44 with EpCAM. Thus, one possibility to explain such a fact is that CD44$^+$ and EpCAM$^+$ CSC strains might locate under the CD133$^+$ strain in the hierarchical linage of hepatic CSCs. In addition, we found that 14 miRNAs were markedly down-regulated in five different CSC subpopulations, i.e. EpCAM$^+$,

$CD133^+$, $CD90^+$, $CD44^+$, and $CD24^+$ cells. miR-192 is the top one with liver-specific expression. Loss of miR-192 significantly increases these five different CSC populations partially through p53/miR-192-5p/PABPC4 axis [142]. Such a signaling axis might be a key mechanism for the origination or formation of hepatic CSCs. Together, many heterogeneous hepatic CSC populations share similar regulatory pathways. It appears that some of hepatic CSCs, if not all, are hierarchically related to each other.

Clinical Implications

In the recent decade, data have been accumulated on the discovery of hepatic CSC subpopulations with different methods as well as their regulatory signaling pathways. CSCs are found to be responsible to therapeutic resistance and tumor relapse after conventional cancer therapies including chemotherapy, radiation therapy, target therapy, and immunotherapy. According to these achievements, researchers have focused on exploring the potential clinical utilization of targeting hepatic CSCs in assisting liver cancer clinical management.

There are two main research areas for targeting hepatic CSCs toward clinical utilization. One is to interfere the key 'stemness' pathways. For example, Wnt/β-catenin pathway is activated in $EpCAM^+$ hepatic CSCs. The inhibition of this signaling pathway by anti-miR-181 inhibitors suppresses the expression of stemness genes and the tumorigenicity ability of $EpCAM^+$ HCC cells [83]. Lupeol is a dietary triterpene found in certain fruits and vegetables and could suppress the PTEN-Akt-ABCG2 signaling pathway. It significantly reduces the malignant features of $CD133^+$ CSCs including self-renewal, chemoresistance, and tumorigenicity [143]. The potential drawbacks or limitations are side effects on healthy stem cells sharing the equivalent signaling pathways with CSCs and the acquisition of resistance mechanisms. The other is the CSC ablation through targeting CSC surface markers. Recently, antibody-drug conjugates with CD133 [144] and CD44 [38] have been tested *in vitro* and *in vivo*, which inhibit $CD133^+$ and $CD90^+CD44^+$ CSC-mediated tumor formation, respectively. Ubenimex is a CD13 inhibitor that reduces the tumorigenicity and self-renewal of $CD13^+$ cells. In combination with 5-FU, ubenimex also significantly suppresses $CD13^+$ tumor growth *in vivo* [31]. A recent study demonstrated that CSCs positive for the isoform 5 of α2δ1, a composing subunit of voltage-gated calcium channel, could be identified from recurrent HCC patients. Monoclonal antibody 1B50-1 exerts a promising therapeutic ability via specifically targeting $\alpha2\delta1^+$ CSCs in the recurrent HCC tumor [36]. CD47 blockade suppresses HCC tumor growth in mice and increases chemo-sensitivity of HCC cells [35,145]. The potential drawbacks or limitations are the toxicity associated with antibody-drug conjugates and the left-over of other hepatic CSC populations.

The anti-CSC methods provide the hope of improving prognosis of HCC patients. Meanwhile, there are also challenges that we need consider in order to achieve a better translational value of targeting hepatic CSCs in clinical interpretation. Firstly, it is likely that different hepatic CSCs co-exist in one tumor bulk and/or trans-differentiation might occur among different hepatic CSC populations and non-CSCs. Thus, targeting one dominant CSC population possibly allows other CSC populations to remain in the diseased liver. These residual CSCs would potentially initiate a new tumor mass. In this vein, to fully understand the hierarchical relationship of these hepatic CSCs might allow a better co-utilization of anti-CSC methods or developing much effective methods, with the hope of largely improving the prognosis of patients with HCC. Secondly, the percentage of hepatic CSCs in the bulk of tumor is varied and generally small. The current effective HCC treatments including surgery, transplantation, and even chemo/radiotherapy are mainly designed to eradicate the tumor bulk. Therefore, anti-CSC therapy alone might not reach a satisfied therapeutic goal. Meanwhile, the proper time of introducing anti-CSC methods and proper ways to combine with primary HCC treatment methods need to be considered, which however remains less perceived. Thirdly, we have no effective methods to monitor the anti-CSC treatment effects for HCC patients. We use primary HCC tissues from patients to characterize hepatic CSCs via FACS analysis. However, it is unreasonable to obtain tissue biopsies from HCC patients to evaluate the alteration of CSC populations after anti-CSC therapies. Serum biomarker detection hosts a great value for evaluation, whereas it remains largely undiscovered whether small CSC population would be able to produce the detectable amount of specific molecules. In addition, the complex and flexible interaction between CSC and CSC niche is also a challenge for anti-CSC method to effectively eliminate CSCs. More efforts on overcoming these challenges are needed to achieve the goal of increasing long-term benefits for patients with liver cancer by targeting CSCs.

Future Directions

Until now, hepatic CSCs can be identified by many cell surface markers, SOX9 or Nanog tracing, and sorting SP cells or cells having a high ALDH activity. The activation of regulatory pathways is common or unique among different CSC populations. Certain groups of hepatic CSCs are closely related based on their co-expression and regulatory pathways, such as $EpCAM^+$ and $CD133^+$ CSCs, as well as $CD133^+$ and $CD44^+$ CSCs, whereas some CSC biomarkers might not even be co-expressed. However, it is inconclusive on the lineage hierarchy of various hepatic CSCs and more efforts are required to answer clearly whether and how these CSCs are lineage-related or simply representing different CSC subgroups.

This review specifically summarized the currently known association of these CSCs from different aspects, with the hope of assisting researchers to explore the potential lineage hierarchy among these hepatic CSCs. In the future, it will be great to use single-cell sequencing methods to systematically compare the profiles of all hepatic CSC subpopulations under different conditions. Systematically, tracing hepatic CSCs will also allow us to investigate whether one hepatic CSC population could trans-differentiate another one. It is certainly worth to continuously investigate the cellular origins of different hepatic CSC populations. Moreover, there is also an urgent need to develop effective methods to monitor populations and functions of hepatic CSCs in patients. Together, these efforts will provide direct clues on the potential lineage hierarchy among hepatic CSCs. In addition, many other factors are also needed for the maintenance of CSCs, such as angiogenesis, hypoxia, immune evasion, etc. It will be necessary to take them into consideration for CSC-related studies and explore their potential specific contributions to the formation and stemness features of CSCs.

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References

1. Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, Minden M, *et al.* A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature* 1994, 367: 645–648.
2. Uckun FM, Sather H, Reaman G, Shuster J, Land V, Trigg M, Gunther R, *et al.* Leukemic cell growth in SCID mice as a predictor of relapse in high-risk B-lineage acute lymphoblastic leukemia. *Blood* 1995, 85: 873–878.
3. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci U S A* 2003, 100: 3983–3988.
4. Fang D, Nguyen TK, Leishear K, Finko R, Kulp AN, Hotz S, Van Belle PA, *et al.* A tumorigenic subpopulation with stem cell properties in melanomas. *Cancer Res* 2005, 65: 9328–9337.
5. Hermann PC, Huber SL, Herrler T, Aicher A, Ellwart JW, Guba M, Bruns CJ, *et al.* Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. *Cell Stem Cell* 2007, 1: 313–323.
6. Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, Wicha M, *et al.* Identification of pancreatic cancer stem cells. *Cancer Res* 2007, 67: 1030–1037.
7. Collins AT, Berry PA, Hyde C, Stower MJ, Maitland NJ. Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res* 2005, 65: 10946–10951.
8. Bapat SA, Mali AM, Koppikar CB, Kurrey NK. Stem and progenitor-like cells contribute to the aggressive behavior of human epithelial ovarian cancer. *Cancer Res* 2005, 65: 3025–3029.
9. Alvero AB, Chen R, Fu HH, Montagna M, Schwartz PE, Rutherford T, Silasi DA, *et al.* Molecular phenotyping of human ovarian cancer stem cells unravels the mechanisms for repair and chemoresistance. *Cell Cycle* 2009, 8: 158–166.
10. Ho MM, Ng AV, Lam S, Hung JY. Side population in human lung cancer cell lines and tumors is enriched with stem-like cancer cells. *Cancer Res* 2007, 67: 4827–4833.
11. Eramo A, Lotti F, Sette G, Pilozzi E, Biffoni M, Di Virgilio A, Conticello C, *et al.* Identification and expansion of the tumorigenic lung cancer stem cell population. *Cell Death Differ* 2008, 15: 504–514.
12. O'Brien CA, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature* 2007, 445: 106–110.
13. Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C, De Maria R. Identification and expansion of human colon-cancer-initiating cells. *Nature* 2007, 445: 111–115.
14. Dalerba P, Dylla SJ, Park IK, Liu R, Wang X, Cho RW, Hoey T, *et al.* Phenotypic characterization of human colorectal cancer stem cells. *Proc Natl Acad Sci U S A* 2007, 104: 10158–10163.
15. Ma S, Chan KW, Hu L, Lee TK, Wo JY, Ng IO, Zheng BJ, *et al.* Identification and characterization of tumorigenic liver cancer stem/progenitor cells. *Gastroenterology* 2007, 132: 2542–2556.
16. Yamashita T, Ji J, Budhu A, Forgues M, Yang W, Wang HY, Jia H, *et al.* EpCAM-positive hepatocellular carcinoma cells are tumor-initiating cells with stem/progenitor cell features. *Gastroenterology* 2009, 136: 1012–1024.
17. Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J, Dirks PB. Identification of a cancer stem cell in human brain tumors. *Cancer Res* 2003, 63: 5821–5828.
18. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, *et al.* Identification of human brain tumour initiating cells. *Nature* 2004, 432: 396–401.
19. Clevers H. The cancer stem cell: premises, promises and challenges. *Nat Med* 2011, 17: 313–319.
20. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global Cancer Statistics. GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018, 2018: 394–424.
21. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin* 2019, 69: 7–34.
22. Chuang SC, La Vecchia C, Boffetta P. Liver cancer: descriptive epidemiology and risk factors other than HBV and HCV infection. *Cancer Lett* 2009, 286: 9–14.
23. Bridgewater J, Galle PR, Khan SA, Llovet JM, Park JW, Patel T, Pawlik TM, *et al.* Guidelines for the diagnosis and management of intrahepatic cholangiocarcinoma. *J Hepatol* 2014, 60: 1268–1289.
24. Palmer WC, Patel T. Are common factors involved in the pathogenesis of primary liver cancers? A meta-analysis of risk factors for intrahepatic cholangiocarcinoma. *J Hepatol* 2012, 57: 69–76.
25. Cancer Genome Atlas Research Network. Electronic address wbe, Cancer Genome Atlas Research N. Comprehensive and integrative genomic characterization of hepatocellular carcinoma. *Cell* 2017, 169: 1327–1341.e23.
26. Chaisaingmongkol J, Budhu A, Dang H, Rabibhadana S, Pupacdi B, Kwon SM, Forgues M, *et al.* Common molecular subtypes among Asian hepatocellular carcinoma and cholangiocarcinoma. *Cancer Cell* 2017, 32: 57–70 e53.
27. Cardinale V, Renzi A, Carpino G, Torrice A, Bragazzi MC, Giuliante F, DeRose AM, *et al.* Profiles of cancer stem cell subpopulations in cholangiocarcinomas. *Am J Pathol* 2015, 185: 1724–1739.
28. Leelawat K, Thongtawee T, Narong S, Subwongcharoen S, Treepongkaruna SA. Strong expression of CD133 is associated with increased cholangiocarcinoma progression. *World J Gastroenterol* 2011, 17: 1192–1198.
29. Sulpice L, Rayar M, Turlin B, Boucher E, Bellaud P, Desille M, Meunier B, *et al.* Epithelial cell adhesion molecule is a prognosis marker for intrahepatic cholangiocarcinoma. *J Surg Res* 2014, 192: 117–123.
30. Gu MJ, Jang BI. Clinicopathologic significance of Sox2, CD44 and CD44v6 expression in intrahepatic cholangiocarcinoma. *Pathol Oncol Res* 2014, 20: 655–660.
31. Haraguchi N, Ishii H, Mimori K, Tanaka F, Ohkuma M, Kim HM, Akita H, *et al.* CD13 is a therapeutic target in human liver cancer stem cells. *J Clin Invest* 2010, 120: 3326–3339.
32. Sukowati CH, Anfuso B, Torre G, Francalanci P, Croce LS, Tiribelli C. The expression of CD90/Thy-1 in hepatocellular carcinoma: an *in vivo* and *in vitro* study. *PLoS One* 2013, 8: e76830.
33. Yamashita T, Wang XW. Cancer stem cells in the development of liver cancer. *J Clin Invest* 2013, 123: 1911–1918.
34. Liu S, Li N, Yu X, Xiao X, Cheng K, Hu J, Wang J, *et al.* Expression of intercellular adhesion molecule 1 by hepatocellular carcinoma stem cells and circulating tumor cells. *Gastroenterology* 2013, 144: 1031–1041 e1010.
35. Lee TK, Cheung VC, Lu P, Lau EY, Ma S, Tang KH, Tong M, *et al.* Blockade of CD47-mediated cathepsin S/protease-activated receptor 2 signaling provides a therapeutic target for hepatocellular carcinoma. *Hepatology* 2014, 60: 179–191.
36. Zhao W, Wang L, Han H, Jin K, Lin N, Guo T, Chen Y, *et al.* 1B50-1, a mAb raised against recurrent tumor cells, targets liver tumor-initiating cells by binding to the calcium channel alpha2delta1 subunit. *Cancer Cell* 2013, 23: 541–556.
37. 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.
38. Yang ZF, Ho DW, Ng MN, Lau CK, Yu WC, Ngai P, Chu PW, *et al.* Significance of CD90+ cancer stem cells in human liver cancer. *Cancer Cell* 2008, 13: 153–166.
39. Kawai T, Yasuchika K, Ishii T, Miyauchi Y, Kojima H, Yamaoka R, Katayama H, *et al.* SOX9 is a novel cancer stem cell marker surrogated by osteopontin in human hepatocellular carcinoma. *Sci Rep* 2016, 6: 30489.
40. Shan J, Shen J, Liu L, Xia F, Xu C, Duan G, Xu Y, *et al.* Nanog regulates self-renewal of cancer stem cells through the insulin-like growth factor pathway in human hepatocellular carcinoma. *Hepatology* 2012, 56: 1004–1014.

41. Cao L, Zhou Y, Zhai B, Liao J, Xu W, Zhang R, Li J, *et al.* Sphere-forming cell subpopulations with cancer stem cell properties in human hepatoma cell lines. *BMC Gastroenterol* 2011, 11: 71.
42. Chiba T, Kita K, Zheng YW, Yokosuka O, Saisho H, Iwama A, Nakauchi H, *et al.* Side population purified from hepatocellular carcinoma cells harbors cancer stem cell-like properties. *Hepatology* 2006, 44: 240–251.
43. Dolle L, Best J, Empsen C, Mei J, Van Rossen E, Roelandt P, Snykers S, *et al.* Successful isolation of liver progenitor cells by aldehyde dehydrogenase activity in naive mice. *Hepatology* 2012, 55: 540–552.
44. 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.
45. Tanaka K, Tomita H, Hisamatsu K, Nakashima T, Hatano Y, Sasaki Y, Osada S, *et al.* ALDH1A1-overexpressing cells are differentiated cells but not cancer stem or progenitor cells in human hepatocellular carcinoma. *Oncotarget* 2015, 6: 24722–24732.
46. Toh TB, Lim JJ, Hooi L, Rashid M, Chow EK. Targeting Jak/Stat pathway as a therapeutic strategy against SP/CD44+ tumorigenic cells in Akt/beta-catenin-driven hepatocellular carcinoma. *J Hepatol* 2020, 72: 104–118.
47. Jang YY, Collector MI, Baylin SB, Diehl AM, Sharkis SJ. Hematopoietic stem cells convert into liver cells within days without fusion. *Nat Cell Biol* 2004, 6: 532–539.
48. Wang X, Willenbring H, Akkari Y, Torimaru Y, Foster M, Al-Dhalimy M, Lagasse E, *et al.* Cell fusion is the principal source of bone-marrow-derived hepatocytes. *Nature* 2003, 422: 897–901.
49. Petersen BE, Bowen WC, Patrene KD, Mars WM, Sullivan AK, Murase N, Boggs SS, *et al.* Bone marrow as a potential source of hepatic oval cells. *Science* 1999, 284: 1168–1170.
50. Fausto N. Liver regeneration and repair: hepatocytes, progenitor cells, and stem cells. *Hepatology* 2004, 39: 1477–1487.
51. Lin S, Nascimento EM, Gajera CR, Chen L, Neuhofer P, Garbuzov A, Wang S, *et al.* Distributed hepatocytes expressing telomerase repopulate the liver in homeostasis and injury. *Nature* 2018, 556: 244–248.
52. Lu WY, Bird TG, Boulter L, Tsuchiya A, Cole AM, Hay T, Guest RV, *et al.* Hepatic progenitor cells of biliary origin with liver repopulation capacity. *Nat Cell Biol* 2015, 17: 971–983.
53. Theise ND, Nimmakayalu M, Gardner R, Illei PB, Morgan G, Teperman L, Henegariu O, *et al.* Liver from bone marrow in humans. *Hepatology* 2000, 32: 11–16.
54. Zeng C, Zhang Y, Park SC, Eun JR, Nguyen NT, Tschudy-Seney B, Jung YJ, *et al.* CD34(+) liver cancer stem cells were formed by fusion of hepatobiliary stem/progenitor cells with hematopoietic precursor-derived myeloid intermediates. *Stem Cells Dev* 2015, 24: 2467–2478.
55. Kubota K, Soeda J, Misawa R, Mihara M, Miwa S, Ise H, Takahashi M, *et al.* Bone marrow-derived cells fuse with hepatic oval cells but are not involved in hepatic tumorigenesis in the choline-deficient ethionine-supplemented diet rat model. *Carcinogenesis* 2008, 29: 448–454.
56. Rountree CB, Barsky L, Ge S, Zhu J, Senadheera S, Crooks GM. A CD133-expressing murine liver oval cell population with bilineage potential. *Stem Cells* 2007, 25: 2419–2429.
57. Corbeil D, Roper K, Hellwig A, Tavian M, Miraglia S, Watt SM, Simmons PJ, *et al.* The human AC133 hematopoietic stem cell antigen is also expressed in epithelial cells and targeted to plasma membrane protrusions. *J Biol Chem* 2000, 275: 5512–5520.
58. Rountree CB, Ding W, He L, Stiles B. Expansion of CD133-expressing liver cancer stem cells in liver-specific phosphatase and tensin homolog deleted on chromosome 10-deleted mice. *Stem Cells* 2009, 27: 290–299.
59. 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.
60. Yin S, Li J, Hu C, Chen X, Yao M, Yan M, Jiang G, *et al.* CD133 positive hepatocellular carcinoma cells possess high capacity for tumorigenicity. *Int J Cancer* 2007, 120: 1444–1450.
61. Ma S, Tang KH, Chan YP, Lee TK, Kwan PS, Castilho A, Ng I, *et al.* miR-130b promotes CD133(+) liver tumor-initiating cell growth and self-renewal via tumor protein 53-induced nuclear protein 1. *Cell Stem Cell* 2010, 7: 694–707.
62. Bralet MP, Pichard V, Ferry N. Demonstration of direct lineage between hepatocytes and hepatocellular carcinoma in diethylnitrosamine-treated rats. *Hepatology* 2002, 36: 623–630.
63. Dumble ML, Croager EJ, Yeoh GC, Quail EA. Generation and characterization of p53 null transformed hepatic progenitor cells: oval cells give rise to hepatocellular carcinoma. *Carcinogenesis* 2002, 23: 435–445.
64. Holczbauer A, Factor VM, Andersen JB, Marquardt JU, Kleiner DE, Raggi C, Kitade M, *et al.* Modeling pathogenesis of primary liver cancer in lineage-specific mouse cell types. *Gastroenterology* 2013, 145: 221–231.
65. Mu X, Espanol-Suner R, Mederacke I, Affo S, Manco R, Sempoux C, Lemaigre FP, *et al.* Hepatocellular carcinoma originates from hepatocytes and not from the progenitor/biliary compartment. *J Clin Invest* 2015, 125: 3891–3903.
66. He G, Dhar D, Nakagawa H, Font-Burgada J, Ogata H, Jiang Y, Shalapour S, *et al.* Identification of liver cancer progenitors whose malignant progression depends on autocrine IL-6 signaling. *Cell* 2013, 155: 384–396.
67. Liu M, Chen L, Ma NF, Chow RK, Li Y, Song Y, Chan TH, *et al.* CHD1L promotes lineage reversion of hepatocellular carcinoma through opening chromatin for key developmental transcription factors. *Hepatology* 2016, 63: 1544–1559.
68. Moraes DA, Sibov TT, Pavon LF, Alvim PQ, Bonadio RS, Da Silva JR, Pic-Taylor A, *et al.* A reduction in CD90 (THY-1) expression results in increased differentiation of mesenchymal stromal cells. *Stem Cell Res Ther* 2016, 7: 97.
69. Okabe M, Tsukahara Y, Tanaka M, Suzuki K, Saito S, Kamiya Y, Tsujimura T, *et al.* Potential hepatic stem cells reside in EpCAM+ cells of normal and injured mouse liver. *Development* 2009, 136: 1951–1960.
70. Dajani R, Fraser E, Roe SM, Yeo M, Good VM, Thompson V, Dale TC, *et al.* Structural basis for recruitment of glycogen synthase kinase 3beta to the axin-APC scaffold complex. *EMBO J* 2003, 22: 494–501.
71. Ha NC, Tonozuka T, Stamos JL, Choi HJ, Weis WI. Mechanism of phosphorylation-dependent binding of APC to beta-catenin and its role in beta-catenin degradation. *Mol Cell* 2004, 15: 511–521.
72. Tetsu O, McCormick F. Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. *Nature* 1999, 398: 422–426.
73. Yochum GS, Sherrick CM, Macpartlin M, Goodman RH. A beta-catenin/TCF-coordinated chromatin loop at MYC integrates 5′ and 3′ Wnt responsive enhancers. *Proc Natl Acad Sci U S A* 2010, 107: 145–150.
74. Staal FJ, Meeldijk J, Moerer P, Jay P, van de Weerdt BC, Vainio S, Nolan GP, *et al.* Wnt signaling is required for thymocyte development and activates Tcf-1 mediated transcription. *Eur J Immunol* 2001, 31: 285–293.
75. Lei ZJ, Wang J, Xiao HL, Guo Y, Wang T, Li Q, Liu L, *et al.* Lysine-specific demethylase 1 promotes the stemness and chemoresistance of Lgr5+ liver cancer initiating cells by suppressing negative regulators of beta-catenin signaling. *Oncogene* 2015, 34: 3214.
76. Yang W, Yan HX, Chen L, Liu Q, He YQ, Yu LX, Zhang SH, *et al.* Wnt/beta-catenin signaling contributes to activation of normal and tumorigenic liver progenitor cells. *Cancer Res* 2008, 68: 4287–4295.
77. Yamashita T, Budhu A, Forgues M, Wang XW. Activation of hepatic stem cell marker EpCAM by Wnt-beta-catenin signaling in hepatocellular carcinoma. *Cancer Res* 2007, 67: 10831–10839.
78. Merle P, de la Monte S, Kim M, Herrmann M, Tanaka S, Von Dem Bussche A, Kew MC, *et al.* Functional consequences of frizzled-7 receptor overexpression in human hepatocellular carcinoma. *Gastroenterology* 2004, 127: 1110–1122.
79. Tokumoto N, Ikeda S, Ishizaki Y, Kurihara T, Ozaki S, Iseki M, Shimizu Y, *et al.* Immunohistochemical and mutational analyses of Wnt signaling components and target genes in intrahepatic cholangiocarcinomas. *Int J Oncol* 2005, 27: 973–980.

80. Chao HM, Huang HX, Chang PH, Tseng KC, Miyajima A, Chern E. Y-box binding protein-1 promotes hepatocellular carcinoma-initiating cell progression and tumorigenesis via Wnt/beta-catenin pathway. *Oncotarget* 2017, 8: 2604–2616.
81. Zhi X, Lin L, Yang S, Bhuvaneshwar K, Wang H, Gusev Y, Lee MH, *et al.* betaII-Spectrin (SPTBN1) suppresses progression of hepatocellular carcinoma and Wnt signaling by regulation of Wnt inhibitor kallistatin. *Hepatology* 2015, 61: 598–612.
82. Wang C, Wang MD, Cheng P, Huang H, Dong W, Zhang WW, Li PP, *et al.* Hepatitis B virus X protein promotes the stem-like properties of OV6(+) cancer cells in hepatocellular carcinoma. *Cell Death Dis* 2017, 8: e2560.
83. Ji J, Yamashita T, Budhu A, Forgues M, Jia HL, Li C, Deng C, *et al.* Identification of microRNA-181 by genome-wide screening as a critical player in EpCAM-positive hepatic cancer stem cells. *Hepatology* 2009, 50: 472–480.
84. Ji J, Yamashita T, Wang XW. Wnt/beta-catenin signaling activates microRNA-181 expression in hepatocellular carcinoma. *Cell Biosci* 2011, 1: 4.
85. Xia H, Ooi LL, Hui KM. MiR-214 targets beta-catenin pathway to suppress invasion, stem-like traits and recurrence of human hepatocellular carcinoma. *PLoS One* 2012, 7: e44206.
86. Chai S, Ng KY, Tong M, Lau EY, Lee TK, Chan KW, Yuan YF, *et al.* Octamer 4/microRNA-1246 signaling axis drives Wnt/beta-catenin activation in liver cancer stem. *Cell* 2016, 64: 2062–2076.
87. Cai H, Chen Y, Yang X, Ma S, Wang Q, Zhang Y, Niu X, *et al.* Let7b modulates the Wnt/beta-catenin pathway in liver cancer cells via downregulated Frizzled4. *Tumour Biol* 2017, 39: 1010428317716076.
88. Liu J, Ruan B, You N, Huang Q, Liu W, Dang Z, Xu W, *et al.* Downregulation of miR-200a induces EMT phenotypes and CSC-like signatures through targeting the beta-catenin pathway in hepatic oval cells. *PLoS One* 2013, 8: e79409.
89. Zheng Z, Liu J, Yang Z, Wu L, Xie H, Jiang C, Lin B, *et al.* MicroRNA-452 promotes stem-like cells of hepatocellular carcinoma by inhibiting Sox7 involving Wnt/beta-catenin signaling pathway. *Oncotarget* 2016, 7: 28000–28012.
90. Beermann J, Piccoli MT, Viereck J, Thum T. Non-coding RNAs in development and disease: background, mechanisms, and therapeutic approaches. *Physiol Rev* 2016, 96: 1297–1325.
91. Wang Y, He L, Du Y, Zhu P, Huang G, Luo J, Yan X, *et al.* The long noncoding RNA lncTCF7 promotes self-renewal of human liver cancer stem cells through activation of Wnt signaling. *Cell Stem Cell* 2015, 16: 413–425.
92. Zhu P, Wang Y, Huang G, Ye B, Liu B, Wu J, Du Y, *et al.* lnc-beta-Catm elicits EZH2-dependent beta-catenin stabilization and sustains liver CSC self-renewal. *Nat Struct Mol Biol* 2016, 23: 631–639.
93. Chen Z, Yao L, Liu Y, Zhu P. LncTIC1 interacts with beta-catenin to drive liver TIC self-renewal and liver tumorigenesis. *Cancer Lett* 2018, 430: 88–96.
94. Fu X, Lin J, Qin F, Yang Z, Ding Y, Zhang Y, Han L, *et al.* LncAPC drives Wnt/beta-catenin activation and liver TIC self-renewal through EZH2 mediated APC transcriptional inhibition. *Mol Carcinog* 2018, 57: 408–418.
95. Fu X, Zhu X, Qin F, Zhang Y, Lin J, Ding Y, Yang Z, *et al.* Linc00210 drives Wnt/beta-catenin signaling activation and liver tumor progression through CTNNBIP1-dependent manner. *Mol Cancer* 2018, 17: 73.
96. Chen Z, Gao Y, Yao L, Liu Y, Huang L, Yan Z, Zhao W, *et al.* LncFZD6 initiates Wnt/beta-catenin and liver TIC self-renewal through BRG1-mediated FZD6 transcriptional activation. *Oncogene* 2018, 37: 3098–3112.
97. Wang BG, Li JS, Liu YF, Xu Q. MicroRNA-200b suppresses the invasion and migration of hepatocellular carcinoma by downregulating RhoA and circRNA_000839. *Tumour Biol* 2017, 39: 1010428317719577.
98. Gui X, Li H, Li T, Pu H, Lu D. Long noncoding RNA CUDR regulates HULC and beta-catenin to govern human liver stem cell malignant differentiation. *Mol Ther* 2015, 23: 1843–1853.
99. Yuan SX, Wang J, Yang F, Tao QF, Zhang J, Wang LL, Yang Y, *et al.* Long noncoding RNA DANCR increases stemness features of hepatocellular carcinoma by derepression of CTNNB1. *Hepatology* 2016, 63: 499–511.
100. You H, Ding W, Rountree CB. Epigenetic regulation of cancer stem cell marker CD133 by transforming growth factor-beta. *Hepatology* 2010, 51: 1635–1644.
101. Zhang L, Sun H, Zhao F, Lu P, Ge C, Li H, Hou H, *et al.* BMP4 administration induces differentiation of CD133+ hepatic cancer stem cells, blocking their contributions to hepatocellular carcinoma. *Cancer Res* 2012, 72: 4276–4285.
102. Fan QM, Jing YY, Yu GF, Kou XR, Ye F, Gao L, Li R, *et al.* Tumor-associated macrophages promote cancer stem cell-like properties via transforming growth factor-beta1-induced epithelial-mesenchymal transition in hepatocellular carcinoma. *Cancer Lett* 2014, 352: 160–168.
103. Xia W, Lo CM, Poon RYC, Cheung TT, Chan ACY, Chen L, Yang S, *et al.* Smad inhibitor induces CSC differentiation for effective chemosensitization in cyclin D1- and TGF-beta/Smad-regulated liver cancer stem cell-like cells. *Oncotarget* 2017, 8: 38811–38824.
104. Lin B, Chen T, Zhang Q, Lu X, Zheng Z, Ding J, Liu J, *et al.* FAM83D associates with high tumor recurrence after liver transplantation involving expansion of CD44+ carcinoma stem cells. *Oncotarget* 2016, 7: 77495–77507.
105. Liu F, Kong X, Lv L, Gao J. TGF-beta1 acts through miR-155 to down-regulate TP53INP1 in promoting epithelial-mesenchymal transition and cancer stem cell phenotypes. *Cancer Lett* 2015, 359: 288–298.
106. Ji J, Zheng X, Forgues M, Yamashita T, Wauthier EL, Reid LM, Wen X, *et al.* Identification of microRNAs specific for epithelial cell adhesion molecule-positive tumor cells in hepatocellular carcinoma. *Hepatology* 2015, 62: 829–840.
107. Zhou JN, Zeng Q, Wang HY, Zhang B, Li ST, Nan X, Cao N, *et al.* MicroRNA-125b attenuates epithelial-mesenchymal transitions and targets stem-like liver cancer cells through small mothers against decapentaplegic 2 and 4. *Hepatology* 2015, 62: 801–815.
108. Xia H, Ooi LL, Hui KM. MicroRNA-216a/217-induced epithelial-mesenchymal transition targets PTEN and SMAD7 to promote drug resistance and recurrence of liver cancer. *Hepatology* 2013, 58: 629–641.
109. Jiang F, Mu J, Wang X, Ye X, Si L, Ning S, Li Z, *et al.* The repressive effect of miR-148a on TGF beta-SMADs signal pathway is involved in the glabridin-induced inhibition of the cancer stem cells-like properties in hepatocellular carcinoma cells. *PLoS One* 2014, 9: e96698.
110. Li L, Liu Y, Guo Y, Liu B, Zhao Y, Li P, Song F, *et al.* Regulatory MiR-148a-ACVR1/BMP circuit defines a cancer stem cell-like aggressive subtype of hepatocellular carcinoma. *Hepatology* 2015, 61: 574–584.
111. Wan S, Zhao E, Kryczek I, Vatan L, Sadovskaya A, Ludema G, Simeone DM, *et al.* Tumor-associated macrophages produce interleukin 6 and signal via STAT3 to promote expansion of human hepatocellular carcinoma stem cells. *Gastroenterology* 2014, 147: 1393–1404.
112. Lee D, Na J, Ryu J, Kim HJ, Nam SH, Kang M, Jung JW, *et al.* Interaction of tetraspan(in) TM4SF5 with CD44 promotes self-renewal and circulating capacities of hepatocarcinoma cells. *Hepatology* 2015, 61: 1978–1997.
113. Yamashita T, Honda M, Nio K, Nakamoto Y, Yamashita T, Takamura H, Tani T, *et al.* Oncostatin m renders epithelial cell adhesion molecule-positive liver cancer stem cells sensitive to 5-fluorouracil by inducing hepatocytic differentiation. *Cancer Res* 2010, 70: 4687–4697.
114. Wang Y, Wu G, Fu X, Xu S, Wang T, Zhang Q, Yang Y. Aquaporin 3 maintains the stemness of CD133+ hepatocellular carcinoma cells by activating STAT3. *Cell Death Dis* 2019, 10: 465.
115. Won C, Kim BH, Yi EH, Choi KJ, Kim EK, Jeong JM, Lee JH, *et al.* Signal transducer and activator of transcription 3-mediated CD133 up-regulation contributes to promotion of hepatocellular carcinoma. *Hepatology* 2015, 62: 1160–1173.
116. Uthaya Kumar DB, Chen CL, Liu JC, Feldman DE, Sher LS, French S, DiNorcia J, *et al.* TLR4 Signaling via NANOG cooperates with STAT3 to activate Twist1 and promote formation of tumor-initiating stem-like cells in livers of mice. *Gastroenterology* 2016, 150: 707–719.

117. Lee TK, Castilho A, Cheung VC, Tang KH, Ma S, Ng IO. CD24(+) liver tumor-initiating cells drive self-renewal and tumor initiation through STAT3-mediated NANOG regulation. *Cell Stem Cell* 2011, 9: 50–63.
118. Long J, Jiang C, Liu B, Dai Q, Hua R, Chen C, Zhang B, *et al.* Maintenance of stemness by miR-589-5p in hepatocellular carcinoma cells promotes chemoresistance via STAT3 signaling. *Cancer Lett* 2018, 423: 113–126.
119. Wang X, Sun W, Shen W, Xia M, Chen C, Xiang D, Ning B, *et al.* Long non-coding RNA DILC regulates liver cancer stem cells via IL-6/STAT3 axis. *J Hepatol* 2016, 64: 1283–1294.
120. Chen ZZ, Huang L, Wu YH, Zhai WJ, Zhu PP, Gao YF. LncSox4 promotes the self-renewal of liver tumour-initiating cells through Stat3-mediated Sox4 expression. *Nat Commun* 2016, 7: 12598.
121. Horie Y, Suzuki A, Kataoka E, Sasaki T, Hamada K, Sasaki J, Mizuno K, *et al.* Hepatocyte-specific Pten deficiency results in steatohepatitis and hepatocellular carcinomas. *J Clin Invest* 2004, 113: 1774–1783.
122. Galicia VA, He L, Dang H, Kanel G, Vendryes C, French BA, Zeng N, *et al.* Expansion of hepatic tumor progenitor cells in Pten-null mice requires liver injury and is reversed by loss of AKT2. *Gastroenterology* 2010, 139: 2170–2182.
123. Chu TH, Chan HH, Kuo HM, Liu LF, Hu TH, Sun CK, Kung ML, *et al.* Celecoxib suppresses hepatoma stemness and progression by up-regulating PTEN. *Oncotarget* 2014, 5: 1475–1490.
124. Tu WL, You LR, Tsou AP, Chen CM. Pten haplodeficiency accelerates liver tumor growth in miR-122a-null mice via expansion of periportal hepatocyte-like cells. *Am J Pathol* 2018, 188: 2688–2702.
125. Feng X, Jiang J, Shi S, Xie H, Zhou L, Zheng S. Knockdown of miR-25 increases the sensitivity of liver cancer stem cells to TRAIL-induced apoptosis via PTEN/PI3K/Akt/Bad signaling pathway. *Int J Oncol* 2016, 49: 2600–2610.
126. Pu H, Zheng Q, Li H, Wu M, An J, Gui X, Li T, *et al.* CUDR promotes liver cancer stem cell growth through upregulating TERT and C-Myc. *Oncotarget* 2015, 6: 40775–40798.
127. Tien AC, Rajan A, Bellen HJ. A Notch updated. *J Cell Biol* 2009, 184: 621–629.
128. Gordon WR, Vardar-Ulu D, Histen G, Sanchez-Irizarry C, Aster JC, Blacklow SC. Structural basis for autoinhibition of Notch. *Nat Struct Mol Biol* 2007, 14: 295–300.
129. Fan X, Khaki L, Zhu TS, Soules ME, Talsma CE, Gul N, Koh C, *et al.* NOTCH pathway blockade depletes CD133-positive glioblastoma cells and inhibits growth of tumor neurospheres and xenografts. *Stem Cells* 2010, 28: 5–16.
130. Lu J, Ye X, Fan F, Xia L, Bhattacharya R, Bellister S, Tozzi F, *et al.* Endothelial cells promote the colorectal cancer stem cell phenotype through a soluble form of Jagged-1. *Cancer Cell* 2013, 23: 171–185.
131. Moon CM, Kwon JH, Kim JS, Oh SH, Jin Lee K, Park JJ, Pil Hong S, *et al.* Nonsteroidal anti-inflammatory drugs suppress cancer stem cells via inhibiting PTGS2 (cyclooxygenase 2) and NOTCH/HES1 and activating PPARG in colorectal cancer. *Int J Cancer* 2014, 134: 519–529.
132. Hirata N, Yamada S, Shoda T, Kurihara M, Sekino Y, Kanda Y. Sphingosine-1-phosphate promotes expansion of cancer stem cells via S1PR3 by a ligand-independent Notch activation. *Nat Commun* 2014, 5: 4806.
133. Nishina S, Shiraha H, Nakanishi Y, Tanaka S, Matsubara M, Takaoka N, Uemura M, *et al.* Restored expression of the tumor suppressor gene RUNX3 reduces cancer stem cells in hepatocellular carcinoma by suppressing Jagged1-Notch signaling. *Oncol Rep* 2011, 26: 523–531.
134. Zhu P, Wang Y, Du Y, He L, Huang G, Zhang G, Yan X, *et al.* C8orf4 negatively regulates self-renewal of liver cancer stem cells via suppression of NOTCH2 signalling. *Nat Commun* 2015, 6: 7122.
135. Wang X, Wang R, Bai S, Xiong S, Li Y, Liu M, Zhao Z, *et al.* Musashi2 contributes to the maintenance of CD44v6+ liver cancer stem cells via notch1 signaling pathway. *J Exp Clin Cancer Res* 2019, 38: 505.
136. Chiba T, Miyagi S, Saraya A, Aoki R, Seki A, Morita Y, Yonemitsu Y, *et al.* The polycomb gene product BMI1 contributes to the maintenance of tumor-initiating side population cells in hepatocellular carcinoma. *Cancer Res* 2008, 68: 7742–7749.
137. Beachy PA, Karhadkar SS, Berman DM. Tissue repair and stem cell renewal in carcinogenesis. *Nature* 2004, 432: 324–331.
138. Huang S, He J, Zhang X, Bian Y, Yang L, Xie G, Zhang K, *et al.* Activation of the hedgehog pathway in human hepatocellular carcinomas. *Carcinogenesis* 2006, 27: 1334–1340.
139. Cheng WT, Xu K, Tian DY, Zhang ZG, Liu LJ, Chen Y. Role of Hedgehog signaling pathway in proliferation and invasiveness of hepatocellular carcinoma cells. *Int J Oncol* 2009, 34: 829–836.
140. Chen X, Lingala S, Khoobyari S, Nolta J, Zern MA, Wu J. Epithelial mesenchymal transition and hedgehog signaling activation are associated with chemoresistance and invasion of hepatoma subpopulations. *J Hepatol* 2011, 55: 838–845.
141. Xu Y, Chenna V, Hu C, Sun HX, Khan M, Bai H, Yang XR, *et al.* Polymeric nanoparticle-encapsulated hedgehog pathway inhibitor HPI-1 (NanoHHI) inhibits systemic metastases in an orthotopic model of human hepatocellular carcinoma. *Clin Cancer Res* 2012, 18: 1291–1302.
142. Gu Y, Wei X, Sun Y, Gao H, Zheng X, Wong LL, Jin L, *et al.* miR-192-5p silencing by genetic aberrations is a key event in hepatocellular carcinomas with cancer stem cell features. *Cancer Res* 2019, 79: 941–953.
143. 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.
144. Smith LM, Nesterova A, Ryan MC, Duniho S, Jonas M, Anderson M, Zabinski RF, *et al.* CD133/prominin-1 is a potential therapeutic target for antibody-drug conjugates in hepatocellular and gastric cancers. *Br J Cancer* 2008, 99: 100–109.
145. Lo J, Lau EY, Ching RH, Cheng BY, Ma MK, Ng IO, Lee TK. Nuclear factor kappa B-mediated CD47 up-regulation promotes sorafenib resistance and its blockade synergizes the effect of sorafenib in hepatocellular carcinoma in mice. *Hepatology* 2015, 62: 534–545.