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MINIREVIEWS

Research progress and prospects of markers for liver cancer stem cells

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Abstract

Liver cancer is a common malignancy and surgery is the main treatment strategy. However, the prognosis is still poor because of high frequencies of postoperative recurrence and metastasis. In recent years, cancer stem cell (CSC) theory has evolved with the concept of stem cells, and has been applied to oncological research. According to cancer stem cell theory, liver cancer can be radically cured only by eradication of liver cancer stem cells (LCSCs). This notion has lead to the isolation and identification of LCSCs, which has become a highly researched area. Analysis of LCSC markers is considered to be the primary method for identification of LCSCs. Here, we provide an overview of the current research progress and prospects of surface markers for LCSCs.

Key words: Hepatocellular carcinoma; Liver cancer stem cells; Surface markers; CD90; Epithelial cell adhesion molecule

Core tip: Liver cancer is a common malignancy and the eradication of liver cancer stem cells (LCSCs) is proposed as the key to improving the curative effect of treatments. Many surface markers have been reported for LCSCs, but there is still no unified standard. This paper addresses the research progress of markers for LCSCs and discusses the relationship with clinical

syndrome in hepatocellular carcinoma.

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DEFINITIONS OF CANCER STEM CELLS AND LIVER CANCER STEM CELLS

Liver cancer is the fifth most common and third most deadly cancer in the world and hepatocellular carcinoma (HCC) accounts for 90% of liver cancers. Currently, surgical resection and liver transplantation are the sole curative options for the treatment of HCC. However, the 5-year survival rate depends on the stage of the liver cancer at diagnosis. Most patients in the late stage with postoperative recurrence exhibit low sensitivity to radiotherapy and chemotherapy[1]. Therefore, the exploration of liver cancer treatments is currently a highly researched area. Since cancer stem cell theory was first proposed, new approaches have been suggested for the treatment of HCC. Cancer stem cell theory was put forward by Reya *et al*[2] based on the previous points. The theory considers that tumor tissue has a small number of cells called cancer stem cells (CSCs) that self-renew indefinitely and have the potential for multi-directional differentiation to produce the heterogeneity of tumor cells. Most CSCs are in the G0 phase of the cell cycle and have certain resistance to radiotherapy and chemotherapy.

Studies have demonstrated CSCs in leukemia and breast cancer. Other studies have shown that CSCs may also exist in liver cancer tissues[3,4]. Liver cancer stem cells (LCSCs) have been highly researched in liver cancer for more than 10 years, and are considered to be a population of cells with certain stem cell-like characteristics in liver cancer tissue. These stem cell-like characteristics include indefinite self-renewal and the potential for multi-directional differentiation that constantly produces liver cancer cell populations in various stages of differentiation with different biological behaviors. In this manner, LCSCs maintain tumor growth. Furthermore, compared with non-LCSCs in liver cancer tissue, LCSCs have a stronger migration ability and tumorigenicity that is closely related to metastasis and recurrence of liver cancer. LCSCs are also resistant to radiotherapy and chemotherapy, which is one of the reasons for the poor efficacy of these treatments for liver cancer patients[5,6].

An increasing number of researchers believe that the key to improving the curative effect of treatments for liver cancer is the eradication of LCSCs. Treatments of liver cancer may simply kill cancer cells and reduce the tumor volume without eradication of LCSCs. In addition, detection of LCSC-specific surface markers can be used for diagnosis, prognosis evaluation, and monitoring after treatment of patients with liver cancer.

CLASSIFICATION OF LCSC MARKERS

In recent years, numerous studies have confirmed that the development and progression of hematological cancers and many kinds of solid tumors, including liver cancer, depend on CSCs[7]. However, compared with most other solid tumors with CSC-specific surface markers, there is no consensus on the surface markers of LCSCs. New LCSC surface markers are constantly being discovered and debated.

Currently, using cell surface markers to isolate CSCs has proved to be feasible[8]. To select LCSC surface markers, stem cell markers are used as a reference because CSCs and normal stem cells have many similar biological characteristics. Major LCSC markers are listed in Table 1.

CD133

Human CD133 is a five-transmembrane single-chain glycoprotein that belongs to the prominin family, which contains two large extracellular and two small intracellular loops[9,10]. The role of CD133 as a CSC marker in liver cancer has been documented in several studies[11-13]. Suetsugu *et al*[14] reported that CD133+ Huh-7 cells (liver cancer cell line) have a high proliferative capacity *in vitro* and tumorigenic ability *in vivo*. Subsequently, Yin *et al*[15] isolated CD133+ cells from the HCC cell line SMMC -7721, and found that these cells had the highest colony-forming ability *in vitro* and tumorigenic ability *in vivo*. Yao *et al*[16] showed that knockout of the CD133 gene in Huh-7 cells suppresses their tumorigenic ability *in vivo*. Kohga *et al*[17] reported a relationship between CD133 and the invasion and distant metastasis of liver cancer. A recent study[12] showed that CD133+ liver cancer cells are resistant to apoptosis induced by radiation and have a stronger proliferative ability *in vitro* and tumorigenic ability *in vivo*. Some studies have shown that CD133+ liver cancer cells exhibit stronger abilities for colony formation and proliferation, which result in a poorer prognosis compared with CD133- liver cancer cells[18,19]. These findings indicate that CD133+ liver cancer cells may be LCSCs.

However, Salnikov *et al*[20] found that CD133+ and CD133- liver cancer cells have no significant differences in terms of migration, and the total number of CD33+ cells has no correlation with the clinical features of liver cancer patients. Therefore, CD133 as a LCSC marker requires further study.

Table 1 Reported major liver cancer stem cell markers

Marker	Origin	Minimum tumorigenic cells	Clinical relevant characteristics	Related literature
CD133	HuH7 cell, SMMC-7721 cell	1×10^2-1×10^3	With a poor prognosis; Related with invasiveness and distant metastases; Resistance to chemotherapy drugs	[14,15,17,48,59]
CD90	PLC cell, MHCC-97L, HCC tumor, blood samples of 90% patients with liver cancer	5×10^2	Related with tumorigenic ability and metastasis of liver cancer	[23,24]
EpCAM	HuH1 cell, HuH7 cell	1×10^3	The origin of recurrence and metastasis postoperatively; Patients with vascular metastasis and low overall survival rate when peripheral circulation exists	[31]
OV6	SMMC-7721 cell	5×10^3	Drugs resistance; Strong metastasis and invasion	[39]
CD44[1]	MHCC-LM3 cell, MHCC-97L cell	1×10^2	Chemotherapy drug resistance; Related with portal vein metastasis in liver cancer	[41]
SP (ABCG2)	Huh7 cell, PLC/PRF/5 cell	1×10^3	-	[46,60]

[1]Co-expression of CD133+ and CD44+ cell surface markers. SP: Side population; EpCAM: Epithelial cell adhesion molecule.

CD90

CD90 (Thy-1) is a 25-30 kDa glycosylphosphatidylinositol-anchored glycoprotein expressed on many cell types, including T cells, thymocytes, neurons, endothelial cells, and fibroblasts. It is an important regulator of cell-cell and cell-matrix interactions with important roles in nerve regeneration, metastasis, inflammation, and fibrosis[21]. A study[22] has reported that CD90 is a surface marker for liver stem cells and hepatic progenitor cells during liver development. Recently, CD90 has also received attention as a CSC marker for various types of tumor cells including hepatic stem cells.

Yang *et al*[23] reported CD45-CD90+ cells in all liver cancer tissues and 90% of blood samples from liver cancer patients. They also found high expression of CD90 during tumor formation. These findings suggest that CD90 may participate in liver cancer development. Yang *et al*[24] also found that 4×10^3 CD45-CD90+ cells from tumor tissues can form liver carcinoma in Beige/SCID mice. In addition, CD90+ cells can differentiate asymmetrically into CD90+ cells and CD90- cells, but CD90- daughter cells are all CD90- cells. These results show that CD90+ cells have strong proliferation, drug resistance and self-renewal abilities[25]. Recently, Michishita *et al*[26] reported that CD90+CD44+ HCC930599 cells (dog liver cancer cell line) have a stronger proliferation ability *in vitro* as well as self-renewal and tumorigenic abilities than CD90-CD44+ cells. All of these observations suggest that CD90 can be used as an LCSC marker.

The above studies show that CD90 is a potential marker of LCSCs. However, another study[27] reported that CD90+ liver cancer stem-like cells may participate in the late stage of liver cancer, and only appear in hepatitis B infection-related liver cancer. Therefore, CD90 still requires further research as a biomarker for LCSCs.

EPITHELIAL CELL ADHESION MOLECULE

Epithelial cell adhesion molecule (EpCAM), also known as CD326, is a single transmembrane glycoprotein encoded by the tumor-associated calcium signal transducer 1 gene, which belongs to a family of adhesion molecules. It has a molecular mass of 30-40 kDa and consists of three domains: an extracellular domain, a single transmembrane domain, and an intracellular structure domain. EpCAM has proven to be a marker of mature liver stem cells and progenitor cells, and is also a marker of hepatic oval cells[28,29]. Studies have shown that EpCAM participates in the β-catenin/Wnt signaling cascade, in which activation of proto-oncogenic proteins c-myc and cyclinA/E leads to tumorigenesis[30]. Yamashita *et al*[31] first reported that EpCAM can serve as a marker for LCSCs. Chen *et al*[32] found that CD133+EpCAM+ Huh7 cells have strong abilities for multi-directional differentiation, self-renewal, and clonal colony formation. Furthermore, only 500 CD133+EpCAM+ cells are tumorigenic in NOD/SCID mice. In addition, CD133+EpCAM+ cells show high expression of stem cell markers Nanog, Oct4, and Sox2.

In HCC patients, Sun *et al*[33] showed that EpCAM+ cells in peripheral circulation express other reported LCSC markers, CD133 and ABCG2. In NOD/SCID mice, injection of tumor cells showed that 300 EpCAM+CD45- cells were tumorigenic, whereas 1×10^4 EpCAM-CD45- cells were not tumorigenic. Schulze *et al*[34] also suggested the existence of EpCAM+ cells in peripheral circulation of patients with liver cancer.

Their clinical pathologic features tended to be > 400 ng/mL serum α-fetoprotein (AFP), various degrees of blood vessel metastasis, middle and advanced stage, and an overall low survival rate. Guo *et al*[35] followed patients with liver cancer after radical surgery, and found that the 1-, 2-, and 3-year survival rates of patients with EpCAM+ specimens were 85.7%, 51.3%, and 85.7%, respectively. Therefore, EpCAM+ cells may be LCSCs and radical surgery cannot completely kill these cells, which is the root cause of postoperative recurrence and metastasis. Therefore, EpCAM+ cell-targeting therapies are needed for the treatment of liver cancer.

OV6

Hepatic oval cells, called hepatic stem/progenitor cells in the liver Herring pipe, can differentiate into hepatocytes and bile duct cells. OV6 is a marker of hepatic oval cells[36]. In liver cancer induced by gene mutation, hepatic oval cells can become abnormal and differentiate into liver cancer cells or bile duct epithelial cells[37]. Thus, liver stem/progenitor cells may be involved in the development and progression of liver cancer. Recently, Jia *et al*[38] examined various cell surface markers, and found that liver cancer cells were derived from liver stem/progenitor cells. Yang *et al*[39] showed that OV6+ liver cancer cells have a stronger tumorigenic ability and chemotherapy resistance than OV6- cells. In addition, they found that the proportion of CD133+ cells in an HCC cell line ranged from 0.1% to 75%, while the proportion of OV6+ cells was relatively stable at 0.2%-3%. It is interesting to note the CD133+ cells express OV6, which further shows that OV6 can serve as a marker of LCSCs. Using magnetic bead separation, Yang *et al*[40] isolated OV6+ cells from HCC cell lines SMMC7721 and Huh7, and found that 103 OV6+ SMMC7721 cells or 104 OV6+ HuH7 cells were tumorigenic in NOD/SCID mice. They also found that OV6+ HCC cells *in vivo* and *in vitro* had strong invasion and metastatic abilities. These studies suggest that OV6 may be a potential marker of LCSCs.

CD44

CD44 is a transmembrane glycoprotein that mediates adhesion between cells and the extracellular matrix, lymphocyte activation and homing, and plays an important role in the invasion and metastasis of cancer. Zhu *et al*[41] reported that CD133+CD44+ cells have a strong tumorigenic ability in nude mice and high expression of the ATP binding cassette (ABC) transporter superfamily members (ABCB1, ABCC1, and ABCG2) that mediate resistance to chemotherapeutic drugs such as doxorubicin and vincristine. Further study found that CD133+CD44+ cells express genes related to stem cells such as β-catenin and Bmi-1. Hou *et al*[42] showed that CD133+CD44+ cells are the initial cells that produce metastasis to the lung and liver in immunodeficient mice. Analyses of human liver specimens showed that CD133+CD44+ liver cancer cells are associated with metastasis to the liver portal vein. Therefore, CD133 and CD44 can better define LCSCs.

Yang *et al*[23] first reported that CD90+CD44+ liver cancer cells are more aggressive, and both CD44 and CD90 can better define LCSCs. After irradiation of N1S1 rat liver cancer cells, Thompson *et al*[43] found a 22-fold increase in CD44+CD90+ cells and the use of a BEZ235 blocker could avoid thermal stress damage of PI3K-Akt-mTOR signaling pathways, in which CD44+ cells increased while CD90+ cells did not change. Further study of immunodeficient mice injected with liver cancer cells revealed CD44+ cells, but not CD90+ cells, on the edge of the thermal ablation and the edge of the liver cancer lesion. Therefore, after thermal ablation, recurrence was associated with a small number of CD44+ cells. Recently, Fernando *et al*[44] reported that transforming growth factor (TGF)-β treatment of long term-cultured PLC/PRF/5 liver cancer cells can induce resistance to sorafenib. Further analysis found that CD44 expression induced epithelial-to-mesenchymal transition characterized by vimentin protein expression, but the drug resistance was proportional to the number of CD44+ cells. In addition, repeated treatment with sorafenib could enrich CD44+ cells. Therefore, CD44 may be a potential molecular marker of LCSCs.

SIDE POPULATION CELLS

Fluorescence-activated cell sorting can isolate CSCs known as side population (SP) cells from a wide variety of tumor cell lines because the ABCG2 transporter effluxes the fluorescent DNA dye Hoechst 33342. The cell surface protein ABCG2, also called breast cancer resistance protein, is a member of the ABC transporter family, which was first identified in drug-resistant breast cancer cells. Cells expressing ABCG2 will pump out drugs, resulting in multi-drug resistance. LCSCs that are resistant to chemotherapy[45] indicate that ABCG2 is a candidate molecular marker of CSCs, which can be used for cell separation.

Chiba *et al*[46] found 0.25%-0.80% of SP cells among liver cancer cell lines Huh7 and PLC/PRF/5. Compared with non-SP cells, the SP cells had a higher proliferative capacity and considerable ability to resist apoptosis. *In vitro* studies also suggest that transplantation of 103 SP cells into immunodeficient NOD/SCID mice can cause tumors. Therefore, separation of SP cells can be useful when CSC markers are unknown. A recent study[47] reported that SP cells from the HAK-1A liver cancer cell line do not express CD90, EpCAM, CD13 or CD133. In the HAK-1B cell line, compared with non-SP cells, the SP cells have a clonal growth ability, strong tumorigenic ability, fast growth rate, and highly express CD13. However, there are no differences in the chemotherapy resistance,

colony forming ability, or cell cycle.

TARGETED THERAPIES AGAINST LCSC MARKERS

Because LCSCs are related to drug resistance, metastasis, and recurrence of liver cancer, targeting LCSCs as a cancer treatment has become a promising strategy. Some studies have found that treatment measures targeting certain surface markers of LCSCs can inhibit their self-renewal and tumorigenesis, such as disrupting the expression of LCSC surface markers CD133[48], EpCAM[31,49], CD24[50], and CD13[49]. In addition, neutralizing antibodies against CD44 can effectively induce apoptosis of CD90+CD44+ LCSCs[23]. There is a broad prospect to develop targeted drugs against specific surface markers of LCSCs.

Some key signaling pathways in LCSCs are also therapeutic targets. Single target therapy is limited, but targeting both the LCSCs and surrounding environment may be more effective to inhibit the growth and metastasis of HCC.

Determination of specific markers for LCSCs and development of corresponding diagnostic strategies will be useful to detect LCSCs and monitor whether LCSCs have diffused into the blood and bone marrow. Such approaches might accurately predict metastasis and/or recurrence in HCC patients, and enable more individualized treatment plans.

PROBLEMS AND PROSPECTS OF LCSCS

LCSC sources

CSC theory suggests that tumor growth and progression are maintained by a small population of CSCs in tumor tissue. The number of cells with stem cell properties is maintained by CSC self-renewal, while CSCs constantly produce new tumor cells by differentiation.

In terms of the origin of CSCs (including LCSCs), there are two viewpoints. Most researchers believe that CSCs originate from abnormal proliferation and differentiation of stem cells[51-53]. In chronic liver disease caused by a variety of reasons such as viral hepatitis, fatty hepatitis, and metabolic liver disease, liver stem cells are actively proliferating. Under the influence of carcinogenic factors, actively proliferating liver stem cells might undergo malignant changes. In addition, most recognized LCSC surface markers such as CD133, EpCAM, and CD90 are also surface markers of stem cells. Finally, the development of LCSCs has common molecular signaling pathways or regulatory molecules with liver stem cells, such as Wnt, TGF-β, Notch, Hedgehog, Myc, and Bmi1[6]. However, the derivation of induced pluripotent stem cells by Takahashi *et al*[54] changed the understanding of the sources of stem cells. Therefore, some researchers consider that CSCs can also be induced from mature tumor cells under the action of various factors[55,56]. Recently, Holczbauer *et al*[57] reported that liver stem/progenitor cells and mature liver cells can transform into LCSCs by excessive activation of Ras. LCSCs are a dynamic cell population. Therefore, factors in the surrounding environment (such as chemotherapeutic drugs, radiation, oxygen, growth factors, and inflammatory factors) might induce both differentiation of LCSCs to cancer cells and mature cells to LCSCs to maintain the growth and progression of tumors.

FUTURE APPLICATIONS OF LCSC MARKERS

Although many surface markers have been reported for LCSCs, there is still no unified standard. At present, cells isolated by most surface markers have LCSC characteristics, but some markers mutually have less cross expression. Therefore, each marker may represent a subset of LCSCs. For example, Yamashita *et al*[27] reported that EpCAM+ and CD90+ LCSCs represent different cells with different biological characteristics. In addition, CSCs are considered to be a small population of tumor cells, and some markers, such as CD133, EpCAM, CD44, and CD24, can be expressed by 50% or more cells in HCC cell lines. Whether these markers can fully represent LCSCs is unclear, and their sensitivity and specificity require further study. To improve the specificity of LCSC markers, some researchers have advocated the combined use of multiple markers for LCSCs, such as CD90 and CD44[23,24], CD133 and CD44[41], CD133 and ALDH[11], EpCAM and AFP[31], and CD133 and EpCAM[32]. Considering a consensus is yet to be reached for LCSC markers, these markers may represent heterogeneous cells. Which surface markers or which combination of markers has higher specificity still requires further research.

LCSC markers have not been recognized until now, and the current problem is determination of LCSC-specific markers for identification, separation and cultivation of LCSCs. Different LCSC markers may represent different stages of liver stem cell differentiation[58]. Furthermore, LCSCs of different origins may express different markers[31].

REFERENCES

1 **Lau CK**, Yang ZF, Fan ST. Role of stem cells in normal liver and cancer. *Anticancer Agents Med Chem* 2011; **11**: 522-528 [PMID: 21554200 DOI: 10.2174/187152011796011091]

2 **Reya T**, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature* 2001; **414**: 105-111 [PMID: 11689955 DOI: 10.1038/35102167]

3 **Chiba T**, Zheng YW, Kita K, Yokosuka O, Saisho H, Onodera M, Miyoshi H, Nakano M, Zen Y, Nakanuma Y, Nakauchi H, Iwama A, Taniguchi H. Enhanced self-renewal capability in hepatic stem/progenitor cells drives cancer initiation. *Gastroenterology* 2007; **133**: 937-950 [PMID: 17673212 DOI: 10.1053/j.gastro.2007.06.016]

4 **Wu XZ**, Yu XH. Bone marrow cells: the source of hepatocellular

carcinoma? *Med Hypotheses* 2007; **69**: 36-42 [PMID: 17300877 DOI: 10.1016/j.mehy.2006.10.064]
5 **Ji J**, Wang XW. Clinical implications of cancer stem cell biology in hepatocellular carcinoma. *Semin Oncol* 2012; **39**: 461-472 [PMID: 22846863 DOI: 10.1053/j.seminoncol.2012.05.011]
6 **Rountree CB**, Mishra L, Willenbring H. Stem cells in liver diseases and cancer: recent advances on the path to new therapies. *Hepatology* 2012; **55**: 298-306 [PMID: 22030746 DOI: 10.1002/hep.24762]
7 **Visvader JE**, Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. *Nat Rev Cancer* 2008; **8**: 755-768 [PMID: 18784658 DOI: 10.1038/nrc2499]
8 **Ishizawa K**, Rasheed ZA, Karisch R, Wang Q, Kowalski J, Susky E, Pereira K, Karamboulas C, Moghal N, Rajeshkumar NV, Hidalgo M, Tsao M, Ailles L, Waddell TK, Maitra A, Neel BG, Matsui W. Tumor-initiating cells are rare in many human tumors. *Cell Stem Cell* 2010; **7**: 279-282 [PMID: 20804964 DOI: 10.1016/j.stem.2010.08.009]
9 **Yin AH**, Miraglia S, Zanjani ED, Almeida-Porada G, Ogawa M, Leary AG, Olweus J, Kearney J, Buck DW. AC133, a novel marker for human hematopoietic stem and progenitor cells. *Blood* 1997; **90**: 5002-5012 [PMID: 9389720]
10 **Corbeil D**, Röper K, Hellwig A, Tavian M, Miraglia S, Watt SM, Simmons PJ, Peault B, Buck DW, Huttner WB. 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 [PMID: 10681530 DOI: 10.1074/jbc.275.8.5512]
11 **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]
12 **Piao LS**, Hur W, Kim TK, Hong SW, Kim SW, Choi JE, Sung PS, Song MJ, Lee BC, Hwang D, Yoon SK. CD133+ liver cancer stem cells modulate radioresistance in human hepatocellular carcinoma. *Cancer Lett* 2012; **315**: 129-137 [PMID: 22079466 DOI: 10.1016/j.canlet.2011.10.012]
13 **Lan X**, Wu YZ, Wang Y, Wu FR, Zang CB, Tang C, Cao S, Li SL. CD133 silencing inhibits stemness properties and enhances chemoradiosensitivity in CD133-positive liver cancer stem cells. *Int J Mol Med* 2013; **31**: 315-324 [PMID: 23233126 DOI: 10.3892/ijmm.2012.1208]
14 **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]
15 **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 DOI: 10.1002/ijc.22476]
16 **Yao J**, Zhang T, Ren J, Yu M, Wu G. Effect of CD133/prominin-1 antisense oligodeoxynucleotide on in vitro growth characteristics of Huh-7 human hepatocarcinoma cells and U251 human glioma cells. *Oncol Rep* 2009; **22**: 781-787 [PMID: 19724856 DOI: 10.3892/or_00000500]
17 **Kohga K**, Tatsumi T, Takehara T, Tsunematsu H, Shimizu S, Yamamoto M, Sasakawa A, Miyagi T, Hayashi N. Expression of CD133 confers malignant potential by regulating metalloproteinases in human hepatocellular carcinoma. *J Hepatol* 2010; **52**: 872-879 [PMID: 20395004 DOI: 10.1016/j.jhep.2009.12.030]
18 **Ma S**, Chan KW, Hu L, Lee TK, Wo JY, Ng IO, Zheng BJ, Guan XY. Identification and characterization of tumorigenic liver cancer stem/progenitor cells. *Gastroenterology* 2007; **132**: 2542-2556 [PMID: 17570225 DOI: 10.1053/j.gastro.2007.04.025]
19 **Song W**, Li H, Tao K, Li R, Song Z, Zhao Q, Zhang F, Dou K. Expression and clinical significance of the stem cell marker CD133 in hepatocellular carcinoma. *Int J Clin Pract* 2008; **62**: 1212-1218 [PMID: 18479363 DOI: 10.1111/j.1742-1241.2008.01777.x]
20 **Salnikov AV**, Kusumawidjaja G, Rausch V, Bruns H, Gross W, Khamidjanov A, Ryschich E, Gebhard MM, Moldenhauer G, Büchler MW, Schemmer P, Herr I. Cancer stem cell marker expression in hepatocellular carcinoma and liver metastases is not sufficient as single prognostic parameter. *Cancer Lett* 2009; **275**: 185-193 [PMID: 19026485 DOI: 10.1016/j.canlet.2008.10.015]
21 **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]
22 **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]
23 **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]
24 **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]
25 **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]
26 **Michishita M**, Ezaki S, Ogihara K, Naya Y, Azakami D, Nakagawa T, Sasaki N, Arai T, Shida T, Takahashi K. Identification of tumor-initiating cells in a canine hepatocellular carcinoma cell line. *Res Vet Sci* 2014; **96**: 315-322 [PMID: 24534130]
27 **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]
28 **Tanaka M**, Okabe M, Suzuki K, Kamiya Y, Tsukahara Y, Saito S, Miyajima A. Mouse hepatoblasts at distinct developmental stages are characterized by expression of EpCAM and DLK1: drastic change of EpCAM expression during liver development. *Mech Dev* 2009; **126**: 665-676 [PMID: 19527784 DOI: 10.1016/j.mod.2009.06.939]
29 **Schmelzer E**, Zhang L, Bruce A, Wauthier E, Ludlow J, Yao HL, Moss N, Melhem A, McClelland R, Turner W, Kulik M, Sherwood S, Tallheden T, Cheng N, Furth ME, Reid LM. Human hepatic stem cells from fetal and postnatal donors. *J Exp Med* 2007; **204**: 1973-1987 [PMID: 17664288 DOI: 10.1084/jem.20061603]
30 **Maetzel D**, Denzel S, Mack B, Canis M, Went P, Benk M, Kieu C, Papior P, Baeuerle PA, Munz M, Gires O. Nuclear signalling by tumour-associated antigen EpCAM. *Nat Cell Biol* 2009; **11**: 162-171 [PMID: 19136966 DOI: 10.1038/ncb1824]
31 **Yamashita T**, Ji J, Budhu A, Forgues M, Yang W, Wang HY, Jia H, Ye Q, Qin LX, Wauthier E, Reid LM, Minato H, Honda M, Kaneko S, Tang ZY, Wang XW. EpCAM-positive hepatocellular carcinoma cells are tumor-initiating cells with stem/progenitor cell features. *Gastroenterology* 2009; **136**: 1012-1024 [PMID: 19150350 DOI: 10.1053/j.gastro.2008.12.004]
32 **Chen Y**, Yu D, Zhang H, He H, Zhang C, Zhao W, Shao RG. CD133(+)EpCAM(+) phenotype possesses more characteristics of tumor initiating cells in hepatocellular carcinoma Huh7 cells. *Int J Biol Sci* 2012; **8**: 992-1004 [PMID: 22904667 DOI: 10.7150/ijbs.4454]
33 **Sun YF**, Xu Y, Yang XR, Guo W, Zhang X, Qiu SJ, Shi RY, Hu B, Zhou J, Fan J. Circulating stem cell-like epithelial cell adhesion molecule-positive tumor cells indicate poor prognosis of hepatocellular carcinoma after curative resection. *Hepatology* 2013; **57**: 1458-1468 [PMID: 23175471 DOI: 10.1002/hep.26151]
34 **Schulze K**, Gasch C, Staufer K, Nashan B, Lohse AW, Pantel K, Riethdorf S, Wege H. Presence of EpCAM-positive circulating tumor cells as biomarker for systemic disease strongly correlates to survival in patients with hepatocellular carcinoma. *Int J Cancer*

2013; **133**: 2165-2171 [PMID: 23616258 DOI: 10.1002/ijc.28230]
35 **Guo Z**, Li LQ, Jiang JH, Ou C, Zeng LX, Xiang BD. Cancer stem cell markers correlate with early recurrence and survival in hepatocellular carcinoma. *World J Gastroenterol* 2014; **20**: 2098-2106 [PMID: 24616575 DOI: 10.3748/wjg.v20.i8.2098]
36 **Crosby HA**, Hubscher SG, Joplin RE, Kelly DA, Strain AJ. Immunolocalization of OV-6, a putative progenitor cell marker in human fetal and diseased pediatric liver. *Hepatology* 1998; **28**: 980-985 [PMID: 9755234 DOI: 10.1002/hep.510280412]
37 **Pusterla T**, Nèmeth J, Stein I, Wiechert L, Knigin D, Marhenke S, Longerich T, Kumar V, Arnold B, Vogel A, Bierhaus A, Pikarsky E, Hess J, Angel P. Receptor for advanced glycation endproducts (RAGE) is a key regulator of oval cell activation and inflammation-associated liver carcinogenesis in mice. *Hepatology* 2013; **58**: 363-373 [PMID: 23504974 DOI: 10.1002/hep.26395]
38 **Jia SQ**, Ren JJ, Dong PD, Meng XK. Probing the hepatic progenitor cell in human hepatocellular carcinoma. *Gastroenterol Res Pract* 2013; **2013**: 145253 [PMID: 23533383 DOI: 10.1155/2013/145253]
39 **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]
40 **Yang W**, Wang C, Lin Y, Liu Q, Yu LX, Tang L, Yan HX, Fu J, Chen Y, Zhang HL, Tang L, Zheng LY, He YQ, Li YQ, Wu FQ, Zou SS, Li Z, Wu MC, Feng GS, Wang HY. $OV6^+$ tumor-initiating cells contribute to tumor progression and invasion in human hepatocellular carcinoma. *J Hepatol* 2012; **57**: 613-620 [PMID: 22612999 DOI: 10.1016/j.jhep.2012.04.024]
41 **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]
42 **Hou Y**, Zou Q, Ge R, Shen F, Wang Y. The critical role of CD133(+)CD44(+/high) tumor cells in hematogenous metastasis of liver cancers. *Cell Res* 2012; **22**: 259-272 [PMID: 21862973 DOI: 10.1038/cr.2011.139]
43 **Thompson SM**, Callstrom MR, Butters KA, Sutor SL, Knudsen B, Grande JP, Roberts LR, Woodrum DA. Role for putative hepatocellular carcinoma stem cell subpopulations in biological response to incomplete thermal ablation: in vitro and in vivo pilot study. *Cardiovasc Intervent Radiol* 2014; **37**: 1343-1351 [PMID: 24452318]
44 **Fernando J**, Malfettone A, Cepeda EB, Vilarrasa-Blasi R, Bertran E, Raimondi G, Fabra À, Alvarez-Barrientos A, Fernández-Salguero P, Fernández-Rodríguez CM, Giannelli G, Sancho P, Fabregat I. A mesenchymal-like phenotype and expression of CD44 predict lack of apoptotic response to sorafenib in liver tumor cells. *Int J Cancer* 2015; **136**: E161-E172 [PMID: 25053293 DOI: 10.1002/ijc.29097]
45 **Ding XW**, Wu JH, Jiang CP. ABCG2: a potential marker of stem cells and novel target in stem cell and cancer therapy. *Life Sci* 2010; **86**: 631-637 [PMID: 20159023]
46 **Chiba T**, Kita K, Zheng YW, Yokosuka O, Saisho H, Iwama A, Nakauchi H, Taniguchi H. Side population purified from hepatocellular carcinoma cells harbors cancer stem cell-like properties. *Hepatology* 2006; **44**: 240-251 [PMID: 16799977 DOI: 10.1002/hep.21227]
47 **Nakayama M**, Ogasawara S, Akiba J, Ueda K, Koura K, Todoroki K, Kinoshita H, Yano H. Side population cell fractions from hepatocellular carcinoma cell lines increased with tumor dedifferentiation, but lack characteristic features of cancer stem cells. *J Gastroenterol Hepatol* 2014; **29**: 1092-1101 [PMID: 24325739 DOI: 10.1111/jgh.12484]
48 **Tang KH**, Ma S, Lee TK, Chan YP, Kwan PS, Tong CM, Ng IO, Man K, To KF, Lai PB, Lo CM, Guan XY, Chan KW. CD133(+) liver tumor-initiating cells promote tumor angiogenesis, growth, and self-renewal through neurotensin/interleukin-8/CXCL1 signaling. *Hepatology* 2012; **55**: 807-820 [PMID: 21994122 DOI: 10.1002/hep.24739]
49 **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]
50 **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 [PMID: 21726833 DOI: 10.1016/j.stem.2011.06.005]
51 **Friedmann-Morvinski D**, Bushong EA, Ke E, Soda Y, Marumoto T, Singer O, Ellisman MH, Verma IM. Dedifferentiation of neurons and astrocytes by oncogenes can induce gliomas in mice. *Science* 2012; **338**: 1080-1084 [PMID: 23087000 DOI: 10.1126/science.1226929]
52 **Schepers AG**, Snippert HJ, Stange DE, van den Born M, van Es JH, van de Wetering M, Clevers H. Lineage tracing reveals Lgr5+ stem cell activity in mouse intestinal adenomas. *Science* 2012; **337**: 730-735 [PMID: 22855427 DOI: 10.1126/science.1224676]
53 **Tong CM**, Ma S, Guan XY. Biology of hepatic cancer stem cells. *J Gastroenterol Hepatol* 2011; **26**: 1229-1237 [PMID: 21557770 DOI: 10.1111/j.1440-1746.2011.06762.x]
54 **Takahashi K**, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006; **126**: 663-676 [PMID: 16904174 DOI: 10.1016/j.cell.2006.07.024]
55 **Scaffidi P**, Misteli T. In vitro generation of human cells with cancer stem cell properties. *Nat Cell Biol* 2011; **13**: 1051-1061 [PMID: 21857669 DOI: 10.1038/ncb2308]
56 **Vermeulen L**, De Sousa E Melo F, van der Heijden M, Cameron K, de Jong JH, Borovski T, Tuynman JB, Todaro M, Merz C, Rodermond H, Sprick MR, Kemper K, Richel DJ, Stassi G, Medema JP. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. *Nat Cell Biol* 2010; **12**: 468-476 [PMID: 20418870 DOI: 10.1038/ncb2048]
57 **Holczbauer A**, Factor VM, Andersen JB, Marquardt JU, Kleiner DE, Raggi C, Kitade M, Seo D, Akita H, Durkin ME, Thorgeirsson SS. Modeling pathogenesis of primary liver cancer in lineage-specific mouse cell types. *Gastroenterology* 2013; **145**: 221-231 [PMID: 23523670 DOI: 10.1053/j.gastro.2013.03.013]
58 **Mishra L**, Banker T, Murray J, Byers S, Thenappan A, He AR, Shetty K, Johnson L, Reddy EP. Liver stem cells and hepatocellular carcinoma. *Hepatology* 2009; **49**: 318-329 [PMID: 19111019 DOI: 10.1002/hep.22704]
59 **Ma S**. Biology and clinical implications of CD133(+) liver cancer stem cells. *Exp Cell Res* 2013; **319**: 126-132 [PMID: 22999864]
60 **Zen Y**, Fujii T, Yoshikawa S, Takamura H, Tani T, Ohta T, Nakanuma Y. Histological and culture studies with respect to ABCG2 expression support the existence of a cancer cell hierarchy in human hepatocellular carcinoma. *Am J Pathol* 2007; **170**: 1750-1762 [PMID: 17456779 DOI: 10.2353/ajpath.2007.060798]

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