Hierarchical potential differentiation of liver cancer stem cells

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Conflict of interest

None declared

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Abstract

Hepatocellular carcinoma (HCC) is one of the most malignant tumors in Chinese people and offers poor prognosis. Tumor tissue, like normal tissue, is hierarchically differentiated. Thus, minor tumor cell populations able to differentiate, such as stem cells, sustain tumor self-renewal and proliferation. The fact that liver cancer stem cells (CSCs) with different surface markers appear heterogeneous with respect to oncogenesis and drug resistance indicates that subpopulations of surface markers preserve the hierarchical potential of differentiation during proliferation, deterioration and relapse. The epithelial to mesenchymal transition (EMT) is correlated to tumor malignancy and aggression, and hepatocytes bearing EMT have obvious hierarchical differentiation potential with respect to signaling pathways such as transforming growth factor β , Wnt/ β -catenin and microRNA. Therefore, it may be more effective for early diagnosis to monitor HCC recurrence using peripherally circulating CSCs, and these may also offer potential for HCC immunotherapy or for targeting HCC treatment using these markers. Thus, we reviewed the generation, hierarchical differentiation and clinical application of hepatic CSCs.

Key words: tumor heterogeneity, tumorigenesis, liver cancer stem cells, surface molecular markers

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Introduction

A high-grade malignant solid tumor with dismal prognoses, hepatocellular carcinoma (HCC) represents the 4th most common malignant neoplasm in Chinese people.¹ Although studies of HCC are advancing, methods for early and effective diagnosis of HCC require improvement.^{2–3} Once HCC symptoms appear, the cancer is often very advanced and includes intrahepatic or systemic metastasis.⁴ Poorly timed identification, late treatment and postoperative relapse contribute to high HCC mortality.⁵ Although much effort to understand HCC recurrence has been put forth, the causes of therapeutic resistance are unclear. Cancer stem cells (CSCs), which may also be considered tumor initiating cells (TICs), may be the source from which HCC arises. In addition, CSCs may be the chief cause of tumor heterogeneity, which may contribute to drug resistance.^{6–9} The hierarchical differentiation of hepatic CSCs and immunologic escape of self-monitoring are connected to tumorigenic stage.⁶ Meanwhile, signaling pathways and genes that regulate stem cell differentiation may contribute to the control and maintenance of CSC differentiation, such as transforming growth factor β (TGF- β), Wnt/ β -catenin and microRNA.^{8–9} Thus, to better understand the etiology of HCC and address drug resistance, we must understand liver CSCs.

Research of the hierarchical differentiation potential of CSCs

Neoplasms are thought to represent the clonal proliferation of malignant tissue driven by genomic and genetic instabilities that are unrelated to mutation, selection or adaptation.⁶ Recent data points to the importance of identification of genotypes among tumor patients because interpatient heterogeneity may drive treatment advances in oncology and may guide individualized therapies.⁷ However, different genetic subclones have been identified in the same tumor and within the same pathological tumor classification with different genetic phenotypes. Then, over time, these subgroup clones behave differently, or cause tumor or intratumor heterogeneity, which are thought to be critical for tumorigenesis and metastasis.^{8,9}

The generation of heterogeneity is related to the differentiation potential of embryonic stem cells that develop into various functional organs.⁹ Thus, HCC is a stem cell abnormality and their ability to self-renew and differentiate is one of their most critical features, hence their reclassification as CSCs or TICs. These cells can initiate tumor formation after administration into nude mice, and they subsequently differentiate to different functional cancer cells in the lesions from which they are derived.

CSCs have 2 hypothetical contributions to oncogenesis and tumor heterogeneity. First, every tumor cell in the same tumor lesion is created with a potential for differ-

entiation and a specific tumor cell population will proliferate and differentiate to stimulate tumor growth.⁷ Next, the tumor lesion is hierarchically organized as is normal tissue differentiation from embryonic stem cells. Few CSCs can proliferate and sustain long-term tumor growth and the remaining tumor cells are silent due to a silencing of differentiation potential by the hierarchical organization.⁷ Strictly speaking, CSCs have the same genotype as other tumor cells in the same tumor lesion, and are only unique after post-translational modifications and epigenetic events. Investigators have reported that this may be due to associations with the mechanism of normal tissue differentiation from embryonic stem cells.¹⁰ All cancer cells originate from one precursor (the CSC) and in a tumor microenvironment, HCC progenitor cells acquire autocrine IL-6 signaling to stimulate growth and malignant progression for CSCs via upregulation of LIN28 expression.¹¹ Although all types of hepatic lineages can acquire oncogenic reprogramming to CSCs, how this occurs and why requires more study.^{12–13}

Obtaining hepatic CSCs

The hepatic CSC theory has been promoted since 1989, and it suggests that a few specific hepatic cancer cell lesions obtain differentiation potential and cause malignant differentiation of HCCs by altering enzyme structures.¹⁰ However, limited technologies at that time prevented direct data to support the existence of liver CSCs. Now, with immunofluorescence, immunomagnetic beads and flow cytometry, we can procure liver CSCs using liver cancer cell markers.¹⁴ These methods also support research into mechanism of HCC heterogeneity and various hepatic CSC surface markers have been confirmed. At present, 10 hepatic CSC markers such as CD133, CD90, and CD44 have been reported.¹⁵

CD133, or Prominin-1, is highly expressed in fetal liver progenitor cells and is a member of the hematopoietic stem/progenitor cell transmembrane glycoproteins. CD133 is the earliest and most widely used protein for the separation of liver CSC subpopulations.^{16–18} Stephanie reported that CD133-positive liver cancer cells were more tumorigenic than CD133-negative liver cancer cells in vivo and in vitro.¹⁹ Also, stem cell-related genes such as Notch, β -catenin and Oct3/4 are highly expressed in the CD133-positive subpopulation.²⁰

CD90, or Thy-1, is a surface marker of hepatic oval cells and CD45-negative CD90-positive subpopulations exists in hepatic tumor lesions and in the peripheral circulation.²⁰ Yang's group identified CD90-positive CD44-positive and CD45-negative markers for CSCs from liver lesions and the peripheral circulation, but they report that these stem cells have increased tumorigenicity and invasiveness compared to CD133-positive subpopulations.^{21–23}

CD44 is a cell surface adhesion molecule that binds to extracellular ligand to regulate hepatic cancer proliferation, differentiation, metastasis and survival.^{16,24–25} Moreover, CD45-positive subpopulations often express other stem cell markers such as CD133 and CD90.^{21,26} Yang and co-workers suggest that CD44-positive liver CSC subpopulations are more aggressive than CD44-negative subpopulations.²¹ Furthermore, Zhu's group reported that CD44-positive CD133-positive liver CSCs not only excessively express stem cell-related genes, but are more resistant to chemotherapy than CD44-negative CD133-positive cells.²⁶

High expression of CD13, which is regarded to be a marker for liver cancer that is dormant or half dormant, occurs during the G1/G0 phase.^{27–29} Haraguchi's group reported that CD13-positive hepatic cancer cells are more resistant to 5-FU and Adriamycin and have greater oncogenicity.²⁹ Also, CD13 expression was excessively induced by 5-FU and Adriamycin in liver cancer cells.²⁹

Epithelial cell adhesion molecule (EpCAM), or CD326, is highly expressed in embryonic liver cells, bile duct epithelial cells, and hyperplastic bile duct epithelial cells in liver cirrhosis.³⁰ Yamashita et. al. reported that EpCAM is expressed more in liver cancer and the molecular signaling pathways of EpCAM-positive liver cancer cells are closely related to liver progenitor cells.^{31–33} Simultaneously, Terris' group reported that EpCAM-positive AFP-positive liver cancer cells are more tumorigenic, mobile and invasive to the portal vein.³¹ Therefore, EpCAM is thought to be a marker of liver CSCs.

OV6 is a marker of hepatic oval cells and is closely related to hepatic disease.^{34,35} OV6-positive liver cancer cells are said to have more resistance to chemotherapy in vitro³⁶ and they are more aggressive in patients as well as are closely related to the clinical pathological characteristics of liver cancer.³⁷ However, OV6-positive patients with liver cancer are not unusually different with respect to disease-free and overall survival compared to OV6-negative patients with liver cancer.³⁷

SALL4, a member of the zinc finger transcription factor family, is a stem cell marker.^{38,39} Oikawa's group suggested that sal-like protein (SALL4) provokes the proliferation of liver cancer cells in vitro and induces overexpression of various liver CSC surface markers such as EpCAM, ATP-binding cassette-G2 (ABCG2) and CK19.³⁹ Others regard SALL4 to be a marker of liver CSCs, which can be used to predict HCC treatment outcomes.^{40–42} Furthermore, expression of phosphatase and tensin homologue protein was suppressed and the formation of tumors in xenograft models was inhibited by overexpression of SALL4.⁴³ Thus, inhibiting expression of SALL4 can inhibit proliferation and differentiation of liver cancer cells.^{44–48}

Many laboratories have reported that different liver cancer subpopulations sorted by surface markers have diverse features. For example, Zen and colleagues reported that liver cancer subpopulations sorted by ABCG2 have

drug tolerance.⁴⁹ More interestingly, aldehyde dehydrogenase (ALDH) is not a stem cell surface marker but ALDH-positive CD133-positive cells have greater tumorigenicity and invasiveness.⁵⁰ Recently, toll-like receptors 4 and interleukin-6 were referred to as liver CSC markers due to their relationship with inflammation and tumorigenesis.^{11,51} However, these subpopulations marked by different surface proteins maintain orderly differentiation potential during proliferation, deterioration and relapse in liver cancer and these may be the source of cancer heterogeneity. Thus, investigations of the mechanisms of liver CSC surface markers during cancer proliferation, metastasis and relapse may allow the development of agents that can inhibit tumor growth by targeting subpopulations of CSCs with unique differentiation potentials.

Mechanism of tumor heterogeneity produced by liver CSCs

Epithelial-to-mesenchymal transition (EMT) is closely related with the degree of liver cancer malignance and aggression, and liver cancer cells bearing EMT have obvious hierarchical differentiation potential closely associated with signaling pathways such as transforming growth factor β (TGF- β), Wnt/ β -catenin and microRNA.^{37,52,53}

Knock-out of TGF- β inhibits the incidence of liver cancer in nude mice and overexpression of TGF- β not only increases the oncogenicity of liver cancer via highly expressed transcription factors related to EMT in different tumor cells such as TWIST1, TWIST2, SNAI1, SNAI2, ZEB1 and ZEB2, but also makes the cells more likely to metastasize.⁵⁴ Also, E-cadherin is inhibited by overexpression of TGF- β and high expression of TWIST1 has been discovered in CD44-positive CD24-negative breast CSCs with more obvious potential for differentiation and more aggressive behavior.^{55,56} In addition, TGF- β signaling alters the patterns of liver tumorigenesis induced by PTEN inactivation in liver CD133-positive CSCs but how tumor heterogeneity is regulated by TWIST1 is unclear.

Leal and colleagues reported that microRNA 181 (miR-181) is highly expressed in embryonic liver and EpCAM-positive AFP-positive liver cancer subpopulations.^{57,58} Also, the tumorigenicity of liver CSCs is significantly suppressed by inhibition of miR-181 which chiefly functions to regulate the differentiation potential of liver CSCs by activating transcription factors such as caudal homeobox gene 2 (CDX2), transcription factor-GATA6 and by negatively regulating the Wnt/ β -catenin pathway via nemo-like kinase (NLK).^{52,57,58} Moreover, let-7 and Lin28 have been reported to be related to the growth and metastasis of HCC. Lin28 is highly expressed in normal embryonic stem cells and maintains the self-renewal of liver CSCs by inhibiting the combination of let-7 with mature microRNA.⁵⁹ Research into microRNA suggests that positive and negative pathways exist for regulating

the differentiation potential of liver CSCs via different microRNAs.^{58,59} For negative regulation, degraded let-7 by excessively active Lin28 and c-MYC disequilibrate liver CSCs and all cancer cells that accelerate the growth and metastasis of HCC.⁵⁹ Meanwhile, with positive regulation, high expression of EpCAM, which is regarded as a prominent marker of liver CSCs, is mediated by inhibition of TGF- β by downstream transcription factors of miR-18 such as CDX2, GATA6 and NLK.^{53,54} EpCAM intracellular domain (EpICD), which is a lysate from EpCAM, enters the nucleus and induces overexpression of cyclin D1, c-MYC and miR-181 after combining with LIM domain protein 2 (FHL2), β -catenin and lymphoid enhancer factor 1 (Lef-1).⁵⁸ In addition, Liu's group asserted that TGF- β 1 acts through miR-155 to down-regulate TP53INP1 to promote EMT and liver CSC phenotypes.⁶⁰ The Wnt/ β -catenin signaling pathway that regulates tumor heterogeneity is mainly related to microRNA but how this occurs to sustain the balance of liver CSCs and cancer cells has not been elucidated.

Function of liver CSCs for diagnosis and treatment of HCC

Evidence is accumulating that CSCs are a source of cancer cell and tumor heterogeneity.⁶⁻⁹ Yang's group reported that liver CSCs from the peripheral circulation had greater tumorigenicity and confirmed that these were significantly positively related to a 2-year relapse after resection.²³ Various liver CSC-related proteins have been reported to be associated with malignance of liver cancer, such as K19 and c-kit.⁶¹ K19 is a keratin on the bile duct cell and c-kit, or CD117, is a stem cell factor receptor. K19 and c-kit are reported to be associated with a range of vascular invasion, tumor diameters, degree of cancer differentiation and APF.⁶¹ Obtaining liver CSCs from the peripheral blood has been made easier with flow cytometry and immunomagnetic beads.^{14,19}

Liu's group reported that CD90 has a specificity of 91.9% for HCC and a sensitivity of 48.22% for predicting poor differentiation.⁶² In addition, TLR4-positive CSCs were found to be significantly greater in HCC tissues with microvascular invasion and strongly associated with both early recurrence and poor patient survival.⁵¹ Thus, high expression of these markers is significantly associated with poor response to treatment and reduced survival, and measuring and/or monitoring liver CSCs in liver lesions or the peripheral circulation may be an effective tool for surveilling growth, metastasis and relapse of HCC.

Stem cell heterogeneity indicates tolerance for cell death induced by DNA damage due to a survival advantage from their full potential of gene expression⁶⁻⁹ and this was discovered in liver CSCs that are more tolerant to chemotherapy and radiotherapy and have greater relapse and metastasis after diverse interventions com-

pared to quiescent cancer cells. It has been reported that a specific antibody targeted to EpCAM can more effectively inhibit liver cancer growth than conventional chemotherapy.^{31-33,59} In addition, liver CSCs influence sorafenib resistance via Akt/pi3k, WNT, Notch, and IL-6 pathways.⁶³ Therefore, specific inhibitors targeted to surface markers that directly kill liver CSCs or restrain self-renewal and differentiation may hold promise for tumor treatment.⁶⁴⁻⁶⁶

Conclusions

Higher oncogenicity has been reported for hepatic CSC surface markers to suggest a source of tumor heterogeneity but at what stage of differentiation these markers are best monitored is uncertain. Additionally, various liver CSC subpopulations may be more tolerant to radiotherapy or chemotherapy and this may explain the tumor heterogeneity due to hepatic CSC post-translational modifications and epigenetic differences of hierarchical differentiation. Current evidence suggests that measuring peripheral circulating CD90-positive liver CSCs may be useful for early diagnosis and for monitoring potential tumor relapse. Meanwhile, antibodies targeted to liver CSCs with IL-6 or its inhibitors to inhibit self-renewal and survival signaling pathways may hold promise for immunotherapy.

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