HBV X may play a role in generating EpCAM$^+$ CSCs.[17] The role of hepatitis virus infection on the generation of CSCs is still unclear and should be clarified in future studies.

We were unable to confirm the tumorigenicity of CD90$^+$ cells in 13 of 15 HCCs, but we observed abundant CD90$^+$ cells in more-advanced HCCs by IHC (data not shown). Tumorigenic CD90$^+$ cells may emerge at a later stage of hepatocarcinogenesis, and the majority of CD90$^+$ cells in early HCCs may be cancer-associated VECs without tumorigenic capacity. Furthermore, we identified tumorigenic CD90$^+$ cells only from HBV-related HCCs, and a recent study suggested that expression of CD90 was associated with HBV infection.[16] We could not detect the small population of CD90$^+$ HuH7 and Hep3B cells reported on by Yang et al. However, because we identified a small population of CD90$^+$ HuH7 cells after treatment with 5-FU (manuscript in preparation), it is conceivable that different cellular stress statuses may explain the observed differences between our findings and those of Yang et al.

The majority of CSC markers discovered thus far are almost identical to those found in healthy tissue stem cells or embryonic stem cells. However, with regard to the liver, the characteristics of healthy hepatic stem/progenitor cells isolated using different stem cell markers are currently under investigation. A recent article examined the characteristics of EpCAM$^+$ and CD90$^+$ oval cells isolated from 2-acetylaminofluorene/partial hepatectomy or D-galactosamine-treated rats.[18] Interestingly, EpCAM$^+$ and CD90$^+$ oval cells represent two distinct populations: The former expresses classical oval cell markers, such as AFP, OV-1, and cytokeratin-19 (CK-19), whereas the latter expresses desmin and alpha smooth muscle actin, but not AFP, OV-1, or CK-19, which indicates that CD90$^+$ populations are more likely to be mesenchymal cells. Another study has demonstrated that mesenchymal cells can interact with HSCs to regulate cell-fate decision.[19] We found that EpCAM$^+$ and CD90$^+$ cells isolated from liver cancer are distinct in terms of gene- and protein-expression patterns in both primary liver cancers and cell lines. Furthermore, these distinct CSCs can interact to regulate the tumorigenicity and metastasis of HCC. Molecular characteristics of EpCAM$^+$/CD90$^+$ CSCs may potentially reflect the cellular context of healthy stem or progenitor cells.

Although our study strongly indicates that abundant CD90$^+$ cells in a tumor is a risk for distant metastasis in liver cancer, the cell identity and role of CD90$^+$ cells remains elusive. As our IHC, FACS, and xeno-transplantation assays revealed, some CD90$^+$ cells in liver cancer may be cancer-associated VECs or fibroblasts that cannot perpetuate in the xenograft. Recent findings have suggested the importance of stromal cells in tumorigenesis and cancer metastasis,[20-22] so it is possible that these cells may help TECs invade and intravasate into blood vessels, thus playing crucial roles in metastasis.

Another possibility is that CD90$^+$ cells are cancer cells with features of fibroblasts (having undergone EMT) or VECs (having undergone vasculogenic mimicry; VM) that can invade, intravasate, and metastasize cells to distant organs. Recently, two groups reported that a subset of tumor VECs originate from glioblastoma CSCs.[23,24] We successfully confirmed the tumorigenicity and metastatic capacity of CD90$^+$ cells that were morphologically identical to VECs from primary HCCs that could perpetuate in the xenograft. However, a recent study demonstrated that CD90$^+$ HCC cells express glypican-3, a marker detected in hepatic epithelial cells.[25] Further studies are warranted to clarify the nature and role of CD90$^+$ HCC cells.

In our study, CD90$^+$ cells expressed the endothelial marker, c-Kit, CD105, and VEGFR1, and a mesenchymal VEC morphology and high metastatic capacity were confirmed in both primary liver cancer and cell lines. We further confirmed that CD90$^+$ liver cancer cells showed chemosensitivity to imatinib mesylate, suggesting that cancer cells committed to mesenchymal endothelial lineages could be eradicated by the compound. Although imatinib mesylate treatment had little effect on the size of primary tumors originated from both EpCAM$^+$ and CD90$^+$ CSCs, it significantly suppressed lung metastasis *in vivo*. These data are consistent with a recent phase II study demonstrating the tolerable toxicity, but limited efficacy, of imatinib mesylate alone for unresectable HCC patients. Eligibility of imatinib mesylate for advanced HCC patients may be restricted to the HCC subtypes organized by CD90$^+$ CSCs with a highly metastatic capacity and VEC features. Therefore, a combination of compounds targeting EpCAM$^+$ tumorigenic CSCs as well as CD90$^+$ metastatic CSCs may be required for the eradication of HCC and should be tested in the future.

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References

1. Tsai WL, Chung RT. Viral hepatocarcinogenesis. Oncogene 2010;29: 2309-2324.

2. Chiba T, Kita K, Zheng YW, Yokosuka O, Saisho H, Iwama A, et al. Side population purified from hepatocellular carcinoma cells harbors cancer stem cell-like properties. HEPATOLOGY 2006;44:240-251.
3. Haraguchi N, Ishii H, Mimori K, Tanaka F, Ohkuma M, Kim HM, et al. CD13 is a therapeutic target in human liver cancer stem cells. J Clin Invest 2010;120:3326-3339.
4. Ma S, Tang KH, Chan YP, Lee TK, Kwan PS, Castilho A, 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.
5. Yang ZF, Ho DW, Ng MN, Lau CK, Yu WC, Ngai P, et al. Significance of CD90+ cancer stem cells in human liver cancer. Cancer Cell 2008;13:153-166.
6. 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.
7. 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.
8. 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.
9. Yamashita T, Forgues M, Wang W, Kim JW, Ye Q, Jia H, et al. EpCAM and alpha-fetoprotein expression defines novel prognostic subtypes of hepatocellular carcinoma. Cancer Res 2008;68:1451-1461.
10. Yamashita T, Ji J, Budhu A, Forgues M, Yang W, Wang HY, et al. EpCAM-positive hepatocellular carcinoma cells are tumor-initiating cells with stem/progenitor cell features. Gastroenterology 2009;136: 1012-1024.
11. Ma S, Chan KW, Hu L, Lee TK, Wo JY, Ng IO, et al. Identification and characterization of tumorigenic liver cancer stem/progenitor cells. Gastroenterology 2007;132:2542-2556.
12. Heffelfinger SC, Hawkins HH, Barrish J, Taylor L, Darlington GJ. SK HEP-1: a human cell line of endothelial origin. In Vitro Cell Dev Biol 1992;28A:136-142.
13. Ishimoto T, Nagano O, Yae T, Tamada M, Motohara T, Oshima H, et al. CD44 variant regulates redox status in cancer cells by stabilizing the xCT subunit of system xc(-) and thereby promotes tumor growth. Cancer Cell 2011;19:387-400.
14. Ramadori G, Fuzesi L, Grabbe E, Pieler T, Armbrust T. Successful treatment of hepatocellular carcinoma with the tyrosine kinase inhibitor imatinib in a patient with liver cirrhosis. Anticancer Drugs 2004;15: 405-409.
15. Kim H, Choi GH, Na DC, Ahn EY, Kim GI, Lee JE, et al. Human hepatocellular carcinomas with "Stemness"-related marker expression: keratin 19 expression and a poor prognosis. HEPATOLOGY 2011;54: 1707-1717.
16. Lu JW, Chang JG, Yeh KT, Chen RM, Tsai JJ, Hu RM. Overexpression of Thy1/CD90 in human hepatocellular carcinoma is associated with HBV infection and poor prognosis. Acta Histochem 2011;113: 833-838.
17. Arzumanyan A, Friedman T, Ng IO, Clayton MM, Lian Z, Feitelson MA. Does the hepatitis B antigen HBx promote the appearance of liver cancer stem cells? Cancer Res 2011;71:3701-3708.
18. Yovchev MI, Grozdanov PN, Zhou H, Racherla H, Guha C, Dabeva MD. Identification of adult hepatic progenitor cells capable of repopulating injured rat liver. HEPATOLOGY 2008;47:636-647.
19. Wang Y, Yao HL, Cui CB, Wauthier E, Barbier C, Costello MJ, et al. Paracrine signals from mesenchymal cell populations govern the expansion and differentiation of human hepatic stem cells to adult liver fates. HEPATOLOGY 2010;52:1443-1454.
20. Dome B, Timar J, Ladanyi A, Paku S, Renyi-Vamos F, Klepetko W, et al. Circulating endothelial cells, bone marrow-derived endothelial progenitor cells and proangiogenic hematopoietic cells in cancer: from biology to therapy. Crit Rev Oncol Hematol 2009;69:108-124.
21. Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, et al. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. Nature 2007;449:557-563.
22. Mishra PJ, Humeniuk R, Medina DJ, Alexe G, Mesirov JP, Ganesan S, et al. Carcinoma-associated fibroblast-like differentiation of human mesenchymal stem cells. Cancer Res 2008;68:4331-4339.
23. Ricci-Vitiani L, Pallini R, Biffoni M, Todaro M, Invernici G, Cenci T, et al. Tumour vascularization via endothelial differentiation of glioblastoma stem-like cells. Nature 2010;468:824-828.
24. Wang R, Chadalavada K, Wilshire J, Kowalik U, Hovinga KE, Geber A, et al. Glioblastoma stem-like cells give rise to tumour endothelium. Nature 2010;468:829-833.
25. Ho DW, Yang ZF, Yi K, Lam CT, Ng MN, Yu WC, et al. Gene expression profiling of liver cancer stem cells by RNA-sequencing. PLoS One 2012;7:e37159.