Commentary

Cancer stem cells in hepatocellular carcinoma

There are currently two major schools of thought or theories that explain the formation of tumours. According to the classical stochastic theory or clonal-evolution model of carcinogenesis, it is believed that a cancer cell develops as a random process *(stəʊˈkæstɪk* in Greek, means “guessing” indicating randomness) and thus any cell in the body can undergo the required harmful changes in DNA sequences that culminate in the formation of cancer. Eventually there is a clonal-selection of cells such that a clonal population of cells forms the cancer. The other model is the cancer stem cell (CSC) or tumour initiating cell (TIC) theory. The cancer stem cells model proposes that tumours are made of a heterogenous cells comprising a small population of cancer stem cells and a bulk of non-cancer stem cells. These cancer stem cells have properties of self-renewal and differentiation. In this model of cancer biology, the presence of residual CSC gives rise to tumour relapse. This is also true for the local intra-organ or distant metastasis, where the CSC in tumour emboli may act as a hidden reservoir of cancer cells and give rise to distant relapse and metastasis. Since these cells posses distinct characterestics and comprise a very small percentage (1-3%) of the total tumour burden and drive tumour development and tumour sustenance, it is logical to believe that identification and subsequent targeted therapy would go a long way in treating malignancies.

The origin of cancer stem cells has not been identified as yet, although these share several features with normal stem cells. It is not known whether CSCs are the cells that are destined to form CSCs, or whether such cells arise from normal stem cells, or whether these cells are formed as a consequence of genetic alterations in lesser differentiated cells or from mature differentiated cells. As in the case of normal stem cells, distinct morphological features of CSCs have not been identified. Therefore, in view of their phenotypic resemblance, it is difficult to differentiate CSC from other tumour cells under a microscope. It also appears that both CSC and other tumour cells are regulated by similar signaling pathways such as BM11, Hedgehog, bone morphogenetic protein 4 (BMP4), Notch, phosphatase and tensin homolog (PTEN) and Wnt pathways. The CSCs are considered to be resistant to conventional radio- and chemotherapy.

The first evidence of the existence of CSC came from mouse models of leukaemia 50 years ago, and subsequently when it was identified that only a subset of acute myeloid leukaemia (AML) cells were capable of engrafting as AML, when transplanted into an immune compromised host. Thereafter, CSCs have been reported in various tumours in organs such as breast, brain, skin, prostate and colon cancers. It is also known that in some tumours such as colon cancer, the CSCs are rare, while in melammas these are in abundance. These findings provided an entirely new pathway for carcinogenesis.

Various markers have been used to identify CSCs. These include Hoechst 33342 dye staining, aldehyde dehydrogenase activity, CD133, CD90, CD44, CD13, EpCAM, to name a few. The CSCs can be isolated with magnetic columns, fluorescein activated cell sorters or flow cytometers. CSCs can be validated post-isolation by identifying tumour sphere formation in cell culture plates or by demonstration of recapitulated phenotype in engrafted tumour following inoculation in immunocompromised mice.

It has also been identified that the CSCs in different organs and different tumours have different phenotypic markers. Al-Hajj et al. first isolated the CSCs from breast carcinoma with a phenotype of CD44+CD24-/low. Subsequent studies identified...
A LPC or hepatocyte and cholangiocyte indicating the considerable overlap in the markers being expressed in marker identified in CD90+ HCC CSCs (HCCs) etc.

c-STAT3+, Oct4+, CD34+, CD45+, CD90+, CD44+, have a poor prognosis. In addition to the markers and CK19 hepatocytes, expressing both markers of hepatocytes CK7 and CK19. Up to 55 per cent of small dysplastic have found that 28 to 50 per cent tumour cells in a marrow and peripheral blood progenitor cells of extrahepatic origin from the bone proliferative potential, there is a limited population of to these intrahepatic progenitor cells with short term (CK)7 and of cholangiocytes such as CK19. Thus LPCs express markers of hepatocytes such as albumin and cytokeratin (CK)7 and of cholangiocytes such as CK19. In addition to these intrahepatic progenitor cells with short term proliferative potential, there is a limited population of progenitor cells of extrahaepatic origin from the bone marrow and peripheral blood.

Studies based on immunophenotyping of HCCs have found that 28 to 50 per cent tumour cells in a HCC express progenitor cell markers such as albumin, CK7 and CK19. Up to 55 per cent of small dysplastic foci were found to contain progenitor and intermediate hepatocytes, expressing both markers of hepatocytes and CK19ći. HCCs that express CK19 are found to have a poor prognosis. In addition to the markers mentioned, CSCs in HCC are also known to express STAT3+, Oct4+, CD34+, CD45+, CD90+, CD44+, c-Kit+, OV6+, CD326 (EpCAM)+, alpha-feto protein, etc.ći. CD133 a surface glycoprotein, has also been identified as a CSC marker (expressed in 1-5% of the HCCs)ći. Glypican 3 (GPC3) is another sensitive CSC marker identified in CD90+ HCC CSCsći. There is a considerable overlap in the markers being expressed in a LPC or hepatocyte and cholangiocyte indicating the possible source of CSCs in the liver. Self renewal and pluripotency of CSCs in HCC appear to be regulated by EpCAM signalling, Wnt/β catenin, Notch, Hedgehog, spalt-like transcription factor 4 (SALL4) signalling pathways, transforming growth factor (TGF)β signaling, micro-RNAs, etc.ći. Cross-talks between different pathways have been identified and thus EpCAM signaling is known to affect Wnt/β catenin pathwayći.

Matthai and Ramakrishnanći in this issue studied the immunohistochemical (IHC) expression of CSC markers, such as EpCAM, CK19 and neural cell adhesion molecule (NCAM, CD56) in 79 surgical specimens of hepatocellular carcinoma and associated their expression patterns with histological parameters, aetiological factors, clinical tumour behaviour and serum tumour markers, such as alfa-feto protein (AFP) levels. Around 41.8 per cent cases of HCC were positive for EpCAM and 32.9 per cent cases were positive for CK19. The authors interpret that liver CSCs possibly originate from CK19 positive liver progenitor cells. There was no association of CSC expression with aetiological factors and presence or absence of background cirrhosis. EpCAM expression was associated with younger disease onset, poor tumour differentiation, a high mitotic index, microvascular invasion (MVI) and portal vein thromboembolism (PVT), increased intrahepatic metastasis and significantly higher serum AFP levels. CK19 expression was associated with poor tumour differentiation, MVI and PVT. The authors hence concluded that EpCAM could be used in future as a therapeutic target in HCCći.

The prognostic significance of several CSC markers have been elucidated in earlier studies. For example, it has been shown that HCC expressing CD34+CD90+CD133+ is more aggressive than that tumours expressing CD90+ and CD133+ aloneći. A corollary to these observations is the possibility of targeting CSCs as a form of therapy to treat HCCći. Thus, if self-renewing CSCs in a tumour can be targeted and killed, the remaining non-dividing cancer cells can be intervened by other forms of therapy including vascular embolization. The strategy would entail identification of CSCs through markers and their subsequent targeting by appropriate molecules. While the authors of the present study suggest that the expression of EpCAM in CSCs is possibly higher in Indian population than in the Westći, high expression of EpCAM has also been reported from elsewhereći. The identification of these markers would depend on several factors including the sensitivity of the
detection method and the expression of these markers in stem cells. Molecules that target CSCs include those that block various growth and differentiation pathways, those directed against markers, those that alter the microenvironment and those that disrupt CSC protection including resistance to drugs and radiation. Though several examples of such therapy are available in the literature but a majority are experimental\(^{22}\). Anti-EpCAM directed therapies include mouse monoclonal antibody edrecolomab (MoAB 17-1A Panorex\(^{2}\)), human engineered ING-1 (inhibitor of growth family, member 1), human monoclonal adecatumumab (MT201) and rat-mouse hybrid bi-specific (both anti-EpCAM and anti-CD3) trifunctional monoclonal catumaxomab (Removab \(^{6}\))\(^{23}\). Of these, catumaxomab, approved for use in the European Union in April 2009, has been used to treat malignant ascites and epithelial cancers\(^{24}\). Adecatumumab has been used in trials related to treatment of breast and prostate cancers and edrecolomab in colorectal cancers with lymph node metastasis\(^{25}\). None have been used in HCCs. The sonic Hedgehog pathway can be suppressed by siRNA (small interfering RNA), which not only decreases tumour cell proliferation, but also chemosensitizes the cells to 5-fluorouracil (5-FU) and induces cell apoptosis\(^{26}\).

Though HCC has been extensively studied and is one of the cancers that has a vaccine against one of its aetiological agents (hepatitis B virus), the process of hepatic carcinoma development is complex. It needs to be seen whether directed therapy following detection of an isolated CSC marker would yield results comparable to multi-line therapy against several cell molecules with or without conventional chemotherapeutic agents. The possibilities are exciting and the identification of cancer stem cells, their markers and eventual targeted therapies herald an era of immense hope in the management of cancers in future.

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References

1. Lobo NA, Shimono Y, Qian D, Clarke MF. The biology of cancer stem cells. *Annu Rev Cell Dev Biol* 2007; 23 : 675-99.
2. Wang JC, Dick JE. Cancer stem cells: lessons from leukemia. *Trends Cell Biol* 2005; 15 : 494-501.
3. Oishi N, Yamashita T, Kaneko S. Molecular biology of liver cancer stem cells. *Liver Cancer* 2014; 3 : 71-84.
4. Ailles LE, Weissman IL. Cancer stem cells in solid tumors. *Curr Opin Biotechnol* 2007; 18 : 460-6.
5. Peacock CD, Wang Q, Gesell GS, Corcoran-Schwartz IM, Jones E, Kim J, et al. Hedgehog signaling maintains a tumor stem cell compartment in multiple myeloma. *Proc Natl Acad Sci USA* 2007; 104 : 4048-53.
6. Reguart N, He B, Taron M, You L, Jablons DM, Rosell R. The role of Wnt signaling in cancer and stem cells. *Future Oncol* 2005; 1 : 787-97.
7. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature* 2001; 414 : 105-11.
8. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA* 2003; 100 : 3983-8.
9. Uchida N, Buck DW, He D, Reitsema MJ, Masek M, Phan TV, et al. Direct isolation of human central nervous system stem cells. *Proc Natl Acad Sci USA* 2000; 97 : 14720-5.
10. Matsui W, Huff CA, Wang Q, Malehorn MT, Barber J, Tanhehco Y, et al. Characterization of clonogenic multiple myeloma cells. *Blood* 2004; 103 : 2332-6.
11. Prince ME, Sivanandan R, Kaczorzowski A, Wolf GT, Kaplan MJ, Dalerba P, et al. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. *Proc Natl Acad Sci USA* 2007; 104 : 973-8.
12. Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, et al. Identification of pancreatic cancer stem cells. *Cancer Res* 2007; 67 : 1030-7.
13. Dalerba P, Dylla SJ, Park IK, Liu R, Wang X, Cho RW, et al. Phenotypic characterization of human colorectal cancer stem cells. *Proc Natl Acad Sci USA* 2007; 104 : 10158-63.
14. Collins AT, Berry PA, Hyde C, Stower MJ, Maitland NJ. Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res* 2005; 65 : 10946-51.
15. Mishra L, Banker T, Murray J, Byers S, Thenappan A, He AR, et al. Liver stem cells and hepatocellular carcinoma. *Hepatology* 2009; 49 : 318-29.
16. Yamashita T, Wang XW. Cancer stem cells in the development of liver cancer. *J Clin Invest* 2013; 123 : 1911-8.
17. 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.
18. Matthai SM, Ramakrishna B. Cancer stem cells in hepatocellular carcinoma - An immunohistochemical study with histopathological association. *Indian J Med Res* 2015; 142 : 391-8.
19. Gedaly R, Galuppo R, Daily MF, Shah M, Maynard E, Chen C, et al. Targeting the Wnt/β-catenin signaling pathway in liver cancer stem cells and hepatocellular carcinoma cells lines with FH535. *PLoS One* 2014; 9 : e92727.
20. 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 : 10831-9.
21. 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: 1451-61.

22. Oishi N, Wang XW. Novel therapeutic strategies for targeting liver cancer stem cells. *Int J Biol Sci* 2011; 7: 517-35.

23. Gires O, Bauerle PA. EpCAM as a target in cancer therapy. *J Clin Oncol* 2010; 28: e239-40.

24. Linke R, Klein A, Seimetz D. Catumaxomab: clinical development and future directions. *MAbs* 2010; 2: 129-36.

25. Colacchio TA, Niedzwiecki D, Compton C, Warren R, Benson AI, Goldberg R, et al. Phase III trial of adjuvant immunotherapy with MoAb 17-1A following resection for stage II adenocarcinoma of the colon (CALGB 9581). *J Clin Oncol* 2004; 22 (Suppl 14): A55tr No. 3522.

26. Wang Q, Huang S, Yang L, Zhao L, Yin Y, Liu Z, et al. Down-regulation of sonic hedgehog signaling pathway activity is involved in 5-fluorouracil-induced apoptosis and motility inhibition in Hep3B cells. *Acta Biochim Biophys Sin (Shanghai)* 2008; 40: 819-29.