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Existence of Cancer Stem Cells in Hepatocellular Carcinoma: Myth or Reality?

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Abstract

Cancer stem cells (CSC) hypothesis has been disproved in many cancers. In blood cancer, CSC may exist while many epithelial cancers may not have CSC, but have tumor-initiating cells (TICs). Several independent studies have provided strong evidence for the existence of CSCs in brain, skin, and colon cancers^{1–3}, while CSC hypothesis is still controversial⁴. Liver TICs are bipotential meaning they give rise to two different lineage types, hepatocellular carcinomas (HCC) and cholangiocarcinomas (CCs). In the liver cancer field, the origin of HCCs and CCs are extensively debated. Several groups validated that TICs give rise to HCCs and CCs whereas Hepatocytes give rise to HCCs. Several groups have demonstrated that oval cells (or liver progenitor cells) give rise to TICs. However, CSC may be a myth in gastrointestinal cancer while many groups validated liver TICs. The definition of CSCs includes pluripotency while TICs do not have to have pluripotency and only need to have bipotency or multi-potency to give rise to diverse tumor types and tumor-initiation potential in mouse models. CSC hypothesis is therefore still controversial⁴. Cancer tissues contain subpopulations of cells known as tumor-initiating cells (TICs, so-called CSCs) that have been identified as key drivers of tumor growth and malignant progression with drug resistance. Stem cells proliferate via self-renewing divisions in which the two daughter cells differ in their proliferative potential, with one displaying the differentiation phenotypes and another retaining self-renewing activity.

Evidence for CSCs

CSC hypothesis has been proposed in several cancers^{1–3}, while CSC hypothesis is still controversial⁴. TICs share features with embryonic stem cells (ESCs) present in preimplantation blastocyst stage embryos, including the expression of a pluripotency-associated transcription factor network^{5,6}, in contrast to ESCs, TICs fail to properly control the self-renewing cell division that is property of stem cells. Identification of difference between normal tissue stem cells and CSCs will allow for development of novel treatments targeted at recurrent chemoresistant cancers, which would leave healthy tissue stem cells unharmed. A restricted cell population propagates glioblastoma growth after chemotherapy⁷. In untransformed stem cells, self-renewal occurs through asymmetric cell division, in which

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one daughter cell retains the multipotent progenitor status of its parent while the other cell commits to a specialized cell fate.

The CSC theory holds true in some studies of human leukemia. A subset of leukemic cells can cause leukemia when transplanted into immunocompromized mice, the key characteristic of CSCs (tumor initiation property). Cells with CSC characteristics are heterogeneous in human and mouse cancers. Eliminating these cells could eventually cure the patient. Destroying the bulk of a tumor while leaving behind CSCs enables CSCs to continue to self-renew leading to tumor recurrence. In the published literature, the terms “Tumor Initiating Cells” and “Cancer Stem Cells” have been used interchangeably and the difference is more of semantics rather than a conceptual one. Both terms have been used for describing subpopulation of tumor cells possessing the stem cell properties of self-renewal, differentiation, and ability to give rise to the heterogeneous lineages of cancer cells.

Drug resistance and tumor-initiating cells (TICs)

Most cells in a bulk tumor lie idle (resting stage) whereas CSCs divides and replenish the tumor, analogous to the queen bee of the beehive. Chemotherapy kills workers in the beehive while the queen bee replaces them with new ones. Cell plasticity allows normal tumor cells to turn into CSCs. Killing the non-TIC bulk cancer (workers), but not the queen bee (TIC), leads the hive to recovery. In contrast, killing the queen bee (TIC) eradicate bad seeds (queen bee).

To eradicate TICs and prevent recurrence, we should aim to eradicate not just queen but all worker bees too because of the malignant potential and plasticity of other tumor cells unlike the worker bees.

CSCs are controversial

The CSC theory is controversial because the existence of CSCs relies on experiments that involve breaking down a tumor microenvironment, taking out particular cells and then transplanting them. This process does not exactly mirror natural cancer growth. To prove that the transplanted cells are really CSCs, scientists will need to look at individual cells and find direct evidence that the cells do indeed feed tumor growth within the natural environment of the body. Furthermore, immortal strand hypothesis in stem cells was disproved since haematopoietic stem cells do not asymmetrically segregate chromosomes (immortal strand) or retain BrdU⁸. It is controversial whether CSCs are equal to TICs in HCC since several paper disproved CSC concept. TIC is the more accepted term in the field. The definition depends on whether the cells generate a tumor in a xenograft mouse model. In order to enrich the cancer stem cells in vitro, “non-adherent sphere culture” technique is commonly used.^{39,9}

Isolation and surface markers of liver TICs

Recent studies of HCC have centered on TICs, including detection of TICs in HCCs, identification of TIC markers, and isolation of TICs from human HCC cell lines. When compared to CD133– cells, the CD133+ cells isolated from the HCC cell lines showed

higher expression of CD44 (cell adhesion molecule) and CD34, but both CD133 subpopulations displayed similar expression for CD49f (integrin $\alpha 6$), CD90, CD29 (integrin $\beta 1$) and CD117 (c-kit: gastrointestinal stroma tumor), indicating these markers are still not definitive TIC markers (Table 1)¹⁰. The CD90+ cells are not present in the normal liver and, when injected into immunodeficient mice, create tumors repeatedly. In human HCC and HCC cell lines, specifically CD133+ cells, not CD133- cells, had the ability to self-renew, create differentiated progenies, and form tumors¹⁰. Nevertheless, these markers are differentially expressed in other cancers, such as CD44⁺/CD24^{-/low} in breast cancer¹¹, CD34⁺/CD38⁻ in acute myeloid leukemia¹², and CD44⁺/ $\alpha 2\beta 1^{\text{hi}}$ /CD133⁺ in prostate cancer¹³. TIC were identified as a CD117⁺/CD133⁺ hepatic precursors in regenerating liver tissue¹⁴ and a CD45⁻/CD90⁺ subpopulation of tumor cells in HCC¹⁵.

The plasticity of TICs with dysregulated signaling and gene expression promotes chemoresistance. Several oncogenic signaling pathways in TICs of HCC are activated, including activated PI3K/AKT¹⁶, signal transducer and activator of transcription 3 (STAT3)^{17,18}, Notch¹⁹, hedgehog^{20,21} and dysregulated transforming growth factor-beta (TGF- β)^{22,23}. This coincided with the expression of genes associated with stem/progenitor status, such as β -Catenin, NOTCH, BMI, and OCT3/4.

Virus (HBV and HCV), environmental factors (alcohol and obesity), and HCC

Chronic liver damage caused by viral infection and environmental factors (such as alcohol or metabolic syndrome) can result in increased risk for HCC. To develop and improve HCC therapeutic modalities, understanding the molecular mechanisms of hepatocarcinogenesis is required³². In particular, chronic infection with HBV or HCV represents a major risk factor for HCC³³. HCV affects more than 170 million people worldwide^{33–35}. HCV and environmental factors (alcoholism and obesity) may induce cellular transformation by the induction of chronic liver inflammation mediated by the immune cells. This chronic liver inflammation leads to cell deaths, hepatocellular regeneration and the emergence of mutated cells that may be oncogenic (Fig. 1). In addition, HCV may also induce cellular transformation directly via the induction of oxidative stress and gene mutations as well as the alteration of cellular physiology, which may also lead to malignant cellular transformation. Indeed, HCV titer has been shown to exhibit a positive correlation with the amount of alcohol consumption³⁶. We also found that TICs were highly sensitized to leptin exposure *in vitro*, as judged by the phosphorylation of Stat3-Y705³⁷. Differential responses to extrinsic, adipocyte-derived cues may promote the expansion of tumor cell subpopulations and contribute to oncogenesis³⁷. Compelling evidence identifies obesity and hepatitis C virus (HCV) as co-morbidity risk factors for hepatocellular carcinoma (HCC), which contains TICs. TLR4 signaling, activated by alcohol/obesity-associated endotoxemia, induces the stem marker NANOG and liver tumors in the HCV nonstructural protein Ns5a transgenic (Tg) mice but not in wild type or Ns5a Tg mice deficient in TLR4.

are all tumor cells descended from small population of ancestors (as the TIC) or are they a mixed bag?

By using lineage tracing, individual cells are labeled by a colored tag that is passed on to daughter cells. Looking at their color helps to identify the cell's ancestry. A solid tumor depends on a small group of cancer cells with 'stem cell-like' properties. There's a risk that treatments against CSCs would also damage the normal, healthy tissue stem cells that help replenish cells following injury or normal wear and tear. If that were indeed the case, the consequences could be disastrous. TICs play an important role in skin cancer: benign skin tumors (papillomas) and originate from a select few cells³⁸. Dr. Clever's group labeled single intestinal cells by different colors. When benign tumors progress into bowel cancer, they tended to be a single color, implying that they arose from a single cell³⁹.

TICs in HCCs in alcoholic, obese or hepatitis virus-infected patients

HCC is the third leading cause of cancer deaths in the world, with more than 600,000 deaths in 2007. Understanding the mechanisms of genesis of TICs will improve therapeutics for treating hepatocellular carcinoma (HCC). Hepatitis C virus (HCV) and hepatitis B virus (HBV) is a major cause of HCC. Compelling epidemiologic evidence identifies obesity and alcohol as co-morbidity factors that can increase the risk of HCV patients for HCC. Alcoholism is associated with endotoxemia which stimulates expression of proinflammatory cytokines. The HCV-TLR4-NANOG signaling network is established by alcohol/obesity-associated endotoxemia (LPS) which then activates TLR4 signaling, resulting in the induction of the stem cell marker NANOG expression causing subsequent liver tumors.⁴⁰ Liver TICs are sensitized to leptin and exposure of TICs to leptin increases the expression and activity of an intrinsic pluripotency-associated transcriptional network comprised of STAT3, SOX2, OCT4, and NANOG.⁴¹ These findings provide a direct link between the adipose-derived hormone leptin (obesity) and TICs. Stimulation of the pluripotency network may have significant implications for hepatocellular oncogenesis via genesis and maintenance of TICs. This article reviews the oncogenic pathways to generate TICs. Chronic HCV infection results in a high frequency of HCC that displays non-metastatic and multicentric characteristics. The molecular pathways of HCV-induced carcinogenesis may involve the indirect, non-virological factors such as the induction of chronic liver inflammation and regeneration that lead to the emergence of mutated cells with high proliferation rates. It may also involve virological factors such as viral gene products that stimulate the production of ROS and the expression of error-prone DNA polymerases. These different pathways highlight the complicated interplays between the virus and its host in HCV related carcinogenesis (Fig. 1). Taken together, TIC population is the origin of cancer recurrence in drug-treated patients with HCCs.

Summary

TICs (so-called CSCs) are key drivers of tumor growth and malignant progression with drug resistance and the origin of cancer recurrence in drug-treated patients with HCCs. Targeting TICs will open an avenue to help development of new drugs to eradicate recurrence problems.

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Abbreviations

TIC	Tumor-initiating cells
CSC	CSCs
HCV	Hepatitis C virus
HBV	Hepatitis B virus

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Figure 1. Environmental factors and virus infection are involved in genesis of TICs

Environmental factors (Alcohol, obesity, etc.) enhance exposure to livers to LPS to help to fix the oncogenic mutation in genome. TICs are resistant to conventional chemotherapy while TICs survives chemotherapy and cause tumor recurrence, leading to metastatic spread of tumors.

Table 1

Markers for liver tumor-initiating cells of HCCs

Gene name	Other name	Function	Species	Organ	References
CD133	Prominin 1 (PROM1)	Glycoprotein, membrane protrusions	Human, Mouse	Liver, Brain	10,16,24-27
CD49f	Integrin α 6 (ITGA6)	Cell adhesion, cell signaling	Mouse	Liver	10,27
CD90	Thy-1	<u>Glycophosphatidylinositol (GPI) anchor</u>	Mouse	Liver	10
CD44	Hyaluronic acid receptor	Cell adhesion and migration, metastasis	Mouse	Liver, Breast	10,11
CK19	Cytokeratin 19	Biliary lineage marker	Mouse	Liver	28,29
OV-6	Oval cell marker	Early progenitor cells	Human	Liver	29
CD34	Glycoprotein	Cell-cell adhesion factor	Mouse	Liver, Leukemia	30
AFP	α -fetoprotein	Fetal counterpart of serum albumin	Mouse	Liver	31
CD117	KIT	C-kit receptor Cytokine receptor	Mouse	Liver	10