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POINT OF VIEW

Existence of cancer stem cells in hepatocellular carcinoma: myth or reality?

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Abstract The cancer stem cell (CSC) hypothesis has been disproved in many cancers. CSCs may exist in blood cancer, while many epithelial cancers may not have CSCs but tumor-initiating cells (TICs). Several independent studies have provided strong evidence for existence of CSCs in brain, skin, and colon cancers (Mani et al. in Cell 133:704–715, 2008, Joseph et al. in Cancer Cell 13:129–140, 2008, Reya et al. in Nature 414:105–111, 2001), while the CSC hypothesis remains controversial (Magee et al. in Cancer Cell 21:283–296, 2012). Liver TICs have bipotential to give rise to two different lineage types: hepatocellular carcinoma (HCC) and cholangiocarcinoma (CC). In the liver cancer field, the origin of HCC and CC is extensively debated. Several groups have validated that TICs gave rise to HCC and CC. Hepatocytes gave rise to HCC. Several groups have demonstrated that oval cells (or liver progenitor cells) give rise to TICs. However, CSCs may be a myth in gastrointestinal cancer, while many groups have validated liver TICs. The definition of CSCs includes pluripotency, while TICs do not have to have pluripotency and only need to have bi- or multipotential to give rise to diverse tumor types and tumor initiation potential in mouse models. The CSC hypothesis therefore controversial (Magee et al. in Cancer Cell 21:283–296, 2012). Cancer tissues contain subpopulations of cells known as tumor-initiating stem-like 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 division in which the two daughter cells differ in proliferative potential, with one displaying differentiated phenotype and the other retaining self-renewing activity.

Keywords Liver · Tumor-initiating stem-like cells (TICs) · Cancer stem cells (CSCs) · Hepatitis C virus (HCV) · Hepatitis B virus (HBV)

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Evidence for CSCs

Cancer stem cell (CSC) hypothesis has been proposed in several cancers [1–3], while CSC hypothesis is still controversial [4]. Tumor-initiating cells (TICs) share features with embryonic stem cells (ESCs) present in preimplantation blastocyst-stage embryos, including expression of a pluripotency-associated transcription factor network [5, 6]; in contrast to ESCs, TICs fail to properly control self-renewing cell division, which is a property of stem cells. Identification of the difference between normal tissue stem cells and CSCs will allow for development of novel treatments targeting at recurrent chemoresistant cancers while leaving healthy tissue stem cells unharmed. A restricted cell population propagates glioblastoma growth after chemotherapy [7]. In untransformed stem cells, self-renewal occurs through asymmetric cell division, in which one daughter cell retains the multipotent progenitor status of its parent while the other commits to a specialized cell fate.

The CSC theory holds in some studies of human leukemia. A subset of leukemic cells can cause leukemia when transplanted into immunocompromised mice, the key characteristic of CSCs (tumor initiation property). Cells with CSC characteristics are heterogeneous in human and mouse cancers. Eliminating these cells would 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 published literature, the terms "tumor initiating cells" and "cancer stem cells" have been used interchangeably, and the difference is more of semantics than conceptual. Both terms have been used to describe a subpopulation of tumor cells possessing stem cell properties of self-renewal, differentiation, and the ability to give rise to heterogeneous lineages of cancer cells.

Drug resistance and tumor-initiating cells (TICs)

Most cells in bulk tumor lie idle (resting stage), whereas CSCs divide and replenish the tumor, analogous to the queen bee in a 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 to hive recovery. In contrast, killing the queen bee (TIC) eradicates bad seeds (queen bees).

To eradicate TICs and prevent recurrence, one should aim to eradicate not just the queen but all worker bees too, because of the malignant potential and plasticity of other tumor cells, unlike 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, then transplanting them. This process does not exactly mirror natural cancer growth. To prove that transplanted cells are really CSCs, scientists need to look at individual cells and find direct evidence that they do indeed feed tumor growth within the natural environment of the body. Furthermore, the immortal strand hypothesis in stem cells was disproved, since hematopoietic stem cells do not asymmetrically segregate chromosomes (immortal strand) or retain BrdU [8]. It is controversial whether CSCs are equivalent to TICs in HCC, since several studies have disproved the CSC concept. TICs is the more accepted term in this field. The definition depends on whether the cells generate a tumor in a xenograft mouse model. To enrich cancer stem cells in vitro, the "nonadherent sphere culture" technique is commonly used [9, 39].

Isolation and surface markers of liver TICs

Recent studies of HCC have centered on TICs, including their detection in HCC, identification of TIC markers, and isolation of TICs from human HCC cell lines. Compared with $CD133^-$ cells, $CD133^+$ cells isolated from HCC cell lines showed higher expression of CD44 (cell adhesion molecule) and CD34, but both CD133 subpopulations displayed similar expression of CD49f (integrin α6), CD90, CD29 (integrin β1), and CD117 (c-kit: gastrointestinal stroma tumor), indicating that these makers are still not definitive for TICs (Table 1) [10]. $CD90^+$ cells are not present in normal liver and, when injected into immune-deficient 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 tumors [10]. Nevertheless, these markers are differentially expressed in other cancers, such as $CD44^+/CD24^{-/low}$ in breast cancer [11], $CD34^+/CD38^-$ in acute myeloid leukemia [12], and $CD44^+/\alpha2\beta1^{hi}/CD133^+$ in prostate cancer [13]. TICs were identified as $CD117^+/CD133^+$ hepatic precursors in regenerating liver tissue [14], and a $CD45^-/CD90^+$ subpopulation of tumor cells in HCC [15].

The plasticity of TICs with deregulated signaling and gene expression promotes chemoresistance. Several oncogenic signaling pathways in TICs of HCC are activated, including activated PI3K/AKT [16], signal transducer and activator of transcription 3 (STAT3) [17, 18], Notch [19], Hedgehog [20, 21], and deregulated transforming growth factor-beta (TGF-β) [22, 23]. This coincided with 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 required [32]. In particular, chronic infection with HBV or HCV represents a major risk factor for HCC [33]. HCV affects more than 170 million people worldwide [33–35]. HCV and environmental factors (alcoholism and obesity) may induce cellular transformation by induction of chronic liver inflammation mediated by immune cells. This chronic liver inflammation leads to cell death, hepatocellular regeneration, and emergence of mutated cells that may be oncogenic (Fig. 1). In addition, HCV may also induce cellular transformation directly via induction of oxidative stress and gene mutations, as well as 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 consumption [36]. We also found that TICs were highly sensitized to leptin exposure in vitro, as judged by phosphorylation of Stat3-Y705 [37]. Differential

Table 1 Markers for liver tumor-initiating cells of HCC

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-chain α6 (ITGA6)	Cell adhesion, cell signaling	Mouse	Liver	[10, 27]
CD90	Thy-1	Glycophosphatidylinositol (GPI) anchor	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]

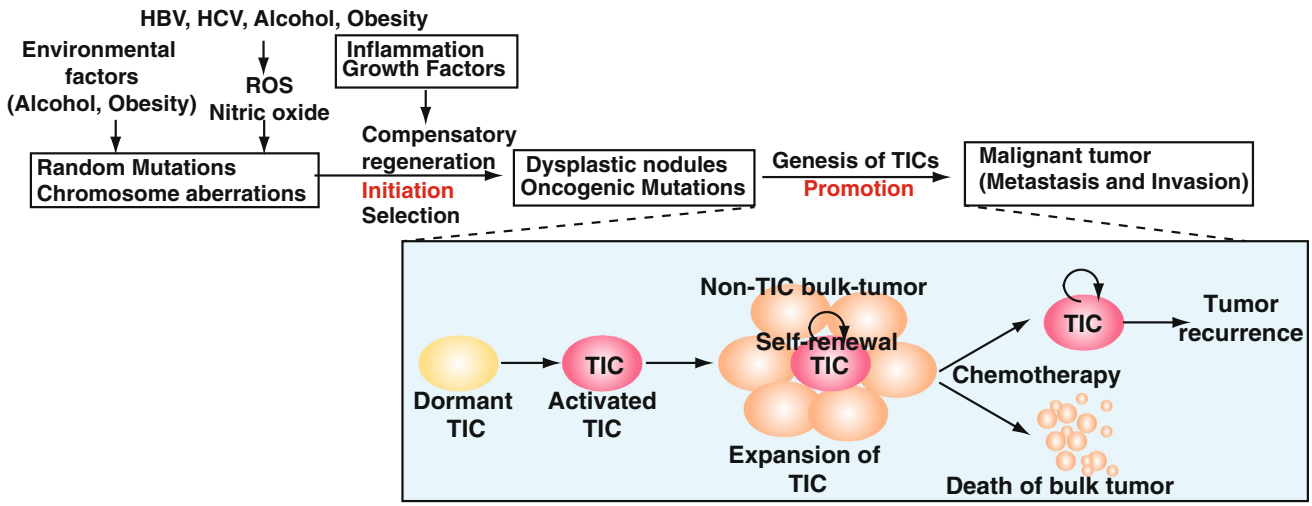

Fig. 1 Environmental factors and virus infection are involved in genesis of TICs. Environmental factors (alcohol, obesity, etc.) enhance exposure of liver to lipopolysaccharide (LPS) to help fix oncogenic mutation in genome. TICs are resistant to conventional chemotherapy, surviving chemotherapy and making tumor recurrent, which leads to metastatic spread of tumors

responses to extrinsic, adipocyte-derived cues may promote expansion of tumor cell subpopulations and contribute to oncogenesis [37]. Compelling evidence identifies obesity and hepatitis C virus (HCV) as comorbidity 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 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 a small population of ancestors (as TICs) or are they a mixed bag?

The established concept of "monoclonality" is summarized below. By using lineage tracing, individual cells were labeled by colored tags that they pass on to their daughter to identify cell ancestry. Dr. Clevers group labeled single intestinal cells with different colors. When benign tumors progressed to bowel cancer, they tended to be of a single color, implying that they arose from a single cell [38]. A solid tumor depends on a small group of cancer cells with "stem cell-like" properties. Treatments against CSCs would also damage normal, healthy tissue stem cells that help replenish cells following injury or in normal homeostasis. TICs play an important role in skin cancer: benign skin tumors (papillomas) all originate from a select few cells [39].

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

HCC is the third leading cause of cancer death in the world, accounting for 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) are major causes of HCC. Compelling epidemiologic evidence identifies obesity and alcohol as comorbidity factors that can increase the risk of HCC in HCV patients, especially in alcoholic or obese patients. Alcoholism is associated with endotoxemia which stimulates proinflammatory cytokines and inflammation. The HCV–TLR4–NANOG signaling network is established, as alcohol/obesity-associated endotoxemia (LPS) which activates TLR4 signaling, resulting in induction of 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 comprising 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 that generate TICs. Chronic HCV infection results in high frequency of HCC that displays nonmetastatic and multicentric characteristics. The molecular pathways of HCV-induced carcinogenesis may involve indirect, nonvirological factors such as induction of chronic liver inflammation and regeneration that lead to emergence of mutated cells with high proliferation rates. They may also involve virological factors such as viral gene products that stimulate production of reactive oxygen species (ROS) and expression of error-prone DNA polymerases. These different pathways highlight the complicated interplay between the virus and its host in HCV-related carcinogenesis (Fig. 1). Taken together, the TIC population is the origin of cancer recurrence in drug-treated patients with HCC.

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 HCC. Targeting TICs will open an avenue to help development of new drugs to eradicate recurrence problems.

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Compliance with ethical standards

Conflict of interest The author declares no conflict of interests.

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