Review

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Epigenetic regulation of cancer stem cells in liver cancer: Current concepts and clinical implications

J.U. Marquardt, V.M. Factor, S.S. Thorgeirsson*

Laboratory of Experimental Carcinogenesis, Center for Cancer Research, National Cancer Institute, NIH, MD, USA

The two dominant models of carcinogenesis postulate stochastic (clonal evolution) or hierarchic organization of tumor (cancer stem cell model). According to the latter, at the germinal center of tumor evolution is a cancer stem cell (CSC) which, similar to normal adult stem cells, possesses the capacity of self-renewal and a differentiation potential.

Over the past few years, compelling evidence has emerged in support of the hierarchic cancer model for many solid tumors including hepatocellular cancers. The CSCs are posited to be responsible not only for tumor initiation but also for the generation of distant metastasis and relapse after therapy. These characteristics are particularly relevant for a multi-resistant tumor entity like human hepatocellular carcinoma and may herald a paradigm shift in the management of this deadly disease. Identification and detailed characterization of liver CSCs is therefore imperative for improving prevention approaches, enhancing early detection, and extending the limited treatment options.

Despite the current progress in understanding the contribution of CSCs to the generation of heterogeneity of tumors, the molecular complexity and exact regulation of CSCs is poorly understood. This review focuses on the genetic and epigenetic mechanisms that regulate and define the unique CSC properties with an emphasis on key regulatory pathways of liver CSCs and their clinical significance.

Introduction

Two general models of carcinogenesis exist: the stochastic and hierarchic cancer model. According to the traditional clonal evolution model, tumor formation is the consequence of accumulating random genetic events in any normal differentiated cell whereas the cancer stem cell (CSC) model postulates that a single CSC gives rise to a hierarchic organization within a tumor [1,2]. Even though the concept of a rare group of cells being responsible for tumor initiation *in vitro* and *in vivo* is not new, the CSC model remained hypothetical until compelling evidence has emerged in the last decade [3–9]. The stochastic and hierarchic cancer models were thought to be mutually exclusive, although current findings favor a likelihood of the complementary co-existence based on the assumption that cancer is a genetically generated disease that is maintained and tightly regulated by epigenetic changes (Fig. 1). Similar to the phenotypic diversity of normal adult tissues that is generated by tissue specific stem cells, the CSC model posits that at the apex of tumor formation is a stem-like cell (commonly referred to as CSC or tumor-initiating cell) that is responsible for the heterogeneity observed within the clonally derived tumors including liver cancer [9,1,10,2]. Despite functional similarities with the adult tissue stem cells, including the fundamental properties of self-renewal and differentiation capacity, the term CSC does not consider the origin of these cells [11].

The CSC model predicts several possible scenarios of how cancer stem cells and tumor heterogeneity may originate [9,12], including (i) differentiation arrest of adult tissue stem cell and/or progenitor cell, (ii) dedifferentiation of mature cell, and (iii) transdifferentiation of a stem cell from a different tissue, e.g. bone marrow. (For a more detailed discussion of the potential origin of CSC, we refer to recent reviews [13–15]). The relative contribution of each scenario may vary depending on factors, such as type of cancer, microenvironment, the contributing mutagen(s), and/or a combination of these factors [13].

Notably, the concept of a hierarchic tumor organization has important clinical implications that include diagnosis, prevention, and most importantly therapy [16]. Thus, defining CSC-specific biomarkers may contribute to early diagnosis while identification of cell of origin (“cell-at-risk”) is required for effective reduction of the CSC numbers. Classical therapeutic regimens target predominantly the proliferating cells, which are unlikely to be CSCs. Similarly, new generation therapies (e.g. sorafenib) seem not to target the CSC as evidenced by frequent tumor relapse and resistance after therapy [17–22]. The eradication of tumors with hierarchic organization would require the development of new therapies directed towards the CSCs. This implies a detailed understanding of the fundamental CSC properties, such as self-renewal, differentiation, chemoresistance, and, most importantly, unraveling the underlying regulatory pathways and molecular, genetic, and epigenetic mechanisms responsible for tumor initiation, seeding of metastasis, and local recurrence which are currently attributed to the CSC [12,23]. This review focuses on the existing evidence for the role of CSC in liver cancer and

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* Corresponding author. Address: Laboratory of Experimental Carcinogenesis, Center for Cancer Research, National Cancer Institute, NIH, 37 Convent Drive, Room 4146A, Bethesda, MD, USA. Tel.: +1 301 496 5688; fax: +1 301 496 0734.
E-mail address: snorri_thorgeirsson@nih.gov (S.S. Thorgeirsson).

provides an overview of the current approaches for the prospective isolation and regulation of CSC. The clinical implications of the CSC model for the management of human HCC as well as critical issues and questions in the field of liver CSC are also addressed.

Key points

- Over the last decade, there is increasing understanding of the hierarchic organization in hepatocellular cancers with the cancer stem cells (CSCs) responsible for tumor initiation, generation of metastasis, and relapse after therapy
- The cancer stem cells (CSCs) rest on the apex of tumor formation and share functional properties ascribed to the normal tissue stem cells,including self-renewal, proliferation, and differentation capacity thereby leading to tumor heterogeneity
- Currently, isolation of liver CSCs relies on their antigenic (e.g. CD133,CD90, EpCAM) or functional (e.g. Side-Population, ALDH1-Activity, Sphere formation and Asymmetric Cell Division) properties
- Eradication of tumors with hierarchic organization would require the development of new therapies specifically directed against the CSCs and their regulatory mechanisms
- Aberrant gene expression in CSCs is linked to genetic and epigenetic deregulation of key signaling pathways controlling stem cell maintenance,self-renewal and pluripotency, such as WNT/β-Catenin, TGF-β, Hedgehog, and MYC
- Activation of these pathways in the CSC reflects the clinical behavior of the tumors and makes CSCs the prime target for efficient CSC eradication

Identification of cancer stem cells in liver cancer

Cancer stem cells are defined by (1) self-renewing capacity; (2) differentiation capacity; and (3) tumor-initiating capacity. Additionally, the seeding of metastasis and tumor relapse are attributed to CSC [12]. A description of the basic properties and respective experimental assessment criteria are provided in Table 1.

Two general approaches for the prospective isolation of CSCs are based on their immunogenic and functional properties. The antigenic approach utilizes a variety of cell surface markers whereas functional isolation relies on the surrogate characteristics, such as anchorage independent growth, chemo-resistance, self-renewal, asymmetric division, and pluripotency. Functional approaches are particularly useful when the specific CSC markers have not been defined as is the case for most CSCs. Given the plasticity of the CSC, it is unlikely that CSCs can be defined by a single marker or functional property. Therefore, a combination of functional and antigenic approaches seems to be the most appropriate for identification and isolation of CSCs.

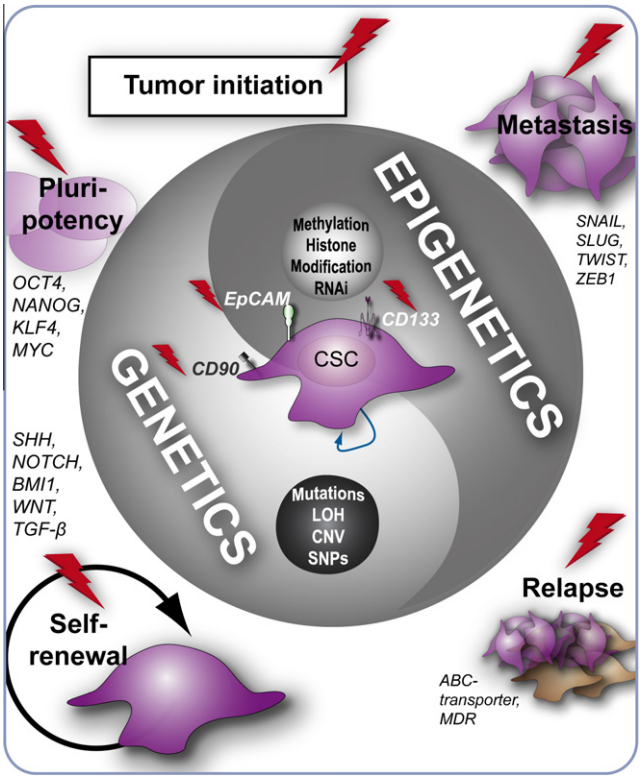

Fig. 1. CSC properties and mechanisms of regulation. The Cancer Stem Cell (CSC) hypothesis places CSCs at the center of neoplastic development. The scheme simplifies our understanding of how CSCs are regulated and emphasizes the contribution of both genetics and epigenetics in tumor initiation, development of metastasis, and tumor relapse. CSCs share the fundamental properties of normal stem cells, including regulatory pathways (representative molecules are shown) and the ability to undergo symmetric (self-renewal) and asymmetric (pluripotency) division. The unique features of CSCs (arrows) provide attractive targets (red arrows) for developing novel therapeutic tools directed specifically at the CSC. RNAi, RNA interference machinery; LOH, loss of heterozygosity; CNV, copy number variations; SNP, single nucleotide polymorphism.

Table 1. Cancer stem cell properties.

Property	Definition	Assay
Self-renewal	The ability to undergo symmetric division and thereby indefinitely replenish itself	Re-plating assays Serial transplantations
Differentiation capacity	The ability to undergo asymmetric division and thereby recapitulate all tumor cell types	Differentiation assays *in vitro* Transplantation
Tumor initiation/ metastasis	The ability to propagate tumor when transplanted into the proper environment	Sphere formation Invasion assays Transplantation
Relapse	The property of resistance to different therapies and the ability to relapse	Chemo/radio-resistance assays

Antigenic markers

A variety of markers have been successfully used to enrich for a cancer stem cell fraction from different tumors including HCC