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Mini-review

Heterogeneity in cancer stem cells

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ABSTRACT

Accumulating evidence suggests that cancer stem cells (CSCs) are heterogeneous populations and their phenotypes are unstable. A number of intrinsic and extrinsic mechanisms contribute to CSC phenotypic variation. The existence of various CSC subpopulations which would lead to a rapid relapse after primary treatments might pose a problem for CSC targeted therapeutics. In order to develop more effective approaches to cancer therapeutics, more CSC-related surface markers or targeting molecules, as well as some novel targeting strategies should be explored. This review summarized the origin and performance of heterogeneity in CSCs and discussed their therapeutic implications.

Introduction

In the past two decades, significant progress has been made in identifying the tumorigenic cancer cells responsible for tumor initiation, maintenance and drug-resistance. This small subset of tumorigenic cancer cells has been termed as cancer stem cells (CSCs) [1,2]. The first prospective identification of CSCs came from studies of acute myeloid leukemia (AML) [3,4], in which leukemic stem cells were defined as $CD34^+CD38^-$ phenotype similar to normal hematopoietic stem cells [4]. Subsequently, CSCs were also isolated and characterized in solid tumors such as breast cancers [5], brain cancers [6], colon cancers [7] and some other tumor types [8–10]. To date, isolation and characterization of CSCs largely depend on surface markers shared with normal stem cells [11]. For example, CD133, which was initially described as a surface marker for human hematopoietic progenitor cells [12], has been widely explored as a reliable marker for CSCs in many tumor types [10,13,14]. Identified by related biomarkers, even in the same tumor mass, there would be CSC subpopulations residing in different fractions [15]. Several studies demonstrated that $CD34^+CD38^-$ was not the only phenotype in leukemic stem cells [16,17]. Similarly, CD166 was reported to be a reliable surface marker for lung CSC enrichment, compared with CD133, CD44 or EpCAM [18], and both $CD271^+$ and $CD271^-$ melanoma cells in NOD/SCID mice could initiate new tumors [19]. The variations ranging from genotype to phenotype of CSCs, which cause heterogeneous in macroscopic feature, microscope appearance and clinical outcomes, define the heterogeneity of CSCs [20]. CSC population is phenotypic heterogeneous among tumor types and even within the same tumor subtype, which are termed as intertumor and intratumor heterogeneity respectively [2,11]. Heterogeneity of CSCs, especially the intratumor heterogeneity, poses the challenge to the targeting therapy in clinic. Herein, we reviewed current progress on heterogeneity in CSCs and discuss therapeutic implications of CSCs.

Sources of heterogeneity in cancer stem cells

The early studies revealed that genetic mutation contributed to tumor initiation and progression [21]. As an inherent factor influencing cell physiological activities, distinct genomic profiles [2] of CSCs originating from different tumors is one of the main explanations for variation of intertumor CSCs. In addition, analogous to an ecosystem, tumors are complicated organizations that individual cells would also interact with the microenvironment where they resided. Accompanying the intrinsic variation among intratumor CSC subclones, microenvironment would be another part of factors that causes intratumor heterogeneity [22,23]. In brief, the heterogeneity of CSCs would be contributed by intrinsic and microenvironmental differences, which share similar explanations with cancer cell heterogeneity [2,24,25]. Intrinsic differences encompass genetic mutations and epigenetic changes, whereas cell–cell interactions, various chemotactic factors, cytokine concentrations and hypoxic conditions are considered to be microenvironmental differences [2,26].

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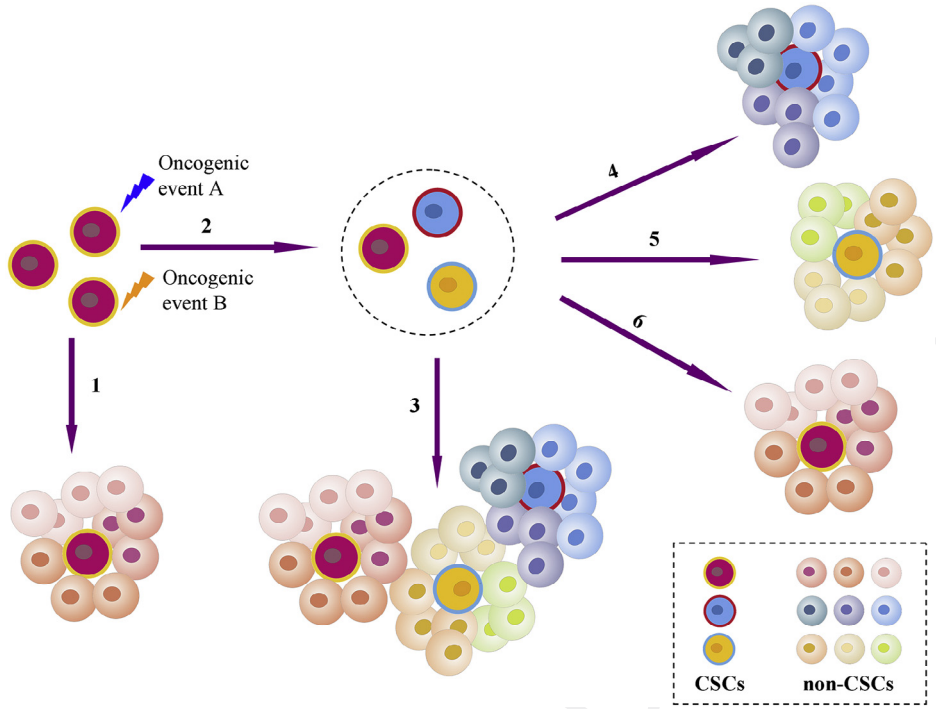

Fig. 1. Schematic illustration of clonal evolution contributing to CSC heterogeneity. It is supposed to have different routes for CSC evolution and differentiation. (1) One initial CSC clone is able to form all populations within a tumor. The CSCs phenotype is homogeneous in this tumor. (2) Different mutations result in multiple CSC subclones. (3) These subclones contribute to tumor growth and CSC heterogeneity exists in an individual tumor. (4–6) More adapting CSC subclones survive under different environmental pressures, which result in CSC heterogeneity among patients or locations.

As one of the intrinsic factors, the instability of CSC genome which includes increased point mutation frequency and chromosomal instability, results in the genetic heterogeneity of CSC [23]. Meanwhile, CSCs undergo clone evolution which brings new subclones [2,25]. These subclones with multiple molecular mechanisms exhibit varying malignant potentials. For example, Emlet and colleagues found that $CD133^{+}$ glioblastoma stem cells with EGF receptor (EGFR) possessed the highest tumorigenic potential [27]. Then, under different environmental pressures, the more adapting and aggressive CSC subclones with adaptable genomic mutation to the selections will become dominant population and contribute to tumor formation (Fig. 1) [2,25].

The advanced sequencing technology and epigenome research also highlight the crucial role of epigenetic modification in the diversity of CSCs, which is considered to be another intrinsic factor governing CSC heterogeneity. The epigenetic reprogramming in cancer cells involve in epigenetic factors adjustment and DNA methylation, as well as altering the chromatin states through chromatin regulators [21,28]. For example, the single-cell RNA-sequencing demonstrated that five primary glioblastoma samples presented different transcriptone profiles [29]. In melanoma, H3K4 demethylase JARID1B could be used as a biomarker to isolate a subpopulation of slow-cycling melanoma cells [30]. MOZ (monocytic leukemic zinc finger) histone acetyltransferase in AML is associated with poor prognosis [31]. The non-coding RNAs, for instance, the microRNAs (miRNAs), also play crucial roles in epigenetic heterogeneity in CSCs. The miR-451 was upregulated in $CD133^{-}$ glioblastoma cells, and the stemness of cell would be weakened when transfected with miR-415 [32]. Altogether, the instability of CSC genomes and the variation of epigenetics co-regulate the heterogeneity of CSCs.

Tumor development is not only manipulated by cancer cell intrinsic factors, but also influenced by the extrinsic compartments which are termed as microenvironment or niche. Microenvironmental differences (e.g., hypoxia, acidosis and reactive oxygen species) are selective pressures, as well as inducers of cell genetic instability [33]. Thus, different microenvironments or niches would contribute to CSC phenotypic and functional heterogeneity [25,33]. For instance, in squamous cell carcinomas, two CSC subclones differing in CD34 expression levels could interchange their phenotype according to different microenvironments [34]. In addition, hypoxic and perivascular niches are also vital conditions for the maintenance of CSC properties [35]. Conley et al. showed that hypoxic niche increased the CSC fraction in breast cancer [36]. Similarly, brain and skin cancer stem cells were found in a perivascular niche where autocrine VEGF could sustain CSCs stemness [37,38]. The diversity of some tumor-related extrinsic components, such as cancer-associated fibroblasts (CAFs), tumor vascular network and immune cells also contribute to CSC heterogeneity [21,39]. In these niches, the secreted factors and the activation of related signaling would regulate the conversion between CSCs and non-CSCs. For instance, hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF) or transforming growth factor-β (TGF-β) secreted from myofibroblast could activate Wnt signaling or Zeb1 promoter transcription, which would induce dedifferentiation of non-CSCs to CSCs [22,40,41]. On the contrary, when the Wnt or Notch signaling was downregulated, miR-27 was upregulated or cells were activated by BMP4, CSCs tend to differentiate to non-CSCs (Fig. 2) [42,43]. All these findings indicated that the heterogeneity of CSCs was the consequences of interaction of intrinsic and extrinsic factors.

Diversity of CSC phenotype

Benefiting from the advent of fluorescence-activated cell sorting (FACS) technology, it is possible to sort phenotypically distinct subpopulations to investigate their functional potential *in vivo*. Using this approach, a number of markers have been found useful for