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Teaser Reviewing the small molecules that showed efficacy against CSCs is of crucial importance to stimulate the design of new promising anticancer compounds.

Chemical approaches to targeting drug resistance in cancer stem cells

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Cancer stem cells (CSCs) are a subpopulation of cancer cells with high clonogenic capacity and ability to reform parental tumors upon transplantation. Resistance to therapy has been shown for several types of CSC and, therefore, they have been proposed as the cause of tumor relapse. Consequently, much effort has been made to design molecules that can target CSCs specifically and sensitize them to therapy. In this review, we summarize the mechanisms underlying CSC resistance, the potential biological targets to overcome resistance and the chemical compounds showing activity against different types of CSC. The chemical compounds discussed here have been divided according to their origin: natural, natural-derived and synthetic compounds.

Introduction

The first link between cancer and stem cells was established during the 19th century based on histological similarities between tumors and embryonic tissues. The observation that teratocarcinomas comprised immature and more differentiated cells led to two key assumptions of the CSC hypothesis: (i) not all tumor cells are identical with regard to their phenotypic and biological behavior and (ii) tumors contain a subpopulation of tumor cells that are endowed with central properties of normal stem cells, such as self-renewal and the ability to produce more differentiated daughter cells. In addition, the CSC model suggests that tumors are organized similarly to normal tissues, with a cellular hierarchy in which CSCs drive tumor growth because they are endowed with increased therapeutic resistance [1,2].

CSC versus the stochastic tumor growth model

The first convincing data demonstrating the existence of phenotypically distinct, hierarchically organized subpopulations of tumorigenic and nontumorigenic cells were obtained in acute

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myeloid leukemia (AML). Extensive studies over decades and substantial technical progress led to the conclusion that leukemic stem cells from patients with AML not only are self-maintaining, but also reconstitute the full spectrum of phenotypes consistent with the CSC model (reviewed in [1]). Meanwhile, similar observations were reported for other tumor types, including chronic myeloid leukemia (CML), breast cancer and glioma (reviewed in [4]). Moreover, in a couple of recent elegant lineage-tracing experiments, the hierarchical organization was confirmed in epidermal and colon tumors by following the fate of individual tumor cells [2]. Moreover, Parada and coworkers demonstrated not only a similar hierarchical organization in a genetically engineered mouse glioma model, but also that the relative quiescent subset of tumor cells with stem-like properties gave rise to new tumor cells after treatment with the standard therapeutic drug temozolomide (TMZ); thus, this subset of cells is responsible for the well-known drug resistance of gliomas [3].

Given that there is still controversy over whether the CSC hypothesis is able to explain fully tumor growth and heterogeneity in all tumor types, occasionally the more neutral terms 'tumor-initiating' (TIC) or 'tumor-propagating' cells are used only to refer to the increased tumorigenicity and the tumor-repopulating capacity observed after xenotransplantation of this immature tumor cell subpopulation. The so-called 'stochastic model' represents an alternative approach to explain tumor growth. In contrast to the CSC model, it claims that all tumor cells are equipotent and stochastically self-renew or differentiate, thus resulting in tumor heterogeneity. For example, in melanomas, the stochastic model rather than the CSC model might better explain the extraordinary high frequency of TICs [2,4]. The occurrence of clonal evolution, which is regarded as a central event in the development of tumor heterogeneity and is defined as the continuous accumulation of mutations responsible for survival advantages in individual tumor cells, is in agreement with both tumor growth models. As an inevitable consequence of clonal evolution, especially for late-stage tumors, deep sequencing approaches revealed a high number of subclones that, in the case of CSC-driven tumors, indicates the generation of different types of CSC within the same tumor that are presumably endowed with different drug-resistance phenotypes. In addition, it strongly implicates the need to target all CSC subtypes simultaneously to achieve more effective tumor treatment [2,4].

Plasticity of CSCs

The putative occurrence of several types of CSC in the same tumor complicates the situation in terms of promising treatment targets. Moreover, there is increasing evidence that the ability of CSCs to contribute to tumor growth is plastic. An intensively studied example of this plasticity is the reversible transition between epithelial and mesenchymal states in carcinomas (EMT), which can have a marked impact on their growth properties. The acquisition of mesenchymal properties is associated with the loss of adhesion molecules and increased migratory properties, and its occurrence in cancer cells results in more aggressive tumors. Although the functional role of EMT has not been fully elucidated, many EMT markers are expressed by cells with *ex vivo* stem cell properties. For instance, the EMT marker Twist-related protein 1 (TWIST1) increases mammosphere formation *in vitro* and growth of secondary tumors after xenotransplantation *in vivo*. Accordingly, several studies on breast, pancreatic and colorectal cancers came to the conclusion that EMT generates cells with CSC characteristics and, thus, these acquire a multidrug resistance phenotype [2].

Importantly, the observation of plasticity in tumor cells does not contradict the CSC model because it can also be found in normal stem cells. For instance, in differentiated nontumor cells, expression of four transcription factors was sufficient to revert them fully to an immature embryonic stem cell-like state, which is in disagreement with an entirely irreversible, deterministic phenotype [1]. Additionally, it has been found that plucking-induced depletion of hair follicle stem cells in skin epidermis is followed by repopulation of more differentiated progenitor cells that reacquire stem cell properties [2].

Another indication that properties of CSCs are not necessarily irreversible comes from studies where CSCs were exposed to therapeutic drugs *in vitro*. The observed adaptive response from sensitive to resistant progenies during cultivation suggests that multidrug resistance also represents a plastic property. However, because most studies analyzing plasticity of CSCs were performed on cultivated cells, it is still not clear to what extent a transition from sensitive to resistant and nontumorigenic to a tumorigenic state occurs [4].

Influence of niches on stem cell properties

Recently, the microenvironment of CSCs and normal stem cells has gained much attention because it is supposed to be an important regulator of stem cell properties and, therefore, an additional source of tissue heterogeneity. It has been shown that maintenance of an immature phenotype, the balance between self-renewal and differentiation, as well as multidrug resistance is not only controlled by intrinsic signals, but can also be markedly influenced by direct interaction with neighboring cells or in an indirect manner by binding to proteins secreted from these cells. This specific microenvironment where CSCs and normal stem cells reside in is called the 'niche'. Although the exact cellular and molecular composition of distinct CSC and normal stem cell niches is still under investigation, two types of niche, the perivascular and the hypoxic niches, seem to be of major importance for the maintenance of stem cell properties and, thus, the preservation of a multidrug resistance phenotype. In the perivascular niche, endothelial cells are one of the main cell types. For instance, in the brain, both normal neural stem cells and glioma CSCs are located close to blood vessels and their immature phenotype is dependent on this niche. In line with this observation, stem cell properties of glioblastoma CSCs can be markedly downregulated through an antiangiogenic treatment resulting in a disruption of the perivascular niche. Similar stemness-promoting effects of the perivascular niche were observed in epithelial tumors [2]. With regard to the hypoxic niche, a crucial step comprises the activation of soluble factors, such as hypoxia inducible factors (HIFs), through lowered oxygen concentration. HIFs were shown to be essential for long-term reconstitution abilities of hematopoietic stem cells, resistance to therapy and tumorigenicity of leukemic stem cells. In line with this, expansion of CSCs has been reported in response to HIFs in brain and pancreatic cancer [2].

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