Mini-review

Cancer stem cells in relation to treatment

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Abstract

The classical cancer therapies, including chemotherapy and radiation therapy, can initially show good results and tumor shrinkage; however, for most cancer patients disease recurrence is a common event. This tumor regrowth following therapy is now thought to depend on a small population of cancer stem cells (CSCs), which, similar to other stem cells, have the capacity for self-renewal and multipotent differentiation. Cancer stem cells have been identified based on cell surface protein expression in many tumor types, and for all diseases studied, this specific cell population is required for serial transplantation in animal models. However, a specific signature of cell surface proteins that can identify cancer stem cells has not been developed for many solid tumors. In this review, we summarize a new technique for identifying and quantifying cancer stem cells in situ, which could be a valuable technique for evaluating the effects of therapies on this cell population. Finally, we conclude by discussing several preclinical treatment strategies that either reprogram cancer stem cells or cause them to be specifically attacked by immune cells. In summary, therapeutic and diagnostic methodologies that can attack and quantify cancer stem cells, respectively, will be valuable tools for eradicating cancer.

Key words: cancer stem cell, self-renewal, heterogeneity, multipotent properties

Introduction

Cancer is a genetic and epigenetic disease that causes uncontrolled proliferation and remains one of the leading causes of death worldwide (1). Initially following conventional treatments such as chemotherapy and radiotherapy, many cancers show good responses; however, some later show recurrences. Cancer is known to show great cellular heterogeneity, and that heterogeneity is produced by small populations of cancer stem cells (CSCs) that have both self-renewal and multipotent properties (2). The role of CSCs was first noted in acute myeloid leukemia (3,4), and the possible involvement of CSCs has since been shown in several solid tumors (5–9). These recent advances have indicated that many cancers possess a differentiation hierarchy that arises from malignant CSCs, which undergo uncontrolled proliferation and produce daughter cells with limited proliferative potential (2) (Fig. 1).

The existence of CSCs is thought to be a cause of resistance to conventional treatments (10–12) (Fig. 2). These small populations of CSCs have been found to possess persistent proliferative potential, which is detectable by various in vitro assays and in vivo animal experiments (13). Accordingly, malignant tumors have been proposed to derive from CSCs with an uncontrolled proliferative potential and a dysregulation of their mechanisms of differentiation (3,13).

Herein, we update the recent progress made in CSCs, such as new methodologies for visualizing them, how CSC maintenance requires histone demethylase modification, and we describe new strategies for targeting them.

Detecting CSCs by surface markers

The isolation of CSCs led to an understanding of why they were more resistant to treatments (14–17). CSCs have several properties that protect them against cytotoxic drugs and DNA damage responses. Several reports support the hypothesis that cancers with higher expression of some stem cell markers are associated with increased recurrence rates and poorer prognoses (18–20). CSC is also reported to contribute the tumor regrowth, examined by lineage-tracing experiment (21).
CSCs identified from solid tumors usually express organ-specific markers (Fig. 3). The cell surface marker profile CD44^+CD24^−/lowLin^− was reported in breast cancer (7). As few as 100 cells with this phenotype were able to form tumors in mice, whereas tens of thousands of cells with the alternate phenotypes failed to form tumors. Furthermore, this tumorigenic subpopulation could be serially passaged from one mouse to another, and each time, the cells within this population generated new tumors containing CD44^+CD24^−/lowLin^− tumorigenic cells, as well as the phenotypically diverse mixed population of non-tumorigenic cells. This indicated that these tumorigenic cells behaved like CSCs. Similar results have also been reported for brain tumors, where brain tumor stem cells were exclusively isolated within the cell fraction expressing the neural stem cell surface marker CD133 (6,22–24). CSC surface markers have also been reported in some gastrointestinal cancers (25–29). Isolating CSCs can be achieved by performing a gene expression profile analysis on a side population of cancer cells with low cell turnover. This population had the ability to extrude dye, which is a reliable method for isolating stem cells, including CSCs (30,31). The two major superfamilies of efflux transporters are the ATP-binding cassette (ABC) transporters and the solute carrier (SLC) transporters. Targeting these efflux transporters in combination with conventional treatments can improve cancer treatment.

**Visualization of CSCs**

Isolating CSCs by flow cytometry is a useful technique for studying cancer cells; however, this procedure stresses them and might alter their biology. Developing a system to visualize CSC in situ will facilitate analyzing real tumor cell behaviors within their local microenvironment. The technique has great advantages for cancer research because it focuses on characteristics of CSCs, such as their quiescence, low protein turnover rate, and decreased 26 S proteasome activity (32) (Fig. 4). A fusion protein of green fluorescent protein (ZsGreen) and the C-terminal degron of ornithine decarboxylase (ODC) are retained (green fluorescence-positive) in CSCs with low 26 S proteasome activity due to decreased protein degradation. In several solid tumors, the fluorescent cells (ZsGreen–ODC-positive) demonstrated features of stemness, such as tumor formation in xenograft models and asymmetric cell division (15–17). The fluorescent cells were also more chemo- and radio-resistant compared with non-fluorescent cells (16,17). The ZsGreen–ODC system has been demonstrated in various solid tumors (33,34), and visualizing CSCs using this system not only allows for stem cell research but also for drug screening to search for novel agents.

**Treatments based on cancer cell reprogramming**

Investigations into how embryonic stem cells develop from zygote to blastodermic vesicle stages have made remarkable progress in elucidating the molecular mechanisms that specify pluripotent differentiation (35,36). Regarding the molecular mechanisms that regulate pluripotency, several transcription factors discovered in multipotent stem cells display mutual cooperation as a result of epigenetic controls (37–40). In a previous study, we analyzed the effects of transcription factors that were previously reported in induced pluripotent...
stem (iPS) cells, as well as cancer-related oncogenes and tumor suppressor genes. The repression of tumor suppressor genes extends the lifespan of embryonic stem cells, increases the induction efficiency of iPS cells and maintains their immortalized state (41–43). These results indicated that the introduction of transcription factors into gastrointestinal cancer cells resulted in the reprogramming of the cells to the pluripotent state and sensitized them to differentiation (44). Thus, these reprogrammed cells were distinct from parental cells. It is hoped that the generation of induced pluripotent cancer (iPC) cells will allow us to test previously uncharacterized cancer treatments using differentiation therapy via the induction of drug susceptibility in cancer cells. Reprogramming cancer cells supports the notion that transduction might cause the differentiation of cells to unique cell lineages. Another goal is to exploit drug discoveries with the aim of producing therapeutc and diagnostic reagents. The four transcription factors, OCT3/4, SOX2, KLF4 and c-MYC, were transfected into cancer cell lines. The cells generated from our study were similar to iPS cells in morphology, and embryonic stem cell-like gene expression and epigenetic modifications (37–40). The reprogrammed cancer cells could be guided to differentiate into cells of epithelial, mesenchymal, neural or adipose lineages by controlling conditions of the culture medium (44). We also demonstrated that iPC cells had the capacity for multipotent differentiation. Originally, we hypothesized that iPC cells would revert to their original phenotypes. However, we found that iPC cells lost the ability to form tumors in mouse xenotransplant models. Furthermore, iPC cells became more sensitive to chemotherapy. In our previous study, it was suggested that the reactivation of tumor suppressor genes by reprogramming may play the role in increased chemosensitivity. These findings suggested that reprogramming and epigenetic modifications are promising methods for cancer treatment regardless of the abundance of harbored genetic mutations.

To clinically make use of this reprogramming strategy of eradicating CSCs, we developed a method of reprogramming murine and human fibroblasts into pluripotent stem cells via a specific combination of microRNAs (miRNAs) (45), which are small non-coding RNAs that silence gene expression to regulate development and differentiation. Because specific miRNAs have been characterized in relation to pluripotency (46–48), we search for miRNAs that could reprogram differentiated cells to pluripotent stem cells.

We started by searching for candidate miRNAs that were highly expressed in murine iPS and embryonic stem cells, and then these candidates were applied to somatic cells derived from transgenic mice with ZsGreen inserted downstream of the Nanog promoter (49). The identification of miRNAs that could reprogram cells to pluripotency was evaluated by the fluorescence (a surrogate for Nanog activation). Using this system, we identified a combination of
miRNAs that could reprogram mammalian cells to pluripotency by demonstrating the ability of the miRNA-induced reprogrammed cells to differentiate into cells of different lineages.

Thus, it is also possible to reprogram cancer cells by administering a combination of miRNAs, as demonstrated by the expression of pluripotency markers. As was the case with the four transcription factors, cancer cell lines reprogrammed with miRNAs also demonstrated decreased tumor-initiating capacity and became sensitive to chemotherapy (50). To confirm the feasibility, safety and effectiveness of administering the miRNAs in vivo, we demonstrated that our miRNA combination suppressed tumorigenesis, suggesting that this therapy could be useful for both preventing and treating cancer (Fig. 5).

Another problem remains to be solved regarding miRNA-based treatments. The presence of RNases in the blood makes them unsuitable for systemic therapy. To prevent RNA degradation, a drug delivery system was designed from super carbonate apatite nanoparticles. These nanoparticles deliver RNAs selectively to tumors because of their preference for the low pH conditions found in tumor microenvironments (51). To further increase the stability and efficacy of this miRNA-based therapy, we are currently exploring the use of synthetic miRNAs.

### Targeting the tumor microenvironment

The heterogeneous tumor and its microenvironment provide various self-protection properties, including mechanisms that enable dynamic interactions with surrounding epithelial cells, infiltrating immune cells, cytokines and chemokines to regulate CSC proliferation and self-renewal. CSCs can be maintained by the tumor microenvironment through the induction of specific features in more differentiated tumor cells (52). The tumor microenvironment plays a key role in regulating the CSC population by direct cell–cell contacts.

### Table 1: Clinical trials of molecular targeting agents focusing on the pathways

<table>
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<th>Pathway</th>
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<th>Notch pathway</th>
<th>PGE2 pathway</th>
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<td>NCT01122901 (glioblastoma)</td>
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Figure 5. Treatment strategy based on cancer cell reprogramming. Cancer cells can be reprogrammed to induced pluripotent stem (iPS) cells by defined transcription factors or microRNAs, as well as normal fibroblasts. The reprogrammed cancer cells (iPC cells) increased the sensitivity for the conventional chemotherapy.

Figure 6. Strategy for the cancer treatment focusing on the CSCs and its tumor microenvironments. The heterogeneous tumor and its microenvironments provides self-protection properties in the tumor. Using stem cell-specific markers or visualizing system can detect the CSCs. For the cancer cells, these targeting therapy and reprogramming-based treatments can improve the conventional treatments for the cancer. Furthermore, targeting its tumor micro-environment also enhance the treatment strategy, leading to the reduction of the recurrence and eradication of the cancer.
and the secretion of various paracrine factors. These microenvironment factors maintain stemness through self-renewal pathways such as Wnt/-catenin, Notch and Hedgehog pathways. Wnt activity regulates the self-renewal of CSCs and drives transit amplifying cell proliferation and differentiation (53). And exosomal Wnt was found to increase Wnt activity and drug resistance in CSC (54). Hedgehog signaling, which is important for embryonic development, patterning and differentiation, is associated in tumor tissues with regulating the self-renewal not only of normal mammary stem cells but also of CSCs (55). Notch signaling controls cell fate during development, and aberrant Notch activation contributes to tumorigenesis (56). Niclosamide, which is a tineacide of the anthelmintic family, has been identified as an inhibitor of Wnt/-catenin and Notch signaling (57). Thus, niclosamide might be useful for inhibiting CSCs. Mesenchymal stem cells of the tumor-associated stroma have been shown to affect cancer cell behaviors and influence the phenotypes of cancer cells. Prostaglandin E2 (PGE2) secreted by mesenchymal stem cells enables tumor progression via creating a CSC niche (58,59). Targeting the CSC microenvironment may stimulate host antitumor responses, which is the strategy of blocking tumor-promoting inflammation with the PGE2 receptor antagonist. Focusing on these pathways, cancer treatments were performed in several clinical trials (Table 1). The best opportunity to demonstrate the effects of targeting CSCs seems to be in the area of combination therapy.

Conclusions

It is generally accepted that the relative quiescence and resistance to drugs resulting from the expression of ABC transporters are associated with a long cellular lifespan. CSCs, through their self-renewal and drug-resistant capacities, may share properties that are conducive to proliferation and differentiation in relation to antitumor cancer therapy. A specific set of markers that covers all CSCs has not been defined. It will also be important to further understand the characteristics of CSCs and normal stem cells. If specific markers for these stem cells can be identified, it will be possible to isolate, identify and analyze these minor populations within tumors. In recent reports, gene expression classifications of colorectal cancers have provided crucial tumor information that recapitulated the poor prognosis (60). Elucidating CSC properties, including the microenvironment and molecular subtypes, could lead to the development of novel and effective cancer treatments (Fig. 6).

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Conflict of interest statement

None declared.

References


