

## The Role of Stem Cells in Cancer Therapy

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### Abstract

Cancer stem cells (CSCs) are a group of tumor cells that have tumor initiation and self-renewal properties and are able to give rise to bulk populations of non-tumorigenic cancer cell progeny by differentiation. They were identified in several human malignancies, and their relative abundance in cancer specimens was correlated with malignant disease progression in human patients. Moreover, recent findings suggest that cancer progression driven by CSCs may contribute to failure of existing therapies to eradicate malignant tumors. Therefore, CSC-directed therapeutic approaches might represent novel strategies to improve clinical cancer therapy especially for those malignancies that are refractory to conventional anticancer agents directed mainly at tumor bulk populations.

**Key words:** Stem Cells, Cancer, Therapy

### Introduction

Cancer represents unregulated proliferation and differentiation of various body cells. The traditional anticancer agents carry many risks that necessitate the search for alternative lines of therapy [1]. Several studies focused on the understanding of cancer stem cells (CSCs) after the existence of CSCs in leukemia was described in 1994. The concept of CSCs explains the phenomenon that malignant status correlates to the existence of CSC-like cells [2]. It is essential to understand the biology of CSC to develop a strategy to eradicate cancer. In various cases, malignant tumor tissues include CSCs, which overcome various anticancer therapies. Numerous genes are involved in cellular processes in CSC cell cycles. It has been assumed that CSCs originate from dysregulated behavior of normal tissue stem cells and is limited to a hematopoietic or germ cells [3]. However, several types of solid tumors appear to include these aberrant CSCs [4-6]. In fact, non-CSCs and normal cells form CSC-like cells [7]. Several gene sets have been identified to have the potential to transform cancer cells into CSCs or

maintain the homeostasis of CSCs. For example, epithelial to mesenchymal transition (EMT) inducers are representative functional markers for the acquisition of a stem-like state in cancer cells [8]. ATP-binding cassette (ABC) transporter proteins in CSC-like cells function to secure genomic stability and prevent apoptosis by efflux of cytotoxic agents [9]. It is important to understand the highly complicated signaling mechanism, which sustains the CSC biology. Inhibiting one of the important pathways for CSC causes the activation of the bypass pathway; therefore, some researchers are seeking a different approach. This approach originates from the ideas of differentiation, which causes leukocyte-initiating cells (LIC) to differentiate into terminally differentiated leukocytes [10,11]. The induction of differentiation has expanded to solid tumors [12]. In this manuscript, we discuss the induction of CSC differentiation based on our recent findings. We have demonstrated the multipotency of CSC in hepatocellular carcinomas (HCC), which implies that CSCs have a potential of multi-lineage differentiation. This strategy would provide novel CSC-targeting therapy.

## Characteristics of cancer stem cells

Recent studies have demonstrated that cancer stem cells or cancer initiating cells with tumor-initiating ability and self-renewal capacity are involved in tumor initiation, metastasis, and relapse [13,14]. It appears that the field of breast cancer contributed considerably to uncovering the biology of CSC [15]. Subpopulations with the cell surface marker CD44+CD24<sup>low</sup> were identified to be tumorigenic cancer cells [16]. EMT is the critical step for acquiring stem cell features [17]. Plasticity of CSCs between epithelial- and mesenchymal-like CSCs is involved in cancer progression by promoting tumor growth and metastasis [18]. In HCC, multiple CSC markers have been identified, including CD13, CD44, CD90, and CD133 [19-21]. Although it remains unclear when CSCs are generated, premalignant lesions of HCCs have CD44- positive CSC-like populations, which can initiate tumor formation by autocrine IL-6 signaling [22]; this suggests that they are required for tumor initiation of HCCs. With regard to the association between CSCs and metastasis, CSCs were found in the bloodstream of patients with HCC [21,23]. Circulating tumor cells (CTCs) are considered to be undergoing metastasis. CTCs are detected in early stages of prostate cancer in patients [24], and clusters of CTCs are more resistant to apoptosis and an increase in metastatic potential [25]. CTCs have been found in different types of cancer patients [26,27]. When combining all of these reports, it is possible that CSCs appear in relatively early stages of a tumor and contribute to both tumor initiation and metastasis.

## Evidence of CSCs

Growing evidence has shown that tumors are derived from and maintained by a rare population of dysregulated stem cells. The CSC hypothesis was first raised by Mackillop et al [28], in 1983. He proposed that there might be a small cluster of cells with similarly special functions to stem-like cells in all the tumors. The first conclusive evidence for CSCs was published in 1997 by Bonnet and Dick. They isolated a subpopulation of leukemic cells that express a specific surface marker CD34, but lack the CD38 marker (CD34+ /CD38- ) [29]. After transplantation into mice with severe combined immune deficiency (SCID), these CD34+ /CD38- cells can form tumors that phenotypically resemble the patient's original tumor [29,30], indicating that they are tumorigenic. At present, this method has become the gold appraisal standard for identification of CSCs [31]. This notion has subsequently been verified in several solid tumors, including cancers of the head and neck [32], lung [33,34], liver [35], ovary [36], colon [37], pancreas [38]. All of these evidences demonstrate that there may be CSCs existing in the tumor tissues, which perform as the driver in the survival process of tumors.

Further evidence of CSCs comes from histology and immunocytochemistry studies. For example, many tumors are very heterogeneous and contain multiple cell types native to the host organ. Heterogeneity is commonly retained by tumor metastases, which implies that the cell that produced them had the capacity to generate multiple cell types. Ginestier et al [39], showed that aldehyde dehydrogenase (ALDH)-positive cells isolated from human breast tumors contained CSCs, as these cells could generate tumors in NOD/SCID mice. Subsequently, Douville et al [40], confirmed that ALDH1 activity can be used to identify and isolate CSCs of the

mammary gland and breast cancer. In addition, ALDH-positive CSCs from the colon [33], brain [44], and liver [45] were also capable of forming tumors in immuno-compromised NOD/SCID mice, whereas ALDH-negative cells did not. OCT4 and SOX2, a class of nuclear proteins [46], are both crucial markers to maintain the pluripotent state of stem cells. In addition, both of them and some other factors are expressed in pluripotent stem cells [47,48]. In 2010, we found that ESC protein markers CD133+ , SOX2 and OCT4 were expressed in a small subpopulation of cells in human primary nasopharyngeal carcinoma (NPC) +. Further study showed that these cells were proliferative. According to label-retaining cell (LRC) trial, adult stem cells can be identified based on their ability to retain nucleoside analog, such as bromodeoxyuridine. In accordance with this principle, Zhang et al [50], found that a few of LRCs existed in human NPC tissues, such as the nasopharyngeal mucosal basal parts and the NPC cell lines. These cells can further develop into tumors after transplantation into the notum of nude mice. In a recent study, laser capture microdissection is used to isolate pure cell populations from NPC and normal nasopharyngeal epithelial tissue samples. Cheng et al [51], confirmed that stathmin, 14-3-3 $\delta$ , and annexin I are related to differentiation degree and/or metastatic potential of the NPC cell lines.

## Origins of CSCs

To date, the cell of origin of CSCs remains to be a pendent and troubled problem around the world. There are two hypotheses for the origin of CSCs [52]. One states that CSCs come from normal adult stem cells through an initial genetic mutation, another states that CSCs originate from already differentiated primary cells or differentiated cells that dedifferentiate. Stem cells existed in normal adult tissues may be the targets of carcinogenesis and tumor transformation. Although the number of stem cells is very small, they can progress continual division for a long time and are more likely to accumulate the molecular mutations that cause tumorigenesis. Thus, they are in a tendency of high deterioration. As mentioned above, the phenotype of tumor initiating cells, CD34+ /CD38- cells, in leukemia is similar to the normal hematopoietic progenitor cells [53]. The evidence from hematopoietic system indicates that the genetic mutations in progenitor cells can reactivate self-renewal, suggesting that CSCs may come from other origins, although normal stem cells are found in many solid tumors.

It has been proposed that CSCs and normal stem cells can interconvert into each other. The more important consequence of this event is that normal stem cells can generate CSCs that ultimately induce a new tumor. Emerging evidence has supported this notion, as CSCs share many properties of normal stem cells. For examples, both have the capacity of self-renewal and non-directional differentiation potential, and many classic cancer related signal transduction pathways also regulate the development of normal stem cells. In this scenario, cancer cells could simply utilize the existing stem cell regulatory pathways to stimulate their self-renewal. In addition, both stem cells and CSCs have telomerase activity and amplified telomere repeats, while most adult human somatic cells lack detectable telomerase. Another theory associates stem cells with the formation of tumors, which is most often related with tissues with a high rate of cell turnover. In these tissues, it has long

been expected that stem cells are responsible for tumor formation. Tissue with fast renewal, such as epithelial tissue and those of the hematopoietic system, are sites with high incidence of cancer. The faster tissues renew, the higher the rate of mutation that will occur during replication and transcription. Although it is not clear which target cells mutate and transform to tumors, experimental data obtained from a variety of tumors show that certain colon cancers and leukemia result from an accumulation of multiple mutations of stem cells [54]. Due to the heterogeneous nature of evidence, it is possible that any individual cancer could come from an alternative origin. Another hypothesis is that the developing stem cells are mutated and then expand such that the mutation is shared by many of the descendants of the mutated stem cell. These daughter stem cells are then much closer to becoming tumors, and many of them have more chance of a mutation that can cause cancer [55]. Taken together, these findings suggest that there may be some linkages between CSCs and stem cells.

Some researchers presume that CSCs may be obtained by the mutation of committed progenitor cells with an ability of self-renewal. For example, leukemia stem cells can be transformed from granulocyte-macrophage progenitors with the assistance of MLL-AF9 fusion protein [56]. Another study also shows that neuronal progenitor cells are likely to be the target of carcinogenic mutations [57]. All of these results indicate that the CSCs may originate from the committed progenitor cells.

#### 4.3. Other Possible Sources

Despite the lack of direct experimental evidence, some studies show that CSCs may be the fusion of stem cells and other cells [58]. These new integration cells obtain the capacity of self-renewal, and are thus effortless to accumulate more mutations for canceration. For example, bone marrow derived cells can fuse with epithelial tissue tumors [59]. Additionally, a recent study [60] concerned with migrating CSCs showed that the development of tumor metastasis might correlate with the dissemination of CSCs, which were mainly caused by the cells at the tumor margins that have undergone epithelial-mesenchymal transition (EMT). The linkages of EMT and the emergence of stem cells have also been reported by Mani et al. [61]. The CSC hypothesis presumes that the path via which CSCs self-renew and generate more differentiated neoplastic progenitor cells through asymmetric replication is hierarchical and unidirectional. However, emerging evidences are beginning to support the notion that relatively differentiated progenitors could switch to dedifferentiate and acquire a stem-like phenotype in response to either genetic manipulation or environmental cues [62], which has been identified sequentially in mammary carcinoma cells [63], A549 lung cells [64], colon cancer cells [65] and glioblastoma cells [66]. Terminally differentiated cells including human somatic cells and skin cancer cells can be artificially induced through specific transcriptional networks to reprogram pluripotent ESCs, called induced pluripotent stem cells (iPSCs), which is a significant breakthrough against the dogma that differentiated cells is irreversible [67-68]. However, these iPSCs are tumorigenic, suggesting that oncogenic transformation of partially differentiated cells can lead to the emergence of CSCs. In addition, more recent studies show several plausible origins of CSCs. For examples, (1) lineage tracing reveals that *lgr5+* cells could generate additional *lgr5+* cells as well

as all other adenoma cell types, thus exhibiting activity of CSCs in mouse intestinal adenomas +; (2) the restricted subpopulation, with properties similar to those proposed for CSCs, propagates glioblastoma growth after chemotherapy [70]; (3) using an inducible genetic lineage tracing system, Gregory Driessens et al. found that the yellow fluorescent protein (YFP) could be expressed in around 1% of basal papilloma epithelial cells in mice, and these YFP-labeled tumor cells were capable of generating all cell types that comprised the tumor [71].

### Implications for Cancer Treatment

Once a cancer has been diagnosed, treatments vary according to cancer type and severity. Surgery, radiation therapy, chemotherapy or hormonal therapy represents traditional approaches designed to remove or kill rapidly-dividing cancer cells. However, there has been hardly any substantial progress with new therapies regarding clinical endpoints, despite significant advances in molecular mechanisms of cancer. Cancer remains a major public health issue. Conventional anti-cancer treatments target the more mature cancer cells that form the bulk of the tumor, but do not target the CSCs, which are relatively quiescent and intrinsically resistant, thus possibly accounting for treatment failures [72]. To target tumors effectively with minimal toxicity, drugs that specifically target the relatively rare CSC subpopulation need to be identified.

Tumor metastasis is a complex process, and is also the main cause of the death of cancer patients in clinic. It is the key to improve the prognosis of patients by removing CSCs selectively with no significant toxicity. Several pieces of instances have been expounded surrounding this conclusion: (1) The maintenance of CSCs viability can be influenced by the microenvironment, thereby appropriate microenvironment exhibits vital importance for CSCs, which brings us a new insight into oncotherapy by changing the survival microenvironment. For example, glioma CSCs have been found congregated close to capillaries in a niche, thus, vasculature-targeted therapeutic strategies could effectively destroy the niche and eradicate the tumor; (2) Growing evidences indicate that CSCs regulate some pathways of normal stem cell self-renewal and the continuing expansion of self-renewal could consult in tumorigenesis. Accordingly, the exploration of self-renewal pathways about defective cancer cells may provide us a new treatment for cancer; (3) Potential approaches to killing CSCs also include inducing tumor cell differentiation in addition to blocking self-renewal signaling and inhibiting cell survival mechanisms. For example, renal CSCs can be differentiated into epithelial cells after treatment with interleukin-15. The differentiated epithelial cells derived from renal CSCs are sensitive to chemo-therapeutic drugs. Knockdown of CD44 caused BCSCs to differentiate into non-breast CSCs with lower tumorigenic potential, and altered the cell cycle and expression profiles of some stem cell-related genes, making them more similar to those seen in non-breast CSCs and resulting in a loss of stemness and an increase in susceptibility to chemotherapy or radiation. As described above, some of the signaling pathways for the differentiation of normal stem cells may be maintained in cancer stem cells. Wnt signaling plays an important role in maintaining the pluripotency of human ESCs and is also implicated in sustaining CSC phenotype by dedifferentiating mechanisms. In

2007, Wei et al. confirm that the Wnt pathway plays a critical role in the self-renewal and maintenance of stem cells. A recent report documents that rapamycin-mediated inhibition of mTOR signaling may prevent CSC self-renewal and circumvent CSC-mediated resistance to cancer therapeutics (3) Some studies show that patients with tumors that express high levels of molecules associated with CSCs had a poorer prognosis than patients with tumors that express low levels of these markers. In breast cancer, for example, the most poorly differentiated tumors have the highest burden of CSCs. Subsequent study indicated that metformin not only selectively killed existing CSCs, but also indirectly lowered the CSC burden by inhibiting the conversion of non-stem cancer cells to CSCs. Cell differentiation is regulated, at least in part, by a recently discovered class of molecules-microRNAs (miRNAs), and as a consequence, a potential therapeutic use of miRNAs is to correct these aberrant transcript levels involved in the signaling pathways of cancer cells [73], especially CSCs [74-75]; (4) To overcome the chemotherapy resistance of CSCs through the activity of multiple drug resistance (MDR) transporters. Recent study indicates that salinomycin, a specific inhibitor of P-glycoprotein, can restore a normal drug sensitivity of MDR cell lines and induce CSC death [76]. Another way to control the tumor progression is to induce differentiation of CSCs. Study by Piccirillo et al [77]. showed a reduction of the number of glioma CSCs after treatment with bone morphogenetic proteins. Additionally, the quiescent CSCs were involved in the resistance of CSCs to anti-cancer treatments as discussed above. Therefore, it will be of great importance to explore the means that break the quiescent state of CSCs. Studies by Ishikawa and his colleagues have recently induced AML stem cell cycle entry and increased the sensitivity of these cells by using granulocyte colony-stimulating factor treatment [78]. The CSC concept promises the development of therapeutic strategies beyond traditional anti-proliferative agents. Studies have confirmed that potential approaches to kill CSCs may exploit the survival mechanisms of the CSCs. The biological exploration that correlative with CSCs in solid tumors will bring us new viewpoints to the clinical diagnosis, treatment, CSC-targeted drug researches and the preclinical trials. Moreover, it will be better to predict the results of clinical treatment by assess the behavior of CSCs. At present, although the cancer treatments which target CSCs unveil a new prelude, it is still a problem that how to identify the CSCs, especially to prevent its formation [79].

## Conclusion

CSCs are believed to be the main reason for tumor growth and metastasis. To achieve the maximum effect and eradicate a tumor, the CSCs compartment should be targeted specifically. To date, although many CSCs markers have been found, it is still impossible to take them as candidates for antibody therapy owing to their broad expression in healthy tissue. There is no doubt that the application of CSC theory to study the tumorigenesis mechanisms will lead a major shift in cancer research and the understanding of the essence of cancer, supplying a new way to effectively diagnose tumors and find functional proteins as potential therapeutic targets.

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