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Review Article

Cancer stem cells and tumor transdifferentiation: implications for novel therapeutic strategies

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Abstract: Highly malignant tumors mostly consist of rapidly proliferating cells. However, tumors also contain a few cells in a quiescent state that can be characterized as slow-cycling, expressing markers of stem cells and possessing the ability to initiate new tumors. These quiescent cells, now generally termed 'cancer stem cells' (CSC) (or 'cancer initiating cells'), are capable of regenerating the entire tumor—as it occurs in metastatic spread. This process of tumor initiation by stem-like cells presumably involves differentiation of quiescent CSC into rapidly proliferating tumor cells. An important implication of the presence of slow cycling, quiescent stem-like cells in the tumor and their ability to initiate tumors is that they contribute to the resistance to treatments by conventional chemo- and radiotherapy directed toward killing rapidly dividing cells. However, similar to normal stem cells, the CSC could also potentially transdifferentiate into cell lineages other than the original lineage from which the tumor arose. Therefore, transdifferentiation of CSC offers a possible therapeutic strategy which has not yet been fully exploited. In this article, we provide a comprehensive review of the concepts in tumor cell transdifferentiation and discuss the mechanisms of transdifferentiation with emphasis on their relevance to potential novel treatment strategies.

Keywords: Cancer stem cells, transdifferentiation, CSC-targeted therapy, tumor Initiating cell, EMT

Introduction

Cancers are a population of genetically compromised cells that proliferate without control [1]. Targeting rapidly proliferating cells is the rationale behind many conventional cancer therapies. However, these therapies are rarely as successful in the long run as those instances where tumors that can be surgically resected. Chemo- and radiation therapy failure is thought to occur due to the well-recognized tumor heterogeneity, which allows a subpopulation of tumor cells to escape cell death [1].

The clonal evolution model of tumorigenesis suggests that cancer begins with a single transformed cell whose progeny, through subsequent rounds of cell division, acquire mutations that further increase their own malignant potential [2]. According to this model, every cell within a cancer should be able to generate an entire new tumor independent of all other cells within the original tumor. However, it has been shown that only a small fraction of cells within a tumor mass is capable of initiating new tumors [3-5]. Therefore, for metastasis to occur, cells that have the ability to initiate the tumor must reach a suitable destination within the body. These tumor initiating cells must also be able to self-renew to create a population of additional tumor initiating cells within the metastatic lesion. This ability to self-renew also defines stem cells. Such cells with "stem-like" and "tumor initiating" properties are now called "cancer stem cells" (CSC) [6].

Cancer stem cell model

Based on similarities observed between certain cancer cells and embryonic cells, Julius Cohnheim first proposed in 1875 that cancers arise from ''embryonic rests,'' cells leftover from embryogenesis [7]. However, it is only within the past decade, experimental evidence for the concept of cancer stem cells has begun to accumulate [8].

The CSC model defines a hierarchy among cancer cells such that only a small subset is capable of sustaining the tumor and establishing the cellular heterogeneity [6]. CSC exhibit the quintessential stem cell properties of self-renewal and differentiation. There is some evidence that CSC originate from normal tissue stem cells [9, 10]. It has also been demonstrated that CSC can arise from spontaneous dedifferentiation of tumor cells [11, 12]. A third possibility is that suggested originally by Cohnheim, namely that remnants [of pluripotent stem cells] from embryogenesis could acquire malignant properties and contribute to CSC [7]. The above mechanisms may not be mutually exclusive.

Additionally, mechanisms for generating and maintaining CSC appear to also depend on the microenvironment of the tumor [13-17]. The microenvironment plays a major role in preserving the CSC population and in their self-renewal [13]. For example, in primary colorectal cancers stromal myofibroblasts secrete hepatocyte growth factor (HGF), which enhances WNT signaling in neighboring cancer cells and thereby induces stem cell features such as self-renewal in CSC [14]. Endothelial cells were shown to contribute to CSC microenvironment in glioblastoma by providing Notch ligands that facilitate self-renewal [15]. Even transient contact between circulating tumor cells and platelets seems to induce a epithelial mesenchymal transition (EMT) program that causes tumor cells to become CSC [16]. Finally, metastasized CSC arriving in a distant organ can reprogram stromal cells to suit their needs [17].

The CSC model also offers an explanation for the notorious resistance of cancers to conventional chemo- & radiation therapy [18-20]. These therapeutic approaches target the highly proliferative tumor cells, whereas CSC that are quiescent escape killing. [19]. Stresses like hypoxia, nutrient starvation, and inflammation can cause CSC to leave their quiescent state and re-enter the cell cycle. This provides a window of tumor vulnerability to conventional therapies [21]. For example, treatment of acute myelogenous leukemia (AML) stem cells with granulocyte colony stimulating factor (G-CSF) was reported to allow CSC to leave their dormant state [22]. Inhibition of either PML or FOXO, molecules involved in TGFβ signaling, in chronic myelogenous leukemia (CML) stem cells also seems to permit CSC to leave their quiescent state and become susceptible to Imatinib treatment [23, 24]. Therefore, while the CSC model of cancer provides valuable insights into tumorigenesis it also has consequences for disease management.

CSC normally differentiate into dividing tumor cells, and they can also differentiate into structures that support the tumor and its propagation. Transdifferentiation, a process by which tumor cells or CSC acquire features of different cell lineages, could either attenuate the tumor's malignant potential, or allow development of structures that support the tumor's aggressive behavior. CSC transdifferentiation also offers an opportunity for cancer treatment. Differentiating CSC into post-mitotic, terminally differentiated cells offers the potential to abolish the ability of a tumor to recapitulate itself from CSC. We feel this strategy has been inadequately investigated. In this review we provide a rationale for transdifferentiation as a therapeutic option for cancer using examples of selected malignancies.

Markers of CSC

Although each type of cancer may have CSC markers that are specific to that tumor (for example, CD271, in melanoma stem cells [25, 26]); there are a few "pan" CSC markers that are expressed in a range of distinct malignancies. While these markers are highly relevant for studies on the biology of CSC, they also could serve as useful markers to monitor efficacy of differentiation therapy. The prominent among these are CD34, CD133, CD44, EpCAM, and ALDH [6, 27-29]. CD34, one of the first CSC markers to be described [30] is a hematopoietic stem cell marker that is well characterized in acute myeloid leukemia stem cells. Similarly, CD133 has also been widely investigated as a CSC surface marker since its identification as a marker of glioblastoma multiforme stem cells [31]. CD133 has since been described in CSC from osteosarcoma, Ewing's sarcoma, endometrial, hepatocellular, colon and lung carcinomas and ovarian and pancreatic adenocarcinoma [32-35]. CD44 is present in head and neck cancer, prostate, gastric and colorectal carcinoma stem cells [36]. EpCAM is a marker of colon carcinoma and pancreatic adenocarcinoma stem cells [37]. ALDH has been used to identify CSC in melanoma, colorectal, breast, prostate and

squamous cell carcinomas, pancreatic adenocarcinoma, and osteosarcoma [38, 39].

Melanoma has received much attention with regard to existence and frequency of CSC. This tumor contains a high proportion of tumor initiating cells (upto 50%) with a wide spectrum of CSC markers (e.g., CD133, CD271, ALDH1, JARID1B) [5, 38, 40, 41]. Because these "melanoma stem cells" (MSC) are sometimes so numerous, some have argued that the CSC model may not apply to melanoma [40]. There are data from two groups indicating that melanoma lesions contain a CSC subset characterized by CD271 expression [25, 26]. In a severely immunodefficient strain NOD-SCID-IL2Rg$^{-/-}$ mice, both (CD271 + and -) subsets were found to be tumorigenic. However, the CD271^{-} fraction did not recapitulate the original tumor histology [26]. It was also reported that many CD271^{-} melanoma cells display MSC qualities, implying that not all MSC display the same surface marker [40]. A possible limitation in this study was that cells were trypsinized prior to assessing tumorigenesis, which could have destroyed CD271 marker resulting in underestimation of CD271 positivity [6]. The frequency of MSC can vary widely from 2.5% to 41% based on the approach used to assess their tumorigenic potential [25].

Moreover, other studies have suggested that the ability of melanoma cells to transition between invasive and proliferative states as governed by prevailing tumor microenvironment (so-called "phenotype switching") may entirely explain metastasis in melanoma [42]. This cellular property of phenotype switching may explain how a normal melanocyte gives rise to melanoma through de-differentiation [42]. A way to reconcile phenotype switching with cancer stem cell model might be that this intrinsic plasticity of cancer cells in response to microenvironment is primarily a property of CSC [43]. That is, only CSC can "switch phenotypes". This concept requires further investigation.

Regardless of the debate over the existence of CSC, tumor transdifferentiation still presents an attractive therapeutic opportunity. Coaxing cancer cells to transition to a post-mitotic, terminally differentiated state could significantly attenuate tumor progression.

Modulating differentiation of CSC as a therapeutic approach

A question of practical importance with respect to the plasticity of differentiation of CSC is: what are the mechanisms that regulate the cellular fates of CSC? CSC transdifferentiation through pharmacologic intervention offers a promising adjunct to conventional chemotherapy. For example, a differentiation therapy approach using all trans-retinoic acid (ATRA) for the treatment of acute promyelocytic leukemia (APL) has shown some promise [44]. This leukemia differentiates into mature granulocytes after ATRA treatment. Melanoma is one of the most studied cancers with respect to transdifferentiation of CSC. It was reported that CSC in melanoma formed spheroids in culture [45]. When grown under varying kinds of differentiation media, these spheroids could be transdifferentiated into melanocytes, adipocytes, chondrocytes, or osteocytes. Melanoma cells from these spheroids showed increased potential to form tumors *in vivo* after injection into mice. These studies highlight both the concept of CSC in melanoma and the ability of melanoma CSC to undergo transdifferentiation producing diverse cell lineages [45]. Surprisingly, there is currently no FDA approved cancer treatment regimen based on transdifferentiation. Nonetheless, considerable accumulating data point to the validity and potential of this approach (see **Figure 1** and subsequent sections).

Neuronal differentiation

Expression of neuronal proteins and neuron-like differentiation has been long recognized in neoplastic melanocytes [46, 47]. Certain melanoma cell lines that express CSC markers CD133 and ABCG2 [48] also express neuronal progenitor and mature neuronal/oligodendrocyte markers (including MAP2, a marker of post-mitotic neurons) and are able to transdifferentiate into astrocytes under specific growth conditions. Microtubule associated protein 2 (MAP2) is highly expressed in benign melanocytic nevi and early primary melanoma but not in metastatic melanoma [49, 50]. More importantly, forced expression of MAP2 in metastatic melanoma cells inhibits their growth [50].

Cellular differentiation is a highly complex process that includes epigenetic modifications

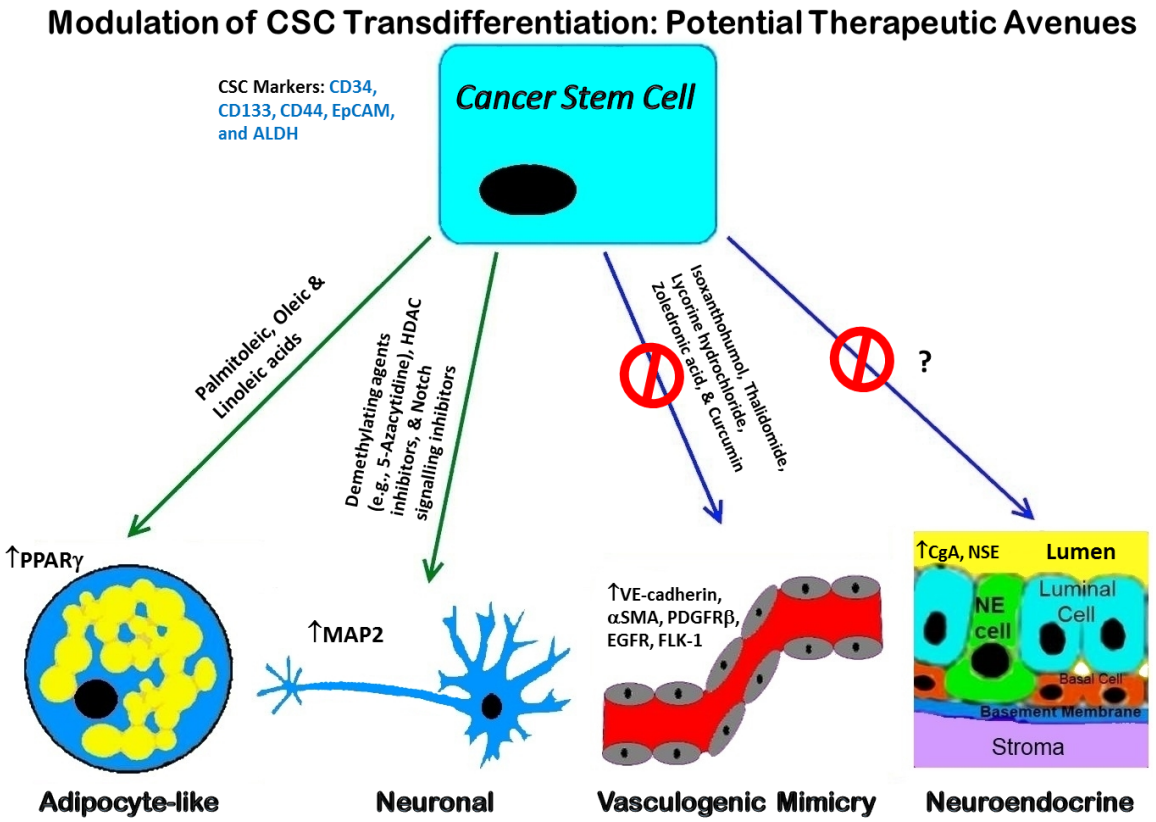

Figure 1. Potential therapeutic avenues to modulate transdifferentiation of cancer stem cells. CSC express various protein markers and are resistant to conventional therapies. Green arrows depict transdifferentiation of CSC into post-mitotic cells, and agents that facilitate these processes are listed. For example, unsaturated fatty acids may drive CSC into terminally differentiated adipocyte-like cells. Similarly, DNA demethylating agents could help melanoma CSC transdifferentiate into neuronal cells. Undesirable types of transdifferentiation are depicted with blue arrows. CSC can transdifferentiate to generate vascular conduits by a process termed "vasculogenic mimicry". A number of agents, tested mostly *in vitro*, but some in animal models, have been shown to prevent this process and need further investigation. Prostate CSC transdifferentiation into neuroendocrine (NE) cells is associated with worse prognosis owing to transition to androgen independence. Therefore, inhibition of NE differentiation of prostate CSC may be desirable. For simplicity, transdifferentiation of small cell lung cancer (SCLC) into NE cells and epithelial mesenchymal transition (EMT) are not shown.

allowing activation of lineage specific factors and repression of stem cell (or precursor cell) factors [51]. Not surprisingly, epigenetic modifications are also reported to regulate tumor cell transdifferentiation [52]. For example, the regulatory sequences of the neuronal marker gene, MAP2, are progressively methylated during melanoma progression, suggesting MAP2 expression is silenced by epigenetic mechanism in metastatic melanoma [53]. Treatment of metastatic melanoma cells with 5-azacytidine induced MAP2 expression [54]. Thus, treatment with demethylating agents such as 5-azacytidine may be useful for melanoma, if used in appropriate combination with other agents [26]. A phase II clinical trial of 5,6-dihydro-5-azacytidine (DHAC) showed limited benefit in malignant melanoma without the side-effect of myelosuppression [55]. Another phase I trial of 5-aza-2'-deoxycytidine (decitabine) plus high dose intravenous interleukin-2 showed regression of melanoma in 31% of patients with significant incidence of neutropenia [56]. In neither trial, the contribution of neuronal (or other) transdifferentiation of melanoma CSC to the anti-tumor effect of the demethylating agent was investigated.

Additionally, histone deacetylase (HDAC) inhibitors have been shown to activate MAP2 expression and induce benign neuron-like differentiation in a metastatic melanoma mouse cell line [57]. HDAC inhibitors also inhibit the growth of uveal melanoma cells both *in vitro* and *in vivo*

and induce melanocyte/neuron-like differentiation of melanoma cells as evidenced by dendritic arborization [58].

It has also been demonstrated that inhibition of Notch signaling upregulates MAP2 gene expression in melanoma through epigenetic mechanism [54]. It is known that Notch signaling is essential to maintenance of neuronal stem cells (NSC) and Notch inhibition results in NSC neuronal differentiation [59]. Thus, Notch inhibition may inhibit melanoma tumor progression through CSC neuronal transdifferentiation. In a recent study, it was shown that treatment with a novel γ-secretase inhibitor (an inhibitor of Notch signaling) reduces tumor initiating potential of melanoma both *in vitro* and *in vivo* [60]. An interesting observation here is that a signaling mechanism that induces differentiation of a normal tissue stem cell-type also appears to be a potential approach to induce transdifferentiation of tumor cells and CSC into post-mitotic cells. Hence, there could be other candidate pathways of tissue stem cell differentiation that could be exploited in a similar fashion.

It was reported more than a decade ago that neoplastic melanocytes express p75NGFR, CD56/NCAM, and GAP-43, markers that are specific to Schwann cell precursors (SCP), but not mature Schwann cells [47]. Specifically, these Schwann precursor-like neoplastic melanocytes were present in melanocytic nevi, which are thought to be precursor lesions to melanoma [47]. Interestingly, these SCP markers are present in only less than 20% of melanomas and they are never present together [47]. It is tempting to speculate that such melanomas arise from aberrant differentiation of SCP. Support for such possibility came recently when it was demonstrated that SCP not only share markers with neoplastic melanocytes, but SCP indeed are the source for melanocytes in the skin [61]. Therefore, it is conceivable that driving melanoma transdifferentiation toward a neuronal phenotype could serve as therapeutic strategy to deplete the pool of CSC in this cancer.

Neuroendocrine differentiation

Much like normal prostate stem cells, prostate cancer stem cells (PCSC) can also give rise to luminal secretory epithelial cells as well as terminally differentiated neuroendocrine (NE) cells [62, 63]. In addition, in prostate cancer, cancerous luminal secretory cells may directly transdifferentiate into NE cells suggesting existence of alternative mechanism for generating this unique population. A higher proportion of NE cells in prostatic tumors is associated with more aggressive behavior and worse prognosis [64].

NE cells are characterized by expression of several neuronal as well as secretory markers, but the most prominent are the neuron specific enolase (NSE) and chromogranin A (CgA) [65]. NE cells do not express androgen receptor making them refractory to anti-androgen therapy [66-68]. Intriguingly, androgen deprivation can itself drive transdifferentiation of PCSC into NE cells [64]. In addition, NE cells can support the survival of other prostatic cells in their vicinity by allowing them to survive in the absence of androgens via secretion of trophic paracrine factors [68, 69]. Alternatively, NE cells can enhance the sensitivity of neighboring prostate cells to androgens such that they can survive in the presence of a much smaller concentration of androgens [70]. The NE cell population is highly resistant to conventional antimitotic agents and these cells also are resistant to undergoing apoptosis [71, 72]. Hence, NE transdifferentiation of prostate cancer stem cells could contribute to the failure of both hormonal as well as cytotoxic therapy in this disease [66-72]. While several approaches are under investigation to interfere with function of NE cells in prostate cancer [64], there are no treatments in the pipeline that could prevent transdifferentiation of PCSC into NE cells.

Neuroendocrine differentiation of small cell lung cancer is another interesting example. One theory for the origin of small cell lung carcinoma (SCLC) is that it arises from failure of lung tissue stem cells to completely differentiate into pulmonary neuroendocrine cells [73]. Aberrant methylation of NE cell lineage determining genes was found in SCLC [73]. At present, SCLC can almost never be fully eradicated with conventional approaches. Therefore, if tumor initiating cells in SCLC can be induced to transdifferentiate into post-mitotic NE cells, it might be possible to prevent recurrence of SCLC after conventional and/or targeted therapy.

Vasculogenic mimicry

As tumors grow, their nutrient and oxygen requirements increase [1]. Classic angiogenesis is a process whereby the tumor secretes factors such as VEGF to recruit host's endothelial cells to form blood vessels for its nutrient needs. Interestingly, it was demonstrated that melanoma stem cells can themselves transdifferentiate and form vascular conduits that supply the tumor with blood [74]. This is referred to as "vasculogenic mimicry" (VM). It is not completely clear whether VM reflects the plasticity of differentiated melanoma cells or transdifferentiation of melanoma CSC. Nonetheless, many different pharmacologic interventions have been reported to inhibit VM in various cancers [75-81]. Among these, thalidomide, endostatin, and lycorine hydrochloride were shown to inhibit VM specifically in melanomas [80, 81]. Other compounds known to inhibit tumor VM either *in vitro* or in an *in vivo* mouse model, include: isoxanthohumol, endostar, zoledronic acid, curcumin, and a group of cell-permeable, phosphatase-stable phosphopeptide mimetic pro-drugs targeted to the SH2 domain of Stat3 [75-79].

Adipocyte-like differentiation

It was demonstrated that specific unsaturated fatty acids, namely palmitoleic, oleic and linoleic acids, induce transdifferentiation in several different human cancers, including melanoma [82]. First, it was observed that conditioned media from human embryonic stem cells would cause adipocyte-like transdifferentiation. Subsequently, it was reported that a similar differentiation could be induced by treating melanoma spheres with unsaturated fatty acids and by upregulation of PPARγ [82]. This mechanistic understanding of transdifferentiation of melanoma CSC into adipocyte-like cells could open new therapeutic avenues.

Epithelial mesenchymal transition (EMT): a transdifferentiation process that generates more CSC

EMT in cancer is the process through which epithelial cells of a tumor acquire invasive properties and migrate away from the primary lesion. Activation of the EMT program can confer on cancer cells the abilities of self-renewal and unlimited differentiation [18, 83, 84]. Hence, EMT can be considered a form of transdifferentiation that generates CSC or tumor initiating cells. Another insight gained from this observation is that the inflammatory stromal conditions that give rise to EMT may be similar to those needed to maintain CSC in their respective niche [1]. EMT in cancer (and ways to combat it) is the subject of extensive research and has been recently reviewed [85, 86]. Thus, one consequence of inhibiting EMT in cancer would be to prevent transdifferentiation of tumor cells back into CSC.

Conclusion

The CSC model of tumor pathogenesis explains why conventional cytotoxic therapies are rarely completely effective and recurrence of disease inevitably occurs. Hence, it is critical to define the precise markers of CSC that are present within specific cancers. As CSC phenotypes appear to be heterogeneous within each type of cancer and perhaps even vary from patient-to-patient, multi-pronged approaches including patient-specific approaches may be needed to eliminate CSC.

While CSC may contribute to the resistance of tumors to conventional therapies, their plasticity to differentiate may prove to be an Achilles' heel. One consequence of plasticity of CSC is their ability to undergo terminal differentiation/ transdifferentiation. The goal is to exploit this vulnerability to deplete the CSC pool by inducing post-mitotic/ terminal differentiation or prevent such differentiation when it serves to enhance the tumor aggressiveness. Tumor transdifferentiation therapy is an area that has been inadequately explored. Accumulating evidence from multiple cancers now points to the potential of differentiation therapies. The time is ripe for a critical evaluation of such evidence and a concerted effort to develop novel treatments. Efforts are already underway to elucidate the mechanisms underlying EMT with the goal of inhibiting mesenchymal differentiation of tumor cells. With new insights gained from the CSC model, and armed with the knowledge that EMT is actually a way of generating CSC, it may eventually be possible to deplete the pool of CSC, prevent their renewal and achieve permanent remission.

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