

Cancer Stem Cells: Distinct Entities or Dynamically Regulated Phenotypes?

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Abstract

The origins of tumor-propagating neoplastic stem-like cells [cancer stem cells (CSC)] and their relationship to the bulk population of tumor cells that lack stem-like tumor-propagating features (i.e., transit-amplifying cancer progenitor cells) remain unclear. Recent findings from multiple laboratories show that cancer progenitor cells have the capacity to dedifferentiate and acquire a stem-like phenotype in response to either genetic manipulation or environmental cues. These findings suggest that CSCs and relatively differentiated progenitors coexist in dynamic equilibrium and are subject to bidirectional conversion. In this review, we discuss emerging concepts regarding the stem-like phenotype, its acquisition by cancer progenitor cells, and the molecular mechanisms involved. Understanding the dynamic equilibrium between CSCs and cancer progenitor cells is critical for the development of therapeutic strategies to deplete tumors of their tumor-propagating and treatment-resistant cell subpopulations. *Cancer Res*; 72(3); 576–80. ©2012 AACR.

Introduction

Most solid malignancies consist of phenotypically heterogeneous neoplastic cells, and only a subset of these cells has the capacity to efficiently propagate tumor xenografts that recapitulate with high fidelity the heterogeneity and pathologic features of the original clinical cancer. These uniquely tumorigenic cells are commonly referred to as cancer stem cells (CSC) because they exhibit a spectrum of biologic, biochemical, and molecular features that are consistent with a stem-like phenotype. These features include growth as nonadherent spheres in a defined stem cell medium, unlimited self-renewal, and the capacity for multipotency and lineage-specific differentiation. These features and evidence that CSCs are particularly resistant to cytotoxic therapeutics support the hypothesis that CSCs are the cell subpopulation that is most likely responsible for treatment failure and cancer recurrence. Understanding the origins and supporting mechanisms of CSCs, and the relation of CSCs to the bulk population of tumor cells that display low self-renewal capacity and a higher probability of terminal differentiation (i.e., transit-amplifying cancer progenitor cells), is obviously of considerable relevance for improving cancer therapeutics.

The CSC hypothesis, in its most prevailing form, currently mirrors normal stem cell biology by emphasizing a hierarchical

unidirectional path through which CSCs self-renew and generate more differentiated neoplastic progenitor cells through asymmetric replication. Emerging evidence from multiple laboratories is beginning to support the existence of a contextually regulated equilibrium between CSCs and transit-amplifying neoplastic progenitors, including the capacity of progenitor cell dedifferentiation to form CSCs. In a recent work (1), we identified a reprogramming-like mechanism through which the c-Met receptor tyrosine kinase via downstream transcription factors (TF; i.e., Sox2, c-Myc, Klf4, Oct4, and Nanog) induces glioblastoma cells to express a stem-like phenotype (1). In this review, we discuss the microenvironmental influences, signaling pathways, molecular circuitries (including transcriptional networks), microRNAs (miRNA), and epigenetic modifications that are currently implicated in the *de novo* generation of CSCs from cancer progenitors (Fig. 1).

Key Findings

Transcription factor networks

Specific transcriptional networks play an essential role in sustaining the growth and self-renewal of embryonic stem cells (ESC) and neoplastic stem-like cells. Expression of a defined and limited set of TFs (i.e., Sox2, c-Myc, Klf4, Oct4, and Lin28) can reprogram mouse and human somatic cells to embryonic-like cells, called induced pluripotent stem cells (iPSC), that efficiently self-renew and display the capacity to differentiate into all tissue types (2). These reprogramming TFs are frequently overexpressed in human cancers, and their expression levels often correlate with tumor progression and poor prognosis. Furthermore, the transcriptomes of high-grade tumors and ESCs are remarkably similar (3). This suggests the existence of dedifferentiation mechanisms through which the expression and function of reprogramming TFs influence the

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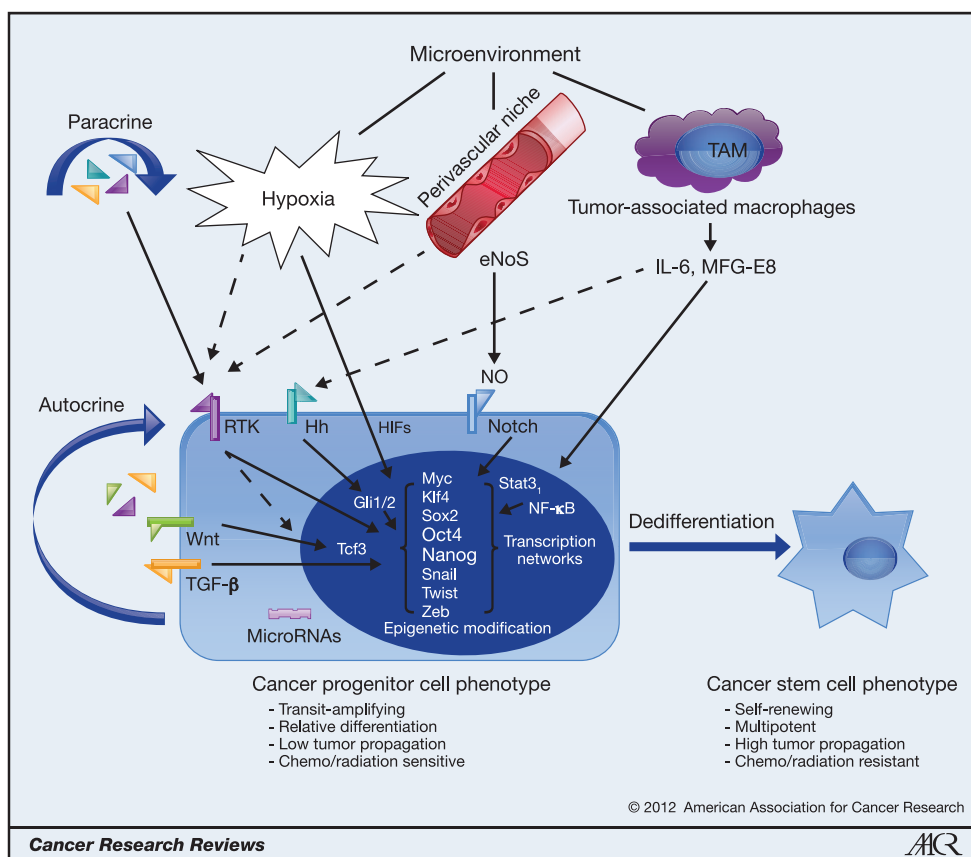


Figure 1. Transit-amplifying cancer progenitor cells acquire stem-like phenotypes by dedifferentiating mechanisms. This schematic model depicts the functional connections among microenvironmental signals, signal transduction pathways, and molecular circuitries, including transcriptional networks, miRNAs, and epigenetic modifications that induce dedifferentiation of cancer progenitor cells into CSC phenotypes. Transcriptional networks involving Oct4, Nanog, and other TFs act as key inducers of dedifferentiation mechanisms. eNOS, endothelial nitric oxide synthase; Hh, hedgehog; NO, nitric oxide; RTK, receptor tyrosine kinase.

malignant phenotype by supporting the formation and/or maintenance of neoplastic stem-like cells. Induced cancer cell reprogramming was recently recapitulated in several types of malignant cells. By expressing defined TFs, including Oct4, Sox2, c-Myc, and Klf4, Miyoshi and colleagues (4) induced gastrointestinal cancer cells to express an ESC-like state. Ectopic coexpression of Oct4 and Nanog in human A549 lung cells also induced a cancer stem-like phenotype characterized by CD133 expression, sphere formation, enhanced tumor-propagating capacity, and drug resistance (5). Enforced Lin28 expression in human colon cancer cells increased expression of the colonic stem cell markers LGR5 and PROM1, and enhanced metastatic potential (6). An alternative transcriptional network that drives the epithelial–mesenchymal transition (e.g., Snail, Twist, and Zeb) has also been found to induce stem cell properties in human mammary carcinoma cells (7). However, critical questions remain to be answered. For example, it has not yet been determined how these multiple reprogramming TFs function cooperatively or which transcriptional events are essential for reprogramming neoplastic progenitors to a stem-like tumor-propagating state. Oct4 alone appears to be sufficient to directly reprogram human and mouse neural stem cells to iPSCs (8), suggesting that Oct4 may initiate the

transcription network. Consistent with this observation, our recent unpublished findings show that Oct4 is the most upstream TF in the reprogramming network driven by c-Met signaling in human glioblastoma cells. Although exogenous Nanog is not necessary for iPSC generation, the induction of endogenous Nanog by Oct4 and other reprogramming factors appears to be necessary to accomplish full somatic cell reprogramming. Consistent with this role in nonneoplastic cells, we found that silencing Nanog inhibits the reprogramming capacity of c-Met signaling in glioblastoma cells (1). Nanog is emerging as a critical TF through which several signaling pathways, including c-Met, hedgehog (Hh), and TGF- β , sustain nonneoplastic and neoplastic cell stemness (1, 9, 10). Of interest, the tumor suppressor p53 reduces the efficiency of iPSC generation from nonneoplastic somatic cells by repressing Nanog induction (11), and loss of p53 enables mammary cancer cells to acquire a stem cell–like transcriptional signature (12).

Stem cell miRNAs

The miRNAs represent a class of small noncoding RNAs (20–25 nucleotides) that regulate a diverse array of biologic processes through posttranscriptional silencing of specific target genes. Recent studies have shown that specific miRNAs, called

stem cell miRNAs (e.g., the miR-302 cluster, miR-372/373, let-7, and the miR-200 family), play a critical role in controlling pluripotency by targeting multiple genes involved in cell cycle regulation, epigenetic modifications, and epithelial–mesenchymal transition. Investigators have successfully generated iPSCs from human and mouse somatic cells by expressing specific stem cell miRNA (13, 14). Lin and colleagues (15) showed that expression of miR-302a alone promotes the dedifferentiation of human skin cancer cells to an iPSC-like state, indicating that miRNAs are also capable of reprogramming cancer cells to a pluripotent ESC-like state. Although the complete spectrum of reprogramming miRNAs and their molecular mechanism of action remain to be fully identified, stem cell miRNAs appear to regulate the stem cell state at least in part by modulating the expression of reprogramming transcriptional networks (6, 16). Several lines of evidence show that stem cell miRNAs, such as miR-145, miR-302 cluster, miR-200 family, and miR-134/296/470, regulate ESC pluripotency by targeting Oct4, Nanog, and Sox2 (16–18). Wellner and colleagues (16) found that miR-200 family members target Klf4 and Sox2 and that repressing miR-200 miRNAs with the zinc finger E-box-binding homeobox protein ZEB1 promotes a stem-like, tumor-initiating phenotype in pancreatic and colorectal cancer cells. Li and colleagues (14) found that miRNA-93 and miR-106b enhance iPSC induction by targeting Tgfr2 and p21. Stem cell miRNAs act through multiple targets and pathways to regulate nonneoplastic and neoplastic stemness and cell differentiation. It remains to be established how and in what context these miRNAs regulate the acquisition and maintenance of the stem cell state in cancer cells.

Microenvironmental signals

Cancer cell subsets localize to specific tumor microenvironments, and increasing evidence is pointing to the influence of microenvironment-specific factors on the plasticity and differentiation of cancer cells, including the subset of CSCs. A broadly relevant example is tumor hypoxia, which through hypoxia-inducible factors induces the stem-like phenotype in cancer progenitors isolated from multiple solid-tumor subtypes. The mechanisms of this process involve the induction of an ESC-like transcriptional program consisting of specific reprogramming factors (Oct4, Nanog, Sox2, and KLF4) and miRNA-302 (19, 20). In one study, hypoxia-inducible factors in combination with the iPSC inducers Oct4, Nanog, and Lin28 reprogrammed differentiated lung cancer cells into stem-like cells that efficiently propagated aggressive tumors in mice (20). Hypoxia also enhances the efficiency of nonneoplastic iPSC formation from mouse embryonic fibroblasts and human dermal fibroblasts (21). In addition to hypoxia, tumor cell stemness is influenced by microenvironmental inflammation. For example, tumor-infiltrating macrophages increase the tumor-initiating capacity and anticancer drug resistance of neoplastic stem-like cell populations through the secretion of interleukin (IL)-6 and macrophage-derived milk-fat globule EGF8, which cooperatively induce tumor cell Stat3 and Hh signaling (22). Of note, IL-6 was found to enhance the conversion of breast cancer progenitor cells to a more stem-like phe-

notype via a positive feedback loop involving NF- κ B, Lin28, and Let-7 miRNA (23). The neoplastic stem-like phenotype is also influenced by paracrine signals derived from other stromal cells. For example, Charles and colleagues (24) found that the perivascular niche promotes glioma cell conversion to a more stem-like state through endothelial-derived nitric oxide-dependent induction of glioma cell Notch signaling. Similarly, hepatocyte growth factor secreted by stromal myofibroblasts was found to activate colorectal cancer cell c-Met and β -catenin transcription and thereby turn nontumorigenic cancer cells into highly tumorigenic stem-like cells (25).

Autocrine/paracrine oncogenic signaling pathways, such as WNT, Notch, Hh, TGF- β , and receptor tyrosine kinases (e.g., c-Met, EGF, and platelet-derived growth factor), are strongly implicated in regulating the CSC pool and pathways involving endogenous reprogramming mechanisms. Several studies support functional interactions between oncogenic signaling pathways and critical stem cell reprogramming TFs. For example, Wnt signaling promotes the reprogramming of murine fibroblasts to pluripotency (26) and maintains the pluripotency of human ESCs and CSCs through the downstream target gene Tcf3, which occupies and regulates the promoters for Oct4, Sox2, c-Myc, and Nanog (27). Hh-Gli signaling was found to have an essential role in GBM stem cell self-renewal via the downstream effectors Gli1 and Gli2, which bind to the Nanog promoter and directly regulate Nanog expression (9). EGFR activation promotes Sox2 expression, which in turn binds to the EGFR promoter and directly upregulates EGFR expression (28). Autocrine TGF- β signaling plays an essential role in the retention of glioblastoma cell stemness by inducing Sox4 and Sox2 expression (29). Our recent findings also show that c-Met signaling induces the glioblastoma stem-like phenotype through a reprogramming network involving Sox2, c-Myc, Klf4, Oct4, and Nanog. Overall, these and similar findings from a diverse array of experimental models, along with correlative clinical data, strongly support the hypothesis that the CSC phenotype is maintained by dedifferentiating mechanisms activated through oncogenic signaling pathways.

Epigenetic modification

Transcriptional networks that determine cell identity and fate are regulated by chromatin architecture and promoter DNA modification. The ectopic expression of the reprogramming TFs Oct4, Sox2, c-Myc, and Klf4 triggers a sequence of epigenetic events, including DNA demethylation and histone acetylation/methylation, that eventually result in heritable induced pluripotency. Promoter DNA demethylation and histone H4 and H3 hyperacetylation during iPSC generation lead to activation of endogenous pluripotency genes, including Oct4 and Nanog (30, 31). DNA methyltransferase 1 inhibitors, such as 5-aza-cytidine and RG108, and histone deacetylase inhibitors, such as valproic acid, trichostatin A, and suberoylanilide hydroxamic acid, accelerate the reprogramming of mouse and human fibroblasts (32). Of interest, these pharmacologic epigenetic modulators also have cell-differentiating and antineoplastic properties, emphasizing the need to better understand the dynamic context-dependent genome-wide epigenetic

mechanisms that regulate cancer cell stemness and tumor-propagating potential.

Implications and Future Directions

The identification of a dynamic, contextually regulated, bidirectional interchange between tumor-propagating CSCs and the much larger population of more-differentiated transit-amplifying cancer progenitor cells in solid malignancies has considerable conceptual and practical implications. Current dominant models of the CSC hypothesis depict tumor-propagating CSCs as a distinct minority cell population from which all other neoplastic tumor cells derive through asymmetric replication. In contrast, the capacity to dedifferentiate transit-amplifying neoplastic cells into new CSCs through autocrine/paracrine signals and endogenous transcriptional reprogramming networks points alternatively to a more dynamically regulated CSC phenotype and possibly to variable degrees of cancer cell stemness. It is currently unknown whether reprogrammed CSCs have a variable stability that is determined dynamically by the relative balance of differentiating and dedifferentiating/reprogramming signals or whether they are relatively stable and maintained through heritable epigenetic mechanisms, as are nonneoplastic iPSCs. Relatively inefficient autocrine/paracrine reprogramming stimuli acting on large populations of transit-amplifying cancer progenitors could be sufficient to generate enough therapy-resistant tumor-propagating cells to substantially affect tumor growth and recurrence. The capacity for endogenous reprogramming has an impact on therapeutic strategies designed to target the

CSC pool within tumors, because any successful strategy will have to target preexisting CSCs and inactivate the endogenous dedifferentiation mechanisms through which additional CSCs are generated. Understanding the contributions of the diverse molecular circuitries, including TF networks, stem cell miRNA expression, and epigenetic modification, will be crucial. Recent discoveries pointing to critical roles for Oct4, Nanog, and their target genes provide an early roadmap toward that end. In addition, elucidating the mechanisms of CSC formation by induced dedifferentiation may make it possible to generate in the laboratory unlimited numbers of patient-specific induced CSCs, differentiated neoplastic progenitors, and tumor xenografts for mechanistic science and cancer drug discovery.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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