

Cancer Stem Cells: A Step Toward the Cure

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This special issue of *Journal of Clinical Oncology* is devoted to the emerging field of cancer stem cells (CSCs). The goal of this article is to introduce general concepts of CSC biology to clinicians, and to provide a framework for their understanding the CSC-based approach to the development of novel diagnostics, therapeutics, and prevention strategies in oncology.

This new approach differs from the traditional one because the latter is based on the long-held concept that tumor formation and growth are due to increased proliferation of cells in cancers compared with normal tissues. This traditional view is supported by molecular oncology research during the last 50 years, which showed that tumors undergo a series of genetic events (mutations) that result in the activation or overexpression of genes promoting proliferation (eg, oncogenes), the silencing of genes involved in inhibition of proliferation (eg, tumor suppressor genes), and the development of the ability of cancer cells to elude apoptosis. The consequence is the development and unchecked growth of tumors and their progression to metastases. Based on this traditional concept of tumor growth, it has been disappointing that therapies designed to kill proliferating cells often fail to cure cancer patients.

Furthermore, during tumor development, tissues accumulate a series of mutations over years or even decades, but, because of tissue renewal, most cells in tissues are lost or eliminated in just a brief time, typically days or weeks, and with their loss, any mutations they have acquired would also be lost. Thus, new mechanisms need to be considered to explain human tumorigenesis.

One explanation is that tumorigenic mutations occur in cells that are *few* in number but reside *long term* in tissues. But that is not sufficient to explain tumorigenesis. To be truly tumorigenic, this population of rare, mutated cells would have to self-renew, clonally expand, and acquire additional mutations. It is now widely believed that these long-lived, uncommon cells are tissue stem cells (SCs) or cells derived from them that acquire the ability to self-renew. When mutated, they can become CSCs. Both normal SCs and CSCs may account for only a small fraction of cells (~1%) in any given tissue or tumor. By definition, SCs are self-renewing. Mounting evidence presented in the articles in this special issue suggests that CSCs initiate and drive tumor growth.

This is nothing less than a paradigm shift.¹ It is a shift to the view that, first, cancers originate in tissue stem or progenitor cells through dysregulation of the self-renewal process. Second, throughout tumorigenesis, CSCs drive tumor growth. Third, current chemotherapeutic agents and radiation therapy largely target proliferating and differentiated cells that form the bulk of the tumor but not the relatively

quiescent CSCs. Also, SCs are drug resistant and CSCs appear to be so too. Thus, this quiescence and resistance may account for many treatment failures. Fourth, if this is the case, then the only effective way to treat cancer is to target the CSC population.

Although this view may seem novel, the basic idea that SCs are the cells of clonal origin of malignancies has existed for at least several decades,² if not longer,³ particularly for leukemias⁴⁻⁸ and teratomas.⁹⁻¹¹ Early attempts—beginning in the 1970s—to directly measure SCs in tumors used several approaches.¹² One was mouse-spleen colony assays (eg, injection of lymphoma cells into mice with the appearance of lymphoma colonies in the spleen). A second was the counting of metastatic lung colonies after intravenous inoculation of tumor cells into mice. A third was tumor colony formation in vitro. However, this latter method produced mixed results for human tumors.¹³ SCs have also been known for decades to radiation biologists.^{14,15} More recent evidence, as presented in the articles in this special issue, supports an SC origin for solid tumors as well. In the following paragraphs, we discuss the properties of normal SCs and CSCs, and their possible roles in carcinogenesis and in mediating tumor behavior.

BIOLOGIC PROPERTIES OF SCs

The most well-known property of SC populations is their ability to self-renew. Self-renewal is particularly important in tissues with high turnover, such as the GI tract and bone marrow, as well as in tissue repair after injury. Self-renewal involves the ability of normal SC populations to precisely maintain their numbers through a combination of symmetric and asymmetric SC division.^{16,17} The property of self-renewal is also a hallmark of populations of CSCs. However, in this case, mechanisms involved in self-renewal are dysregulated and seem to lead to CSC overpopulation. The underlying mechanisms for replacing lost SCs (eg, during tissue repair) or in generating excess SC numbers (eg, in tumor development) are believed to relate to increases in symmetric SC division (which produces two SC daughters) relative to asymmetric SC division (which produces one SC daughter and one non-SC daughter). Indeed, we documented this concept quantitatively for colon cancer development using mathematical modeling.¹⁷ We showed that only increased symmetric division of cancer SCs could account for the biologic observation that there is an exponential increase in SCs, non-SC proliferative cells, and nonproliferative cell populations in the development of colorectal cancer. Our modeling also showed that only increased symmetric SC division could account

METHODS TO IDENTIFY AND ISOLATE SCs

for the known long lag phase in colon cancer development, which is typical in the development of many cancers.

Although SCs have the capacity for self-renewal, in fact they are relatively quiescent; that is, they have proliferative capacity but are often not cycling. Indeed, they have been shown to have significantly longer cell cycle times than proliferating non-SCs.¹⁸ This is presumably due to the arrest of SCs at a G₀-like cell cycle phase or checkpoint.

Another property of SCs is their potential for multilineage differentiation. This is consistent with the CSC hypothesis because cancers typically contain multiple differentiated cell types that correspond to cells in the tissue from which the cancer arose. This suggests a SC origin for these tumors because only multipotent SCs (or perhaps SC-like progenitor cells) could have given rise to these different cell types. Indeed, it has been known for more than a century that tumors are composed of heterogeneous populations of partially differentiated cell types that resemble those in the normal organ.¹⁹ This observation gave rise to the idea that tumors are functioning as aberrantly developed complex organs.²⁰ This is consistent with the concept that CSCs, like normal SCs, give rise to a hierarchical organization of cell populations that underlie organogenesis.²¹ This hierarchy includes CSCs that produce committed progenitor cells that, in turn, produce rapidly proliferating cells, finally resulting in the generation of fully differentiated cells (Table 1). This is discussed further in this issue in the articles by Sell and Leffert²² on liver CSCs and by Lee et al²³ on pancreatic CSCs.

The development of various organs during embryogenesis is known to involve specific signaling pathways. Key pathways include sonic hedgehog, Notch, PTEN, BMI-1, WNT, and p53. Indeed, there is growing evidence that these signaling pathways regulate self-renewal of normal SC populations.¹⁶ These pathways are known to become dysregulated during development of tumors, which is believed to lead to dysregulation of SC self-renewal and contribute to neoplastic proliferation.²¹

Progress in the field of SC biology, including CSC biology, has been long hampered by difficulties in identifying, isolating, and characterizing SCs. However, considerable progress has been made recently. At first, SC populations could only be identified functionally using their ability to undergo self-renewal and differentiation into multiple lineages. More recently, CSCs and even normal SCs from certain tissues have been identified and isolated. This became possible in large part due to the identification of SC markers (Table 2), their use in combination with fluorescence-activated cell sorting, and the ability to show that specific cell subpopulations form tumors in xenograft assays in immunocompromised mice.⁴ The CSCs and the tumors they form demonstrate the capacity for serial passage (transplantability) in immunocompromised mice and for generation of multiple cell lineages in a histopathologic pattern resembling the original tumor. This approach has been used to isolate CSCs from various cancer types (Table 2), as discussed in the corresponding articles in this issue. Interestingly, many CSC markers are shared across multiple tumor types. They also may be expressed on normal tissue SCs in organs from which these tumors originated.

CSCs have also been identified by in vitro sphere-forming assays.²⁴ It has been demonstrated that the ability of cells to form colonies in multicellular spheroids or spherical cell aggregates under nonadherent culture conditions is a characteristic of many cells having self-renewal capacity. These spheroids can be disaggregated and passaged multiple times with retention of sphere-forming ability. Sphere-oid formation in vitro was used to study CSCs in breast (see article in this issue by Kakarala and Wicha²⁵) and brain (see article in this issue by Dirks²⁶). The practicality, ease, and low cost of this assay makes it adaptable for performing high-throughput screens for identifying drugs that target CSCs and for measuring the effects of radiation on

Table 1. Hierarchical Organization and Properties of Stem, Proliferating, and Non-Proliferative Cell Populations

Characteristic	Stem Cell-Like Populations	Proliferating Cell Population	Differentiated Cell
	Stem cells → Progenitor cells → Rapidly proliferating cells → Terminally differentiated cells		
Hierarchical organization			
Cell populations	SCs and PCs	RPCs	Terminally differentiated, apoptotic and terminally injured cells
Population maintenance (self-renewal)	Maintenance is independent of input from other cell populations	Maintenance depends on input from the SC and PC populations	Maintenance depends on input from SC, PC, and RPC populations
Capacity for differentiation	Can produce a variety of differentiated cell types	Committed to producing specific differentiated cell types	Already differentiated
Proliferative capacity	PCs with the capacity for cell division over the lifetime of the host organism	PCs with capacity for cell division over the short term	Non-PCs with zero capacity for cell division
Fraction of overall cancer cell population	Small (~1%)	Large	Large
Proliferation status	Cells that are quiescent or slowly proliferating	Rapidly proliferating	Non-proliferating
Role in tissue renewal	Continually drives tissue renewal	Amplifies population size during tissue renewal	Zero capacity for tissue renewal
Role in tissue generation	Responsible for tissue generation during embryonic development and regeneration after injury	Amplifies population size during tissue generation	Zero capacity for tissue generation during development and regeneration after injury
Sensitivity to chemotherapy and radiation	Resistant	Sensitive	Sensitive

Abbreviations: SCs, stem cells; PCs, progenitor cells; RPCs, rapidly proliferating cells.

Table 2. Markers to Identify and Isolate Stem Cells From Various Malignancies

Cancer	Stem-Cell Markers										
	CD44	CD24	CD133	ALDH1	ESA	B1	α6	CD138	CD34	CD166	CD20
Breast	+	–	+	+	+	+	+				
Colon	+		+	+	+					+	
Prostate	+		+	+		+	+				
Head and neck	+			++†							
Pancreatic	+	+	+	+	+						
Lung			+								
Brain			+								
Liver			+								
Melanoma	+		+			+					+
Multiple myeloma				+				–	+		+
Leukemia	+			+					+		

NOTE. Definitions for the human CD (cluster destination) molecules are: CD44, hyaluronate receptor (p-glycoprotein 1); CD24, heat stable antigen; CD133, prominin 1; ALDH1, aldehyde dehydrogenase 1A1; ESA, epidermal surface antigen (Flotillin/2); B1, integrin B1 chain; α6, integrin α6 chain (CD49F); CD138, heparin sulfate proteoglycan fibroblast growth factor receptor (syndecan proteoglycan 1); CD34, hematopoietic progenitor cell antigen (GP105-120); CD166, activated leukocyte cell adhesion molecule; CD20, B-lymphocyte cell-surface antigen B1, membrane-spanning 4-domains, subfamily A, member 1.

*Unpublished data: BM Boman, MA Wicha, and E Huang.

†Unpublished data: M Prince and MS Wicha.

CSCs. Targets can be validated using more labor-intensive xenograft models.

ROLE OF CSCs IN CANCER INITIATION, PRIMARY TUMOR GROWTH, AND METASTASES

Our current understanding of human carcinogenesis involves two central ideas. One is that most, if not all, cancers result from the accumulation of specific genetic mutations. The other is that tumorigenesis involves aberrant tissue organization and cell proliferation. The CSC paradigm begins to suggest how these two events are linked. That is, it provides a cellular mechanism that can explain how the genetic and epigenetic changes give rise to the tissue changes. Moreover, evidence suggests that these mechanisms may be operational in all phases of tumorigenesis: initiation, progression, invasion, and metastasis. The idea that cancer initiation involves development of CSC overpopulation was supported by an analysis of tissues from hereditary cancer patients.^{27,28} For fully developed cancers, support comes from recent reports that many cancers contain a small but significant proportion (~1%) of CSCs.²⁹⁻³⁵ Given that the total number of cells is exponentially increased (up to 10⁸ to 10¹³ cells) in fully developed cancers, it can be deduced that the size of the SC subpopulation in those tumors is also exponentially increased. Indeed, this must have occurred during all phases of tumorigenesis.¹⁷ The progressive increase in the CSC population throughout tumorigenesis in the colon will be discussed in this issue in the article by Boman and Huang³⁶ on colon cancer SCs. Regarding metastases, there is a growing body of evidence that suggests that metastases develop when distant organs are seeded with CSCs that arise from a primary tumor.³⁷ This implicates CSCs in the seeding and growth of metastatic lesions. This mechanism is discussed, in this issue, in the article on breast CSCs by Kakarala and Wicha.²⁵

CSCs IN CANCER DIAGNOSIS, TREATMENT, AND PREVENTION

As noted, current systemic cancer therapies frequently fail to eradicate advanced tumors. Failure of these therapies to effectively target cancer SCs may account for this failure.³⁸ First, most current agents destroy rapidly

proliferating cells—cells that represent the bulk of tumor cell populations. If cancer SCs drive tumorigenesis, current chemotherapeutic approaches may be ineffective because cancer SCs, like all SCs, are relatively quiescent; that is, they proliferate only infrequently. Second, SCs tend to be more resistant to chemotherapeutic agents and radiation than are more mature cell types from the same tissue. This is believed to be due to the presence of multidrug resistance, antiapoptotic proteins, and enhanced DNA repair mechanisms. Since this appears to hold true for cancer SCs, we would predict that they would also display resistance to therapeutics. This is discussed, in this issue, in the article by Eyler and Rich.³⁹ Third, most current therapies do not target the signaling pathways that regulate self-renewal, which appear to be either mutated or epigenetically dysregulated. These pathways, including sonic hedgehog, Notch, PTEN, BMI-1, WNT, and p53, are discussed in this issue in the article by Fan and Eberhart.⁴⁰ There is evidence that these pathways are involved in regulation of self-renewal, and effective therapies will need to target symmetric SC division.¹⁷ Targeting these pathways, it should be noted, will probably not be enough—an ideal agent would have to selectively target cancer SCs over normal SCs. Without this selectivity, the effectiveness of treatment might be limited by systemic toxicity.

Control of SC numbers would also be important in cancer chemoprevention. The ideal cancer chemopreventive agent would also selectively target CSCs over normal SCs. This suggests that the development of agents that eliminate or control CSC populations may be an effective strategy for cancer prevention as well as the development of more effective cancer therapies. Indeed, experiments using antibodies against an CSC marker, CD44, eliminated human acute myeloid leukemic SCs in immunodeficient mice transplanted with human acute myeloid leukemia.⁴¹

It is also likely that treatment of patients with CSC-targeted therapeutics will require new clinical end points for monitoring therapeutic efficacy. This is the case because one is targeting only a small fraction of cells within the tumor, not the bulk of the tumor. In addition, responses may take a much longer time than is typically seen with agents that are cytotoxic to the rapidly dividing cells that constitute the bulk of the tumor. Rational approaches might also include

using cytotoxic chemotherapies to target the proliferating bulk of the tumor in addition to a CSC-directed therapy directed at eliminating this critical cell population. An important end point will likely involve assessing changes in the size of the CSC population in response to treatment. One way to do this might be by monitoring the burden of CSCs in the circulation. Of course patient survival remains the ultimate clinical end point, which will involve appropriate clinical trials.

Markers for CSCs could be used for predicting treatment responses by identifying the presence of specific CSC subtypes that are selectively sensitive to specific agents, particularly biologic agents such as antibodies against specific CSC targets. Microarray and proteomic profiling of CSCs will likely lead to identification of new markers, as well as potential therapeutic targets. Indeed, a recent microarray study identified a genetic signature associated with the BMI-1 driven SC self-renewal pathway.⁴² Other gene expression studies have identified genes associated with prognosis in breast cancer, and these signatures contained the aldehyde dehydrogenase 1 (ALDH1) SC marker.^{43,44} This approach to identify new markers is discussed, in this issue, in the article by Glinsky.⁴⁵ CSC markers may have prognostic value by allowing assessment of the size of the CSC population within any given tumor. Early evidence suggests that the greater the size of the CSC population, the poorer the prognosis, as reflected by tumor aggressiveness and/or patient survival. This is discussed, in this issue, in the article by Kakarala and Wicha.²⁵

MATHEMATICAL MODELING OF CSC DYNAMICS AND THERAPEUTIC APPROACHES

Mathematical modeling will be especially useful in understanding the role of CSCs in cancer development and growth, as well as response to therapy. For example, modeling provided a quantitative basis for the ideas that CSCs drive tumor growth, curative anticancer therapy must eradicate or control CSC populations,^{28,46} and that future therapeutic approaches need to target symmetric division of CSCs.¹⁷

Future modeling studies will be important because SCs are involved in homeostasis of normal tissues, which are complex in their structure and in their dynamics, and which involve many interacting cellular pathways. Moreover, the development of tumors is a complicated biologic process, and this will add to the overall complexity. Indeed, a recent article advocates the use of mathematical studies on SCs as a requisite to advance the field.⁴⁷

Mathematical modeling will also be required to understand responses to treatment when using anti-CSC therapies, especially in predicting how treatments might affect the dynamics of tumor growth and overall tumor responses to treatment. This is exemplified by recent modeling studies that have provided explanations for the role of CSCs in development of resistance to imatinib therapy in patients with chronic myeloid leukemia.⁴⁸⁻⁵¹ Such modeling will also likely be useful in developing treatment and dose schedules, as well as criteria for

monitoring therapeutic responses. The use of mathematical modeling is discussed further throughout this issue, especially in the article by Michor.⁵²

OVERTURE TO THIS SPECIAL ISSUE

To fully appreciate the CSC-based approach to cancer treatment, diagnosis, and prevention requires a discussion of different cancer types and the CSCs that drive tumor growth in each type. The rest of this *JCO* special issue is devoted to that end. This includes 13 articles that focus on the roles SCs may play in each type of malignancy. Together, these articles illustrate the commonality of CSCs in multiple tumor types. This suggests that approaches involving similar techniques and molecular markers may be applicable across tumor types. Furthermore, because pathways of CSC self-renewal and cellular survival also seem to be preserved across different tumor types, strategies designed to target CSCs may also have broad clinical utility. This suggests the need to develop pathway-based rather than tumor type-based approaches to CSC-based therapy. This issue also includes five articles covering select research and clinical topics related to CSCs and clinical oncology, including molecular profiling and biomarkers, mathematical models, chemotherapy resistance, radiation resistance, and imaging approaches. All of these articles provide evidence that SCs or their self-renewing progeny may be the cells of origin of specific cancer types, and demonstrate that significant progress has been made in identifying and characterizing each of those CSC populations. The goal of this issue is to inform clinicians and clinical investigators about progress in the field of CSCs. If the hypotheses central to this emerging field prove to be correct, then they represent an important paradigm shift in our understanding of carcinogenesis. The great promise of this work is that it may lead to more effective strategies for cancer prevention and treatment. Given that CSC-targeting agents are now entering clinical trials, we will soon have an indication as to whether this is the case.

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