Are Cancer Stem Cells Ready for Prime Time?
A flood of new discoveries has refined our definition of cancer stem cells. Now it’s up to human clinical trials to test if they can make a difference in patients. The Scientist. Suling Liu, Hasan Korkaya, and Max S. Wicha | April 1, 2012

In the 30-year battle waged since the initiation of the “war on cancer,” there have been substantial victories, with cures for childhood malignancies among the most important. Our ever-expanding understanding of cellular and molecular biology has provided substantial insights into the molecular underpinnings of the spectrum of diseases we call cancer. Yet, while researchers view this as tremendous progress, many patients have seen only limited improvement. In fact, the relatively modest gains achieved in treating the most common malignancies have caused some to say that we are actually losing the war on cancer.¹

Based on new intelligence, oncologists are making informed battle plans to attack a particularly pernicious enemy—the cancer stem cell. Controversial though they are, cancer stem cells are an incredibly promising target. If treatment-resistant cancer, and the metastases that transplant the cancer throughout the body, could be attributed to the actions of a single cell type, it could explain many of the treatment failures and provide a novel way to attack the disease.

The idea that cancers are driven by cells with “embryonic features” is an old one. Many cancers regress to a less differentiated state, expressing proteins that are usually expressed only in the embryo or during early development. It is only in the past 20 years or so, however, that additional observations led to the hypothesis that these embryonic-like cells were a separate subpopulation that fuelled tumor expansion, much the same way that stem cells churn out the cells that make up a particular organ.

A number of groups, including our own, have identified cancer stem cell markers enabling the isolation and characterization of these cells. In addition, the development of in vitro and mouse functional assays has led to a veritable explosion of research on cancer stem cells from both blood-derived malignancies and solid tumors.²³ However, the limitations of these markers and assays have generated heated debate regarding which tumors follow a stem cell model, and which do not. New data from our lab and from others is helping to clarify some of these areas of debate with the goal of better understanding how these cells can be identified and characterized.
Clarifying the debate

A cancer stem cell (CSC) is defined as a cell that has the ability to self-renew, dividing to give rise to another malignant stem cell, as well as to produce the phenotypically diverse, differentiated tumor cells that form the bulk of the tumor. Evidence for CSCs was first documented in leukaemia, where it was clear that only a small subset of cancer cells was capable of perpetuating the cancer upon serial transplantation from one mouse to another. Extensive knowledge of normal blood stem cells facilitated our recognition and understanding of leukaemia stem cells. Evidence for CSCs in solid tumors has been more controversial, because it is more technically challenging to divide a solid mass into individual cells without damage or alteration, and knowledge of the properties of normal-tissue stem cells in these organs is more limited. However, some of the areas of contention may be resolved by continuing research into the biology of these CSCs.

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One of the points of confusion in CSC biology is the question of where these cells come from. Do they arise from normal stem cells that have become cancerous through mutation, or do they arise from partially differentiated tissue-progenitor cells that have acquired the ability to self-renew? Recent evidence suggests CSCs may arise from either source.

A second misconception is that the definition of CSCs precludes the possibility that cancers arise from sequential mutations that accumulate over many cell generations and are selected for through a Darwinian process—the so-called “clonal evolution model.” Some have proposed that the “CSC model” is a competing theory of carcinogenesis. In fact, both models may be correct. There is evidence that CSCs may also be genetically unstable, resulting in clonal evolution that generates several distinct CSC clones in a tumor.

While the identification of CSC markers and the development of in vitro and mouse models have led to important advances in the field, each of these markers and models has limitations that have fueled debate. Markers used to isolate cancer stem cells, such as CD44, CD24, CD133, aldehyde dehydrogenase (ALDH), and Hoechst dye exclusion, have proven useful for identifying these cell populations in tumor samples. However, expression of these markers is highly dependent on experimental conditions such as culture medium and oxygen concentration. Similarly, in vitro assays that rely on the ability to form spherical colonies in suspension can be useful, but are notoriously inaccurate. Since the definition of CSCs is ultimately an operational one, the most reliable assay for these cells has been their ability to initiate tumors when transplanted into mouse models. Because the immune system will reject any implanted foreign tissue, researchers have had to use immunosuppressed mice to test for human CSCs. In some tumor types, such as melanoma, the proportion of cells capable of initiating tumors is dependent on the degree of immunosuppression in the mouse models utilized. However, the more immunosuppressed mouse models may actually overestimate the true frequency of CSCs.

Recent studies have indicated that CSCs have the ability to evade immune surveillance, even when the same immune cells can detect and destroy bulk tumor cells. If this is truly the case, then highly immunosuppressed models of cancer may not reliably simulate the behaviour of the immune system in the microenvironment of a patient’s tumor. Indeed, CSCs isolated from transgenic mouse tumors have been transplanted successfully into mice with intact immune
systems, demonstrating the existence of CSCs and providing further support for relevance of these cells in patients.

Another misconception is that the cancer stem cell hypothesis requires that CSCs be rare. In fact, studies suggest that the percentage of CSCs may vary significantly in different types of cancer, as well as within each cancer type. In acute leukaemia, CSCs appear to be rare, constituting less than 0.1 percent of the total cancer cell population. In some solid tumors, such as breast cancer, their numbers are reported to represent approximately 1–10 percent, while in some tumors, such as melanoma, they may be even more common, leading some investigators to propose that some cancers follow a stem cell model while others don’t. While this is debatable, identifying populations of CSCs in tumors in which these cells are abundant, or in the majority, may be of less importance, since any effective therapy would primarily target the CSCs.

**Developmentally informed**

Some researchers have focused on defining CSCs from a genetic and developmental perspective. Researchers including Robert Weinberg and colleagues from the Whitehead Institute for Biomedical Research have noted that when cancer cells adopt a genetic program responsible for the epithelial-to-mesenchymal transition (EMT), they convert to a CSC phenotype.\(^4\) EMT is known to developmental biologists as the transition from a non-motile, epithelial-like cell to one that can detach from the surrounding tissue and migrate. Cellular migration is important in development, and it is also a defining characteristic of aggressive tumors that metastasize to new sites in the body. Both inflammatory immune responses and a hypoxic tumor microenvironment induce EMT in cancers. It is also increasingly recognized that EMT plays an important role in therapeutic resistance.\(^5\)

![Infographic: The Two Faces of Metastasis](image)

**Infographic: The Two Faces of Metastasis**

In contrast, other studies have suggested that the EMT state, although associated with tumor invasion, is characterized by cellular quiescence, or an inability to replicate, creating a paradox. How can cells which are associated with aggressive metastatic behaviour be quiescent? Recent observations by our group and others have suggested an additional mechanism that could explain both observations: CSCs may in fact flip-flop between an EMT state and its converse, the mesenchymal-to-epithelial transition (MET), in which cells re-attach to the matrix and become highly proliferative, thus generating tumors at sites of metastasis.
These results suggest that CSCs, such as those found in breast cancers, have plasticity and can exist in two alternative states: an EMT-like state of CSCs expressing surface markers CD44 but not CD24 (CD44⁺CD24⁻), and an MET-like population expressing the CSC marker ALDH. Previous studies taken together with our current work suggest that CSCs located inside the primary tumor mass exist predominantly in the MET state in which they are highly proliferative and express ALDH. In contrast, tumor cells that migrate into the circulation and metastasize are characterized as CD44⁺CD24⁻—highly invasive but quiescent EMT CSCs.

This scenario is supported by studies showing that in women with breast-cancer-derived, bone micrometastases express the EMT CSC markers CD44⁺CD24⁻. These micrometastases are largely quiescent, as indicated by their lack of expression of markers of cellular proliferation such as Ki67. In order to enter a proliferative state, EMT CSC cells must undergo an MET transition in which they lose their invasive characteristics and acquire self-renewal capacity.

Clinical implications of cancer stem cell models

The effectiveness of the majority of cancer chemotherapeutic agents has been judged by their ability to cause tumor regression, as ascertained by direct measurement or through radiographic imaging. Since tumor size is largely determined by bulk cell populations, however, it follows that tumor regression reflects changes in this population rather than in the rarer CSCs, which may be the real drivers of tumor growth and metastasis. This could explain why in many cancers tumor regression does not translate to increased patient survival. There is substantial evidence in preclinical models that most CSCs are relatively resistant to chemotherapeutic agents and radiation therapy. In addition, CSCs may display resistance to molecularly targeted therapeutics. For example, one of the greatest advances within targeted therapeutics has been the development of imatinib (Gleevec) for chronic myelogenous leukemia (CML). Almost all patients treated with this molecularly targeted tyrosine kinase inhibitor enter clinical remission. However, disease quickly recurs following discontinuation of the drug, and CML cancer stem cells have been demonstrated to be resistant to this agent. This has led to experimental approaches that target CSCs using agents such as sirtuin inhibitors or interferon.

Another targeted therapy is trastuzumab (Herceptin), the development of which has represented a major advance in therapy for breast cancers that overexpress the human epidermal growth factor receptor 2 (HER2). Unfortunately, only some 20 percent of women with breast cancer have this genetic alteration. In 2008, however, researchers from the University of Pittsburgh published clinical trial results showing that women who were HER2-negative also appeared to benefit from treatment when the drug was part of the chemotherapy cocktail given after surgery to prevent recurrence. This puzzling finding could have huge implications for the majority of breast cancers that are currently not being treated with the drug. When we looked further into HER2 expression patterns, we found that this receptor also increased the self-renewal of breast CSCs.

This may provide an explanation for the remarkable efficacy of the drug, which blocks HER2. Our ongoing preclinical studies indicate that trastuzumab can kill the CSCs in tumors that express this receptor only on their CSCs, and would thus be classified as HER2-negative.

New Horizon for Cancer Treatment
The cancer stem cell (CSC) hypothesis offers explanations for many of the frustrating failures of cancer therapy in the clinic. The resistance of CSCs to chemotherapy, radiation, and many targeted therapies, may explain why cancers come back after the tumor mass has been removed and the patient has gone into remission. As such, CSCs offer a new target for attack.

**Chemo catch-22:** Although chemotherapy is still considered to be the most effective treatment for many cancers, the drugs may act on a tumor’s surrounding tissue in a way that spurs the production of more stem cells. In fact, increases in CSC numbers have been observed in tumors after chemo or radiation. These treatments can create inflammation in the tissue surrounding the tumor as well as hypoxia, or loss of oxygen, which activates Wnt signaling. Inflammatory mediators such as IL-8, IL-6, and Wnt signaling spur CSCs to self-renew or increase in number, thus driving tumor growth.

Antiangiogenic agents are another treatment whose administration may need to be rethought in light of what we now know about CSC biology. The development of antiangiogenic agents such as bevacizumab (Avastin) and sunitinib (Sutent) represented an area of significant promise in cancer. However, recent clinical trials have produced relatively disappointing results. Although these agents delay tumor progression, they do not significantly increase patient survival. Our group has demonstrated that in mouse models, these antiangiogenic agents actually increase CSC populations through generation of tumor hypoxia, or low oxygenation, which drives the proliferation of CSCs by triggering the Akt and Wnt pathways. This suggests that, to be clinically effective, these agents may require additional therapies capable of targeting CSC populations.

Over the past decade, a number of developmental pathways that regulate the self-renewal of normal stem cells have been elucidated. These include the developmental pathways Wnt, Notch, and Hedgehog, and the cell division and proliferation pathways PI-3K, NF-kB and Jak/STAT. Interestingly, these pathways are dysregulated in many human cancers, leading to uncontrolled self-renewal of CSCs. These pathways may provide excellent targets for developing drugs against CSCs.

In addition to the regulation of CSCs by intrinsic signals, elements in the tumor microenvironment or niche also play a role in regulation of the stem cells. In tumors, this niche contains a variety of cellular elements, including inflammatory cells, fibroblasts, endothelial cells, and mesenchymal stem cells. Iterative crosstalk between cancer stem cells, their differentiated progeny, and the microenvironment regulates cellular function through paracrine cell signaling. Some of these interactions include the Wnt, Notch, and Hedgehog pathways. In addition, inflammatory cells, fibroblasts, and mesenchymal stem cells may interact with CSCs and increase their production and replication via cytokine loops. Several inflammatory cytokines, including IL-6 and IL-8, have been demonstrated to increase breast cancer stem cell self-renewal in mouse models and in vitro. In addition, chemotherapy-induced cellular...
toxicity increases local IL-8 production, which may contribute to increased cancer stem cell populations following chemotherapy. High serum levels of IL-6 and IL-8 in patients with advanced breast cancers have been associated with development of metastasis and poor outcome. These studies suggest that developing strategies to interfere with these loops may provide a novel way to target CSCs. Interestingly, statins, which have anti-inflammatory effects, have been reported to decrease breast cancer risk.

We have also recently demonstrated that blocking IL-8 with antibodies or drugs targets breast CSCs in mouse models and inhibits tumor growth and metastasis. Repertaxin, a drug that blocks the IL-8 receptor, was developed to prevent graft rejection and has been reported to be relatively nontoxic in phase I clinical trials. We have recently begun a clinical trial combining Repertaxin with chemotherapy in women with advanced breast cancer.

In the past 5 years there has been an exponential increase in CSC research. This research has helped to resolve a number of controversies regarding identification of these cells and their role in driving tumor growth and mediating treatment resistance. Despite these advances, the CSC field is still in its relative infancy, and many questions and challenges remain. More than a dozen biotechnology and pharmaceutical companies are now vigorously pursuing CSC research. As a result, a number of early-phase clinical trials targeting CSCs are in progress. These studies and the later-stage efficacy trials that follow them should indicate whether successful targeting of CSCs significantly improves outcomes in cancer patients. If this is found to be the case, it may usher in the beginning of a new era of cancer therapy.

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References


