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The Cancer Stem Cell Hypothesis: Challenges and Implications

Over the past ten years, the cancer stem cell (CSC) hypothesis has produced an enormous amount of interest, optimism, and debate in the cancer research community. The biological principle at the heart of the CSC hypothesis is simple: in cancers there exist a small, distinct population of cells, "cancer stem cells", that have the ability to self-renew. Consequently, these CSCs continually replenish tumors and sustain their growth, even after traditional cancer treatments like chemotherapy, radiation, and surgery. Thus, optimistically, if scientists are able to identify true CSCs, they may be able to develop novel clinical therapies that irreversibly destroy cancer at its source (1).

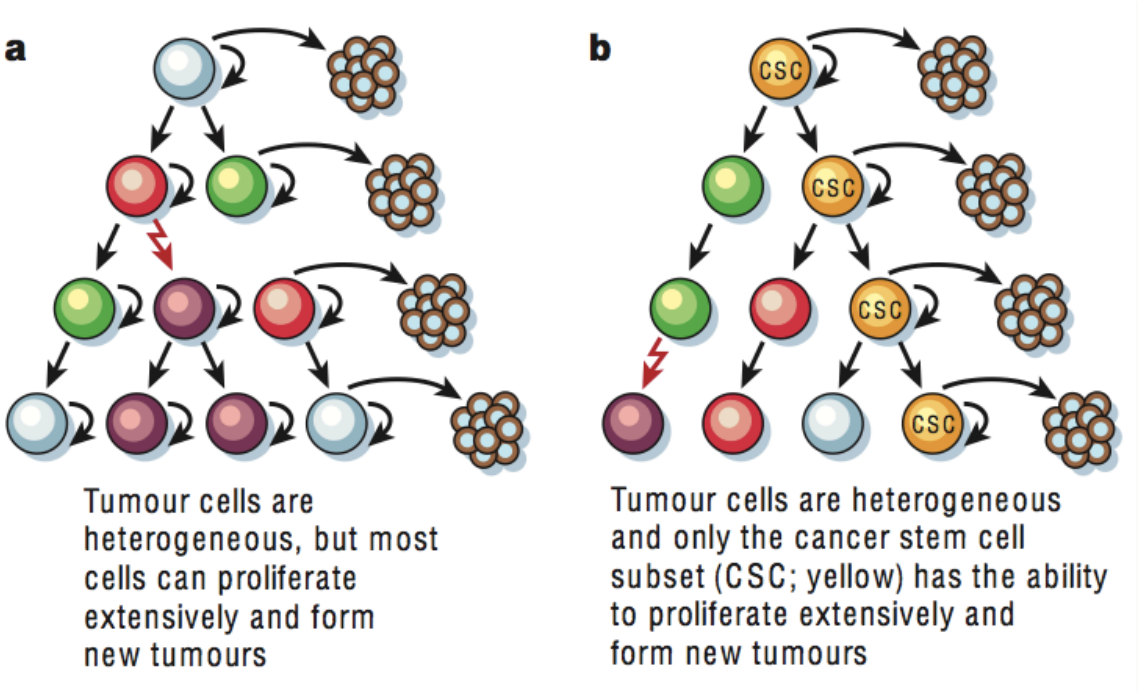
While the conceivable clinical importance of the CSC hypothesis is compelling for many, there is not universal agreement on the value or even existence of CSCs. Nevertheless, a thorough analysis of recent literature does indicate that the CSC model rests on solid experimental foundations. Important areas of current CSC research include studying if all forms of cancer have CSCs, understanding the mechanisms by which CSCs renew, and learning which genes are mutated or used differently in CSCs. The ultimate goal for CSC researchers is to eliminate CSCs in humans without interfering with normal, tissue-specific stem cells that work to replenish the brain, bone marrow, intestines, skin, and other organs. By specifically targeting CSCs, cancer therefore would effectively be prevented from relapsing and metastasizing, drastically improving health outcomes for chronically ill patients.

First and foremost, it is important to outline current treatment strategies of cancer and their limitations in order to examine in detail the contribution that novel CSC-based therapies may have on the field. The most common forms of cancer treatment are chemotherapy, radiation therapy, and a variety of surgical procedures, which are selected from based on the form of cancer a patient has and the stage to which it has developed. Chemotherapy, first used in the 1950's, is the administration of powerful drugs and chemical agents to kill cancer cells in mass. While often effective in reducing tumor volume, chemotherapy is unable to distinguish between cancer cells and potential CSCs. Additionally, chemotherapy, due to its lack of specificity, can cause destruction of other healthy, fast-growing cells like hair and blood cells. Another important disadvantage of chemotherapy is the large number of uncomfortable side effects associated with treatment, including hair loss, nausea, immunosuppression, and infertility. Radiation therapy or radiotherapy is the medical use of ionizing radiation targeted at tumor sites to destroy cancer cells. Like chemotherapy, radiotherapy cannot differentiate between cancer cells and potential CSCs and can lead to its own set of negative side effects, including radiation-inducing pneumonitis (a pneumonia-like condition) and fibrosis. Finally, surgical procedures attempt to physically extricate tumors from patients. However, often due to lack of access (especially if the cancer has metastasized) surgery is ineffective (2).

Each of these treatment options attempts to destroy or remove tumor cells arbitrarily without seeking to identify potential source CSCs. As a consequence, chemotherapy, radiotherapy, and surgery all, despite shrinking or apparently destroying tumors, are associated with tumor recurrence at a high, unpredictable frequency. For this

reason, CSC therapies are promising, as they would solve the widespread problem of tumor relapse following treatment.

With respect to the validity of the CSC hypothesis, the scientific evidence of the existence of CSCs in various cancers has accumulated significantly in recent years. CSCs are tumor-driving cells that are like regular stem cells in that they are able to both differentiate into more cells like themselves and also into various cell types that presumably do not have stem cell properties. Stem cells have the capacity to undergo unlimited cell divisions. What distinguishes CSCs from normal adult stem cells, however, is that the cells that comprise cancerous tissue have lost homeostatic mechanisms that maintain normal cell numbers. Therefore, normal tumor cells can proliferate extensively and form new tumor cells, but only CSCs are able to give rise to new CSCs. See figure taken from Reya *et al* (3):



Bonnet and Dick published the first evidence of the existence of CSCs in 1997 (4). In their experiment, fluorescence-activated cell sorting (FACS) was utilized to isolate cells from acute myeloid leukemia (AML), a form of cancer with a low survival rate in humans, and these cells were found to be able to initiate leukemia upon transplantation into immunodeficient mice. The cells had two markers that are now synonymous with cancer stem cells, specifically the high expression of antigen CD44 and the low expression of antigen CD24. This "xenotransplantation" approach is now widely accepted as a necessary criterion for defining CSCs. Results indicated that the percentage of CSCs in AML was on the order of 1 out of every 10,000 cancer cells. By discovering such a low percentage of CSCs, Bonnet and Dick thus provided an explanation for why traditional cancer researchers had failed to generate tumors in experimental animals in the past, even after the introduction of many thousands of cancer cells. These past experiments had failed to transport the necessary, tumor-causing CSCs and thus no tumor had grown (4).

An issue of debate has been explaining the observed rarity of cancer occurrence in mice following transplantation of human cancer cells. On one hand, as Bonnet and Dick postulated, the reason why not all cancer cells could form cancer in mice was directly attributed to these cells not being CSCs, which are the only cell form that can give rise to tumors independently. In 2007, however, a research group led by Andreas Strasser countered that it was not the rarity of tumor-initiating cells that was being observed, but rather the effects of interspecies environmental interactions that explained why cancer cells so rarely lead to the proliferation of tumor cells (5). Therefore, the large amount of evidence generated using the xenotransplantation approach was potentially null, given it

had been carried out by transplanting human cancer into mice models. In other words, the instances in which transplanted cancer cells led to tumor formation in mice (previously explained as caused by the transplantation of CSCs), were in reality just a reflection of the chance instances at which human cells had been able to grow in mice: human cells of any form are only rarely able to grow in mice (6).

One counter argument to Strasser's theory, that it is host environment and not cell type that explains the rarity of cancer occurrence following transplantation, is that CSCs do not necessarily need to be rare. Consequently, the most aggressive forms of cancer can actually be due to the presence of a large percentage of CSCs in the total tumor volume (6). Nevertheless, Strasser's work dos highlight the importance of playing close attention to the environment in which scientific experiments are conducted, particularly if it is nonhuman, since discovering the existence of true CSCs in humans is the ultimate goal. With regards to choosing an appropriate animal model to measure CSC representation, the ideal model should, therefore, match to the best extent as possible CSC behavior in humans.

Within the past ten years, researchers have also produced abundant evidence of CSCs that drive solid tumors using xenotransplantation approaches. In 2003, Al-Hajj *et al* isolated tumorigenic cells from human breast carcinoma via FACS to purify populations of cells able to form tumors in immunodeficient mice (7). Since the work of Al-Hajj *et al*, a variety of solid tumor stem cells have been discovered, including cancer of the brain, colon, intestines, ovary, pancreas, and prostate. Interestingly, in the case of brain cancer, CSCs were found to confer higher resistance to radiation as compared to normal cancer cells, further explaining the occurrence of relapse of cancer following radiotherapy. The

wide range of forms of cancer in which CSCs are present has large clinical implications, as now researchers have the ability to focus treatment strategies of patients with a variety of cancers on a specific subset of cells rather than on all cells present within the tumor. Furthermore, given the greater understanding of CSCs in a variety of forms of cancer, the longstanding problem of treating metastasized cancer now has the potential to be addressed.

With respect to the origins of CSCs, tumor-driving cells seem to arise from when mutations or epigenetic changes arise in normal adult stem cells, which already have an innate ability to divide and differentiate into various cell forms. In CSCs, the internal controls that keep in check the cells rates of proliferation are no longer controlled. These controls are present in the stromal niche, a grouping of supporting cells and substances in which stem cells reside (6). Therefore, some argue CSCs are likely normal cells that were previously tightly regulated but no longer are. Another possible explanation for the origin of CSCs is that they may represent specialized cells from adult tissues that have acquired a stem-cell-like state through a series of mutations.

In either case, it is has recently been observed experimentally that CSCs are less expendable and better protected than their more abundant and replaceable tumor cell counterparts. These studies help explain why current cancer treatments like chemotherapy, radiotherapy, and surgery are often ineffective in preventing tumor relapse. To demonstrate this idea using an individual case study, in 2009, an investigation performed at the Stanford Institute for Stem Cell and Regenerative Medicine led by researchers Maximilian Diehn and Robert Cho found an association between reactive oxygen species (ROS) levels and radioresistance in cancer stem cells (8). Specifically, it

was shown that similar to normal tissue stem cells, subsets of CSCs in some tumors contain lower ROS defenses compared to their non-tumor-causing progeny, which may contribute to tumor resistance from radiotherapy. Protection of CSCs from radiation therefore was attributed to increased expression of proteins that bind to and deactivate ROS. As a result, CSCs were reported to be about twice as likely as other tumor cells to survive a course of ionizing radiation. Suggestive of the conceivable clinical implications of this research, blocking the activity of an important antioxidant, glutathione, made CSCs more susceptible to ionizing radiation and cell death (8).

As further evidence of the implications of the CSC hypothesis, large drug makers are taking increased interest in CSC therapies. In 2008, GlaxoSmithKline collaborated with OncoMed Pharmaceuticals of Redwood City, CA to license 4 antibody candidates that target CSCs. As of 2008, there are 40 companies devoted to CSC research and development and that number has likely risen dramatically to date (9).

In the design of new drugs that work to target CSCs, it is necessary to understand the cellular and genetic mechanisms that regulate cell proliferation. Work has begun to develop genetic signatures and markers characteristic of stem cells. For example, *Bmi-1*, *Tie-2*, *Shh*, *Notch*, and *Wnt/β-* are genes and signaling pathways that have important regulatory functions for stem cells. Using microarray and genome-wide techniques, trends in genetic and epigenetic blueprints for cancer stem cells can be identified. However, to detect true signatures, pure populations are necessary. For rare CSCs, this is especially needed, given their expression levels would be much smaller than that of surrounding non-CSCs that make up the tumor. Even if a CSC-specific signature is identified, it must be validated by a functional assay (such as an *in vivo* self-renewal

assay) before the signature can be deemed useful in identifying CSCs in different tumor types (10).

In evaluating the potential for CSCs to revolutionize cancer therapy in the upcoming years, there are still major barriers that CSC researchers need to overcome. The first important barrier is the animal models that researchers utilize when attempting to understand what cancer cells to target as CSCs. While animal studies have proved useful in the development of cancer treatments in the past, they do not provide a complete model of human disease. In particular, since the CSC hypothesis has implications to address the issue of the recurrence of tumors following traditional treatment, often-utilized mice are often of insufficient use to CSC researchers because their life span is generally less than two years, which is not directly relatable to the life cycle of cancers in humans (11).

Additionally, the fact that the CSC hypothesis suggests that CSCs comprise a small percentage of total tumor cells serves as an impediment to CSC-based drug development. The efficacy of cancer treatments during the initial stages of testing is often measured by the amount of tumor mass they are able to kill off. As a result of the relatively small mass of CSCs, chemotherapies that kill a large number of cells indiscriminately but leave CSCs untouched are preferentially selected for during drug testing.

In spite of these challenges, in the end, the CSC hypothesis still remains a source of hope for patients of cancer that suffer from recurrent tumors and metastasis of their cancer – both areas where little progress has been made in the past few decades. While many in the medical community remain cautious about embracing the CSC hypothesis,

more studies that confirm the existence of CSCs and explain their origin and mechanistic behavior will undoubtedly lead the debate forward. In the end, the true test for CSC researchers will be to demonstrate unequivocally that destroying identified and specifically targeted CSCs will improve the survival outcomes of human patients with cancer.

Works Cited:

1. Gupta et. al., Cancer stem cells: mirage or reality?. *Nature Medicine*. 2009; **15**: 1010-1012.
2. "Types of Treatment" *National Cancer Institute at the National Institutes of Health*. Retrieved December 4, 2011 from URL: http://www.cancer.gov/cancertopics/treatment/types-of-treatment
3. Reya et. al., Stem cells, cancer, and cancer stem cells. *Nature*. 2001; **414**: 105-111.
4. Bonnet D, Dick J., Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nature Medicine*. 1997; **3**: 730-737.
5. Kelly et. al., Tumor Growth Need Not Be Driven by Rare Cancer Stem Cells. *Science*. 2007; **317**(5836): 337.
6. Goldman B., "Cancer stem cell sightings and slightings" *Nature Reports Stem Cells*. 2007. Retrieved December 5, 2011 from URL: http://www.nature.com/stemcells/2007/0709/070927/full/stemcells.2007.93.html
7. Al-Hajj et. al., Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci*. 2003; **100**(7): 3983–3988.
8. Diehn et. al., Association of reactive oxygen species levels and radioresistance in cancer stem cells. *Nature*. 2009; **458**(7239): 780-3.
9. Schmidt C., Drug makers chase cancer stem cells. *Nature Biotechnology*. 2008; **26**: 366-367.
10. Clarke et. al., Cancer Stem Cells—Perspectives on Current Status and Future Directions: AACR Workshop on Cancer Stem Cells. *Cancer Res*. 2006; **96**(9339).
11. Tan et. al. The cancer stem cell hypothesis: a work in progress. *Laboratory Investigation*. 2006; **86**: 1203–1207.