

Discovering and Exploiting Cancer Stem Cells

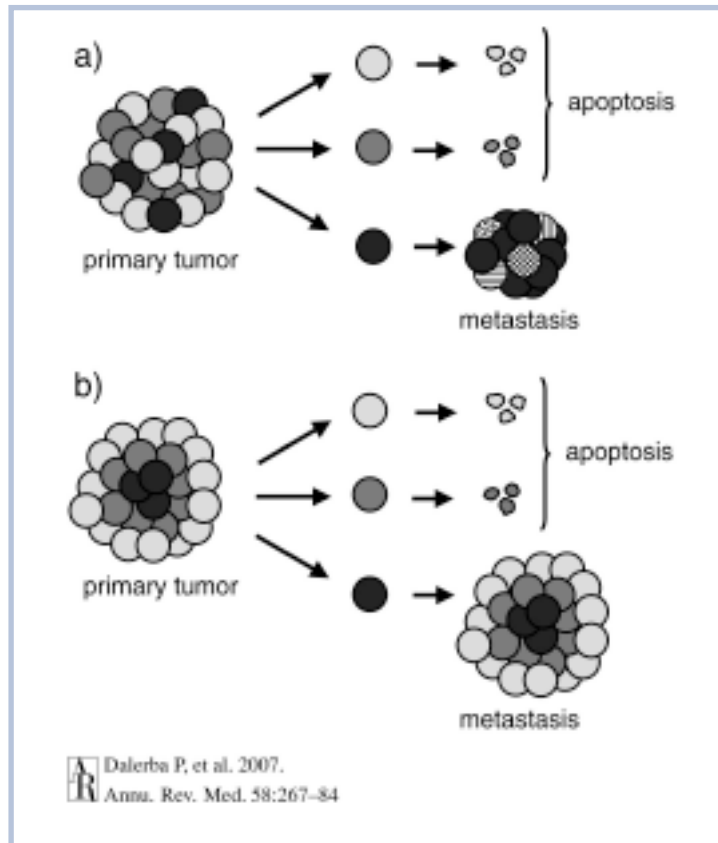
Introduction

The discovery of cancer stem cells has transformed how researchers and clinicians are thinking about cancer, which kills more than half a million Americans each year. Cancer stem cells, while rare, appear to be the source of all the cells in the cancers they cause. They are fertile new ground for exploring how the disease arises, how it grows and spreads, and how it may be contained or even eliminated. Some researchers believe cancer stem cells to be the key to defeating many forms of cancer.

Cancer stem cells are closely related to and may often originate with adult stem cells. Under normal circumstances, the regular turnover of cells in developed tissues is offset by the work of adult stem cells, which can divide to make more stem cells or progenitor (immature) cells. The progenitors then differentiate into the mature cells needed to maintain the organ or to respond to an injury, hormones, or other external signals. Cancer stem cells can produce more of their kind or progenitors that multiply and differentiate to become the malignant cells that make up the bulk of a cancer. Cancer stem cells are remarkably potent: Transplanting as few as 10 of them into disease-free, immunologically compromised mice can create a new cancer.

The cancer stem cell hypothesis is a departure from traditional models of oncogenesis, which proposed that genetic alterations transform mature, differentiated cells into cancer cells. Cancer stem cells, however, help explain two of the most challenging and demoralizing aspects of cancer: remission and recurrence. Often, radiation or chemotherapy halts the malignancy, sometimes to the point where it can no longer be detected, yet the disease returns. Cancer stem cells may be especially resistant to eradication for two reasons. First, current chemotherapies selectively target rapidly dividing cells, but stem cells tend to divide at a slow rate. Second, normal stem cells congregate in niches—specific physical areas within an organ that protect stem cells. Cancer stem cell niches have been found in the brain and proposed elsewhere, making radiation and chemotherapy treatment even more complicated. If even a small number of cancer stem cells survives an assault, they can once again give rise to full-fledged cancer, as metastases result from the surviving cancer stem cells that travel through the body. See Figure 1.

FIGURE 1:
Impact of the cancer stem cell model on the design and evaluation of antitumor treatments. (a) Current antitumor treatments may kill the majority of cells and reduce tumor size, but tumor tissues can regenerate from surviving cancer stem cells. (b) Anticancer treatments that target cancer stem cells may not cause rapid tumor shrinkage but could eradicate the disease in the long run by eliminating the tumor's ability to grow. Source: Dalerba, et al., *Annu. Rev. Med.* 58:267–284, 2007



The therapeutic implications are vast. As we learn the specific vulnerabilities of cancer stem cells and create drugs to exploit them, we will add a new front in the war on this category of disease. Cancer treatments may become more effective and less toxic, and survival rates may increase.

From Discovery to Application

Zeroing in on the characteristics of cancer stem cells began with the discovery of the first definitive cancer stem cell, which brought together new technology with old theory. Rudolph Virchow, the father of modern pathology, suggested in 1855 that leukemia arises from residual embryonic tissues. Beginning in the 1960s, researchers noticed that a small minority of cancer cells could initiate a tumor. At the same time, the first adult stem cells were discovered in bone marrow. The progression from a hematopoietic (blood) stem cell to the range of mature blood cells has become the best-understood cellular differentiation pathway in mammals.

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As that research proceeded, immunologists developed an extensive system of classifying cells based on their surface proteins (called cell surface markers). Technological advances also played a key role: By the late 1990s, commercially available machines could take a mixed pool of cells, labeled with fluorescent tags to particular surface proteins on these cells, and rapidly sort them into functionally homogeneous pools. In 1997, researchers found that a small minority of cells (0.02%) isolated from patients with acute myelogenous leukemia met the criteria for cancer stem cells.

The questions researchers need to answer as they move from discovery to application fall into three main categories:

1. How common are cancer stem cells?
2. What is the role of cancer stem cells in the origin and growth of cancer?
3. What elements of cancer stem cells' metabolism are selectively vulnerable?

How Common Are Cancer Stem Cells?

The techniques used to find leukemia stem cells were quickly adapted to solid tumors. In the decade since leukemia stem cells were first isolated, similar self-renewing, multipotent, tumor-producing cells have been found in cancers of the breast, colon, and central nervous system. Furthermore, there are tantalizing signs that other subsets of tumor cells also possess at least some characteristics of cancer stem cells. Normal adult stem cells in the lung and prostate gland have been identified as sources of carcinomas. It seems increasingly likely that many, if not all, cancers have stem cells at their core.

The challenge is finding them. The blood disease leukemia has been the easiest to study, partly because blood is more accessible than most tissues and the cell surface markers in blood are more fully classified. To study solid tumor cells, researchers and surgeons must be geographically close to one another and coordinate carefully so that tissue samples move rapidly from the operating room to the research laboratory. Solid tumor assays have been developed to study cancer formation both in the laboratory and in animal models. More recently, the issue of "stemness" has arisen, and researchers are looking for a set of genes that may serve as universal stem cell markers.

What Is the Role of Cancer Stem Cells in the Origin and Growth of Cancer?

Do cancer stem cells represent normal developmental mechanisms run amok, much as cancer often seems to be normal tissue growth that has suddenly become malignant? The degree to which cancer stem cells recapitulate or try to recapitulate the developmental pathways of the tissue from which they arose is still unclear. Overexpression of the transcription factor Cdx4 leads to acute myelogenous leukemia in a mouse model of the disease, probably by boosting the expression of the HOX gene family, which is also involved in normal hematopoiesis (the formation of blood cells). And while the precise molecular events that transform a stem cell into a cancer stem cell are unknown, several studies have noted that pathways needed for self-replication in normal hematopoietic stem cells are also active in leukemia stem cells.

Because of their longevity and because they go through more division cycles than most cells, somatic stem cells (cells other than germline cells) are more likely to accumulate genetic defects associated with cancer. The Philadelphia chromosome, a genetic rearrangement associated with chronic myelogenous leukemia, is found in multiple blood cell types of patients with the disease, indicating that the original rearrangement happened in a multipotent precursor, likely to be a hematopoietic stem cell. Stem cells may also be where a congenital defect that predisposes someone to cancer is joined by a new mutation that alters the regulation of the stem cells' growth.

Some of the same molecular signaling pathways used in normal development, and in some cases already identified with oncogenesis, play important roles in cancer stem cells. For example:

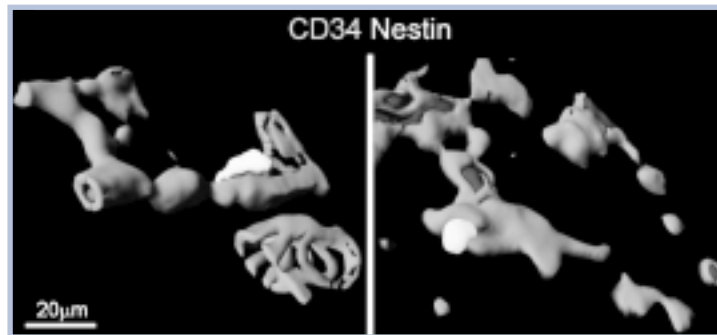
- The Notch pathway, whose activation by external signaling molecules is essential in hematopoietic stem cell self-renewal and the formation of T cells (a subset of white blood cells), is active in T-cell acute lymphoblastic leukemia even in the absence of the signal, due to a mutation in one of the Notch receptor genes.
- Bmi1, a gene whose activity is required for stem cell immortality, acts on a particular spot on chromosome 9 that encodes for tumor suppressor proteins. These interact with the p53 and Rb genes, whose products are involved with the regulation of the cell cycle. It has been well documented that these two genes are central to the disruption of normal cell cycle checkpoints (the stage where a cell verifies that everything is normal before deciding to continue dividing versus dying) and normal cell-growth control.

- Telomerase is an enzyme that acts on telomeres, which are regions at the end of eukaryotic chromosomes that, for many cells, shorten each time the cell divides. This shortening may contribute to cell senescence (inability to divide). Telomerase extends the life of a cell by maintaining telomeres, is expressed in both stem cells and tumor cells, and may indicate a mechanism for the capacity of these cells to replicate.

To add to an already complicated landscape, some cancer stem cells do not appear to arise directly from somatic stem cells but from progenitor cells that have moved a step or two down the pathway to differentiation. For example, as chronic myelogenous leukemia progresses, some of the cancer cells appear to have arisen through the cancerous transformation of an intermediate cell, the granulocyte monocyte progenitor. This adds another set of cell types with different properties that researchers must investigate to unravel the mysteries of cancer development.

As a cancer moves from genesis to growth, the cancer stem cell remains as a reserve of new cancer capacity. New evidence from one form of brain cancer suggests that cancer stem cells may mimic their normal counterparts by congregating in stem cell niches where they are physically shielded from chemical and radiological attack, and where a stem-cell-friendly microenvironment is maintained. In addition, just as normal stem cells can migrate through the bloodstream and engraft into new sites, cancer stem cells also have the ability to migrate, though, in this case, forming new loci of cancer: metastases.

FIGURE 2:
A three-dimensional reconstruction from microscope images indicates brain cancer stem cells (green, identified by the presence of Nestin) nestled against the endothelium of blood vessels (red, identified by the CD34 surface marker). Source: Adapted from Calabrese, C., *et al. Cancer Cell*. 11:69–82, 2007



What Elements of the Stem Cell's Metabolism Are Vulnerable?

The metabolism of cancer stem cells appears to be quite different from that of tumor cells, as measured by patterns of gene expression and surface markers compared between normal stem cells, cancer stem cells, and tumor cells. The array of genes expressed in cancer stem cells appears to overlap partially with those expressed in both normal stem cells and differentiated cells. The narrow zone of expression unique to cancer stem cells may be the most fruitful for therapeutic exploitation. A small-scale clinical trial is currently underway to test whether inhibitors of the Notch pathway, which is permanently active in T-cell acute lymphoblastic leukemia, have an effect on the disease. A sophisticated understanding of the differential expression of genes in cancer cells may be the most important initial step in developing specific drugs against cancer stem cells and is an active component of current cancer stem cell research.

However, those exploring cancer stem cells as therapeutic targets also recognize a familiar dilemma. Current cancer treatments selectively attack rapidly growing cells. This is effective against the bulk of the tumor mass, but these cytotoxic drugs also affect normal healthy cells. The side effects are well known. Likewise, cancer stem cells bear a significant resemblance to normal adult stem cells. Will therapies that seek to eliminate cancer stem cells have unacceptable negative effects on normal maintenance of organs and tissues?

The discovery of the first cancer stem cells is barely a decade old and is still being extended from leukemias to other forms of the disease. There is reason to hope that these elusive cells may be the key to durable victories against cancer, and that hope is why research concerning cancer stem cells is a substantial topic in cancer research today. But there is still significant fundamental knowledge to gather before the excitement is converted to widespread benefits for cancer's victims.

For more information about the HSCI Cancer Program, visit the HSCI Web site at www.hsci.harvard.edu.

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