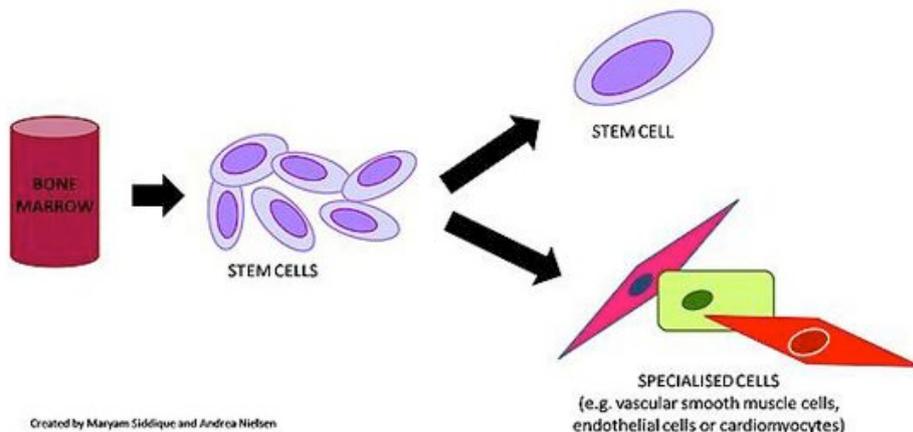


## The Evolving Science of Cancer Stem Cells

<http://www.cancer.gov/ncicancerbulletin/072710/page4>



Stem cells can both self-replicate, as well as produce progenitors that differentiate into other, more mature cell types, such as endothelial cells. Similarly, a cancer stem cell is thought to self-replicate and produce progenitors that generate all of the cell types that make up a tumor.

The theory of the cancer stem cell (CSC) has generated as much excitement and optimism as perhaps any area of cancer research over the last decade. Biologically, the theory goes, these cells are distinct from the other cells that form the bulk of a tumor in that they can self-perpetuate and produce progenitor cells, the way that traditional stem cells do. The progenitors' job is then to repopulate tumor cells eradicated by treatments such as chemotherapy or radiation.

But for all the attention and fanfare CSC research has received, the findings reported to date are far from clear-cut, investigators acknowledge. For example, most of the studies that have identified human CSCs have used mouse [xenograft](#) assays and cells from only a small number of human tumor samples, making it difficult to draw firm conclusions. In addition, other researchers haven't always been able to replicate initially reported findings. (See the sidebar: "Tools of the CSC Trade: Markers and Xenografts.") And while these tumor-initiating cells, as they are also called, have been described as being a rare class, several studies have found that the number of cells that can form tumors in these mouse experiments is [actually quite large](#), suggesting that perhaps CSCs aren't such a privileged breed.

In other words, the idea of just what cancer stem cells are, and their role in different cancers, appears to be changing.

"The [stem cell] model has not been adequately tested in most cancers," said Dr. Sean Morrison, who directs the Center for Stem Cell Biology at the University of Michigan. "I think that there are some cancers that do clearly follow a cancer stem cell model...But it will be more complicated than what's been presented so far."

### An Evolving Idea

Unlike the random or "stochastic" model dominant in cancer research, which holds that nearly any cancer cell has the potential to form a tumor, the cancer stem cell model is one of a hierarchical organization, with the [pluripotent cancer stem cell](#) sitting ready and able to amass all of the components of the original tumor.

It's also thought, with some [experimental evidence](#) to support it, that CSC pluripotency allows these cells to adapt and to resist chemotherapy, radiation therapy, and even current molecularly targeted therapies. If true, then these treatments may not harm the most lethal tumor cells, those that can lead to a recurrence with the production of a new set of progenitors.

Despite numerous studies published in the last 16 years that identified CSCs for different cancers—including [colon](#), [brain](#), pancreatic, and breast cancer—the consensus among researchers seems to be that the evidence is strongest for the first cancer in which a population of tumor-initiating cells [was discovered](#), acute myeloid leukemia (AML), as well as for other blood cancers.

"The reason why it's so much stronger for hematologic malignancies is because hematopoiesis research goes back 40 or 50 years and it's very stem cell-based," said Dr. Jean Wang, a stem cell researcher at the University of Toronto. "Whereas in solid tumors, there's less of a foundation for identifying the normal cellular hierarchies and for [cell-surface] markers that identify different populations of cells like stem cells and progenitors."

Even so, Dr. Wang believes the existence of CSCs is pretty well demonstrated for breast and brain cancers. But, she cautioned, "I don't know if it applies to all cancers. In a lot [of cancers] it does seem to apply. But most of the markers we have right now are still very rough."

Despite the evidence for CSC-like cells in a growing number of cancers, the theory clearly has its skeptics, who point to problems such as shortcomings in the mouse xenograft assay and the variable specificity of the cell-surface markers used to demarcate a CSC from a non-CSC.

"I still feel that it's a concept yet to be proven," said Dr. Barbara Vonderhaar, who, along with colleagues in NCI's [Center for Cancer Research](#), recently [published a study](#) identifying a population of CSC-like cells in estrogen receptor-negative breast cancer. "It's certainly a good idea, but it's only a hypothesis at this point. We still don't have definitive proof that cancer stem cells exist."

The CSC concept is "a work in transition," said Dr. William Matsui, from the Johns Hopkins School of Medicine, whose lab studies the role of stem cells in hematologic cancers. "To me, as a clinical person, the ideal model is one where you can find something that is going to work in humans. We're far from that."

### Case Study: Melanoma

One of the most well-known studies in the CSC literature came from Dr. Morrison's lab in 2008. Earlier studies had suggested that, consistent with the CSC model, there was only a rare population of cells from human melanoma tumors that, when injected into mice with compromised immune systems (called NOD/SCID mice), could form new tumors.

But in a [study](#) published in *Nature*, Dr. Morrison's team tweaked the common experimental approach: they used mice with immune systems that were even more impaired than NOD/SCID mice and waited longer to assess tumor growth. The result: approximately one in four randomly selected single cells taken from a human melanoma sample could form a tumor.

The results "made clear that estimates of the frequency of tumorigenic cells are far more assay-dependent than we realized," Dr. Morrison said. In addition to factors such as the status of the mouse's immune system in the experiments, he continued, "there are probably other variables that have a much bigger influence that we still haven't discovered." (In AML, it's worth noting, use of more immunocompromised mice does not significantly increase the number of cells that can form tumors.)

Researchers from Stanford University earlier this month [reported](#) in *Nature* that they had found a marker, CD271, that identified a somewhat unique population of cells that could produce melanoma in highly immunocompromised mice; anywhere from 2.5 percent to 41 percent of cells in their human tumor samples expressed the marker. In additional experiments using similar mice on which human skin was engrafted, only tumor cells with the marker could produce tumors and metastases in the mice. (In his lab, Dr. Morrison noted, the same marker did not differentiate tumor-forming from nontumor-forming cells.)

The fact that a fairly large percentage of the cells from the nine human melanoma tumors used in the study could initiate a tumor reflects a number of things, wrote lead author Dr. Andrew Boiko and colleagues in the *Nature* paper. Among them, an evolutionary type process selects for the survival of tumor cells that fail to normally differentiate during tumor development.

That might mean that a cancer stem cell isn't necessarily part of the original tumor, but due to various factors or influences—such as interactions with the immune system or hypoxia—certain tumor cells, maybe many of them, can activate a stem cell-like "program."

"I'm a firm believer that the microenvironment, the stem cell 'niche,' is every bit as important as the cell itself," Dr. Vonderhaar said. "I don't know if just any cell can become [a CSC], but there is a hierarchy of cells, and some may be able to function in a stem cell-like manner, and others may not."

The CSC field itself, Dr. Matsui noted, needs to move more quickly beyond just determining whether these cells can grow tumors on their own, "and ask what other properties they might have that contribute to clinical outcomes." Those might include their role in problems such as drug resistance or metastasis.

Some of the controversy surrounding CSCs "is a good thing," Dr. Matsui said, "because it forces us to be more rigorous in our work. The more rigor we can get in the research, the more clinically applicable all of the ideas are going to be."

—Carmen Phillips

#### **Tools of the CSC Trade: Markers and Xenografts**

Although there are several experiments that can be used to identify potential CSCs, a functional *in vivo* experiment with immunocompromised mice has been the "gold standard" assay, Dr. Wang said.

Many of the markers used to identify CSCs came from "studying normal systems," Dr. Matsui explained. In brain tumors, for instance, early research focused on the cell-surface protein CD133, which had been identified as a marker for normal neural stem cells. In addition to brain cancer, CD133 has been used to identify potential CSCs in colon cancer, whereas CD44 has been used to identify breast cancer stem cells, and aldehyde dehydrogenase (ALDH), in combination with other markers, has been used to identify CSCs for breast, prostate, and pancreatic cancer.

Once researchers have identified a marker or multiple markers of interest, they take a human tumor sample and separate them from one another based on the presence or absence of those markers.

The cells are injected into the mice at varying sites to see if the mice that received the potentially tumorigenic cells—those with the proposed stem cell marker(s)—develop tumors, while those that received the nontumorigenic cells do not. If the initial experiment shows the expected tumor development, additional "passages" of the proposed CSCs—this time, fractionated out from the mouse tumors—are done in other mice to more firmly establish the marker-bound cells' tumor-forming capacity.

Other animal-model assays are also being used, including mice that are genetically engineered to develop certain tumors and a [zebrafish model](#).