

MOLECULAR BIOLOGY

Cancer's Perpetual Source?

The discovery of tumor cells that behave like stem cells suggests why cancer may be so hard to eradicate—and how new therapies might be targeted

Beginning about 15 years ago, John Dick's team at the University of Toronto in Canada provided a new clue as to what makes cancer such a formidable foe. They found that only a tiny population of leukemia cells could transmit the cancer from one experimental animal to another. More remarkably, the cells had a property previously seen only in stem cells: the ability to produce an exact copy of themselves each time they divide, thereby maintaining the ability to reproduce in perpetuity. These so-called cancer stem cells, Dick suggested, might be what makes the disease so hard to eradicate with radiation or chemotherapy.

Since then, such cells have been found in many other cancers, including those of the breast, brain, colon, and head and neck. "Cancer stem cells are being identified in virtually every cancer," says Max Wicha of the University of Michigan Comprehensive Cancer Center in Ann Arbor.

Not everyone is convinced that the stem-like cells found in cancers play such a key role in tumor growth and maintenance. But if that idea is correct, "the ramifications could be huge," says Craig Jordan of the University of Rochester School of Medicine and Dentistry in New York state. In that event, therapies that target cancer stem cells may prove more effective than current cancer treatments. Indeed, radiation and many chemotherapeutic drugs wipe out dividing cells, but stem cells may be quiescent most of the time and so

may survive these treatments. "You can reduce tumor bulk 90%, and in no time at all, the tumor will take over again," says Levy Kopelovich of the National Cancer Institute in Bethesda, Maryland, which held a workshop in mid-May on stem cells as targets for cancer prevention.

Cancer researchers are beginning to understand what makes cancer stem cells dangerous. Among other things, they foster the formation of new blood vessels needed to feed tumor growth. Recent work is also uncovering the cellular signaling pathways that control cancer stem cell proliferation, raising hopes of new treatments that selectively kill these cancer seeds. Indeed, some existing cancer drugs and others that may soon be tested in people appear to target the cells, new studies indicate. "By gaining a sophisticated understanding of how normal and cancer stem cells differ, we'll be able to design a new class of drugs that is less toxic," predicts Sean Morrison of the University of Michigan Medical School in Ann Arbor.

Multiple threats

For the first decade after Dick's discovery, the clinical importance of cancer stem cells seemed limited, because leukemias are much rarer than solid cancers. But interest in the cells began to take off in 2003. That year, a team led by Michael Clarke, then at the University of Michigan Medical School, spotted

Blood vessel stimulator. When transplanted into mouse brains, glioma stem cells form larger and more vascular tumors (*middle row*) than do nonstem cells (*top*). As shown by the mouse at right, an antibody to the angiogenesis-promoting protein VEGF greatly inhibits the growth of glioma stem cell tumors; the mouse at left is an untreated control.

cancer stem cells in breast cancers, and Peter Dirks of the Hospital for Sick Children in Toronto and his colleagues identified them in a variety of brain cancers (*Science*, 5 September 2003, p. 1308).

In the past several months, that early trickle of results has turned into a flood. In the 4 January issue of *Nature*, for example, two independent teams, one led by Dick and the other by Ruggero De Maria of the Istituto Superiore di Sanità in Rome, reported the discovery of cancer stem cells in colon cancer, and others have reported finding them in cancers of the prostate, lung, pancreas, head and neck, and the deadly skin cancer melanoma. "Cancer stem cell research has gone from an interesting sidelight to mainstream in a very short time," Jordan says.

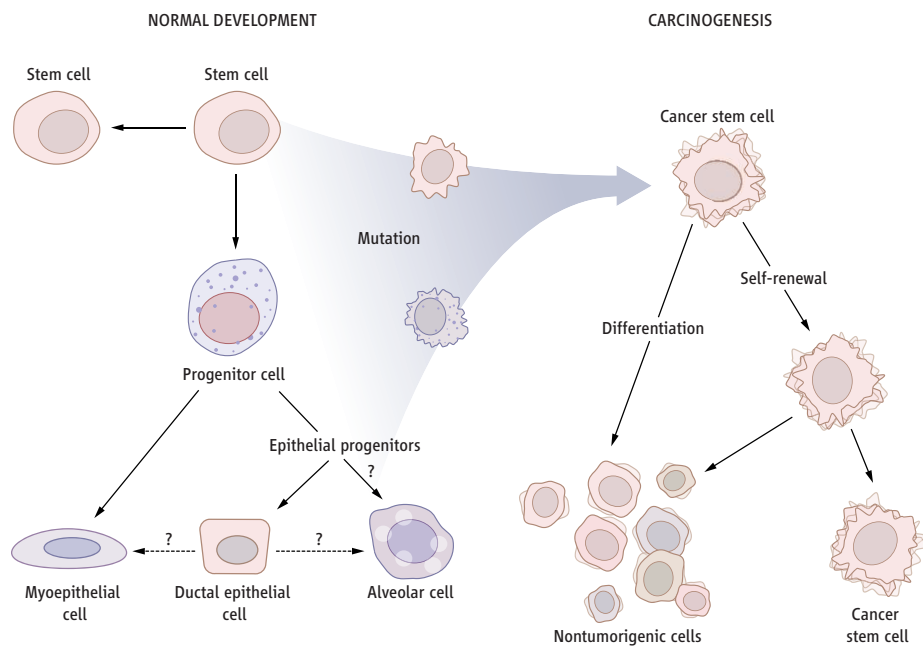
What's more, cancer stem cells display an array of traits that aid in their proposed role of driving and maintaining cancer growth. For example, Jeremy Rich's team at Duke University in Durham, North Carolina, looked at their ability to resist radiation.

In one key set of experiments, these researchers transplanted cells obtained from human glioblastomas, which are highly malignant brain tumors, into mice and then subjected the animals to radiation doses similar to those used to treat human patients. Other than surgery, Rich says, "radiation therapy is the most effective [treatment] for brain tumors, but it is rarely curative."

The transplantation results, which appeared in the 7 December 2006 issue of *Nature*, suggest an explanation. In irradiated tumors, the proportion of cancer stem cells went up from about 2% to about 8%, as indicated by the number bearing the protein CD133, a marker for brain cancer stem cells. This shows that those cells survived the radiation whereas other tumor cells succumbed.

The stem cells overcame the radiation, further analysis suggested, because they repair the DNA damage it induces more effectively than nonstem cancer cells do. "I thought that was a great paper," says Clarke, who's now at Stanford University in Palo Alto, California. "It provided experimental evidence for what we've all been predicting."

A second way that cancer stem cells may pose a threat is by stimulating angiogenesis, the formation of new blood vessels



Dangerous cells. According to current thinking, oncogenic mutations arising either in normal tissue stem cells or in the more developmentally advanced progenitor cells can produce cancer stem cells. When these cells divide, one daughter is an exact copy of the original and retains the ability to divide—and to initiate additional tumors—whereas the other target differentiates to produce nontumorigenic cells.

that support tumor growth. For example, the Duke team recently found that glioma stem cells in culture produce much more of the angiogenesis-promoting protein VEGF than do other glioma cells.

The VEGF discovery suggests that one current drug may be a prototype of therapies that target cancer stem cells. Bevacizumab (Avastin) is an antibody designed to block VEGF action that is already used for cancer therapy. And when the Duke team transplanted human glioma stem cells into mice and treated the animals with the antibody, “the tumors from cancer stem cells shrank dramatically,” Rich says. “It looks like bevacizumab is a kind of anticancer-stem cell therapy.”

And cancer stem cells may have yet another dangerous property: the ability to drive metastasis, the spread of tumors in the body. The presence of tumor cells in the bone marrow of breast cancer patients is a bad prognostic sign, indicating that such patients have a high risk of cancer spread. Last fall, Marija Balic, Richard Cote, and their colleagues at the University of Southern California in Los Angeles reported that roughly 70% of the tumor cells in bone marrow carry the surface markers of breast cancer stem cells. Although it hasn’t been proven that those cells seed metastatic tumors, such a possibility is in line with the idea that cancer stem cells are the tumor-initiating cells. Other

research has shown that genes involved in cell migration and tissue invasion are highly active in breast cancer stem cells.

Searching for vulnerability

In their search for ways to disrupt cancer stem cell activities, researchers are focusing heavily on the signaling pathways needed for their maintenance and development. And several intriguing connections between genes already linked to cancer development have emerged.

At this year’s annual meeting of the American Association for Cancer Research in April, Hasan Korkaya, a member of Wicha’s group at Michigan, reported that reducing expression of *PTEN*, a known tumor suppressor gene, in cultured human breast cancer cells increased cancer stem cell populations by as much as fivefold. Boosting expression of the *HER2* oncogene at the same time doubled that increase. In addition, such cells migrate more in culture, an indication that they may seed metastases. This might help explain why women whose breast cancers have both loss of *PTEN* and extra *HER2* copies usually have a poor prognosis.

Other work also points to *PTEN* loss as a trigger for cancer stem cells. Early last year, Owen Witte’s team at the David Geffen School of Medicine in Los Angeles found that when *PTEN* is deleted in the prostate cells of mice, the number of cells bearing a

stem cell marker called Sca-1 increases, and small premalignant growths form in the prostate. And Linheng Li’s team at the Stowers Institute for Medical Research in Kansas City, Missouri, has found that deleting *PTEN* in cells lining the intestines leads to the production of stem cells that form polyps that can develop into full-blown cancers. Finally, Morrison’s team has found in mice that *PTEN* deficiency promotes the formation of leukemia stem cells while depleting normal hematopoietic stem cells.

This suggests, he notes, that it may be possible to selectively strike cancer stem cells. Indeed, Morrison and his colleagues have evidence that the drug rapamycin, which can help make up for *PTEN* loss, can prevent leukemia development in their mouse model while at the same time restoring normal hematopoietic stem cell function. This is “exciting,” Morrison says, “because it offers the possibility of developing therapies that kill cancer stem cells but are less toxic in normal tissue.” Rapamycin is already used clinically as an immunosuppressant and is being studied in cancer therapy, and the group hopes to begin a clinical trial to test it in patients with acute myeloid leukemia (AML).

Researchers seeking to target cancer stem cells are also looking at several more pathways previously implicated in stem cell maintenance. Among these are the Wnt pathway, which is implicated in intestinal and other cancers, and also the Polycomb and sonic hedgehog pathways.

Some surprising aspects of cancer stem cells may also provide unexpected new targets for therapies. In a recent screen of 1267 compounds in a library of pharmacologically active agents, Dirks and his colleagues identified 160 agents that decreased the proliferation of brain cancer stem cells in lab cultures. Many of these drugs affected unexpected neuronal functions, such as neurotransmission, which were supposed to be properties of mature neurons rather than unspecialized stem cells. The mechanisms that control cancer stem cell growth “may be more diverse than what we see right now,” Dirks says.

Even a folk remedy is showing some promise against cancer stem cells. Rochester’s Jordan, working with Dianna Howard’s team at the University of Kentucky Medical Center in Lexington, has evidence from both cells in culture and a mouse model that the drug parthenolide—the active ingredient in a herbal remedy called feverfew—specifically kills AML stem cells.

In addition to simply screening for compounds that kill cancer stem cells, Dick and his colleagues are going after leukemia stem

cells with an antibody that binds to a protein called CD44 that is highly expressed on the surface of AML cells. When the researchers transplanted human AML stem cells into mice and then administered the antibody, the treatment apparently abolished the tumor stem cells driving the leukemia. Closer examination showed that the antibody prevents AML stem cells from migrating to the spleen and bone marrow, where they would otherwise reside. “The [leukemia] stem cells still need to interact with their niche, and if you interfere with their trafficking, they can’t maintain themselves,” Dick says.

Even if cancer stem cells can’t be killed or their spread blocked, they might be restrained in another way: by inducing them to lose their “stemness” and differentiate into nonrenewing cells. Last December, Angelo Vescovi of the University of Milan–Bicocca in Italy and colleagues reported in *Nature* that bone morphogenetic proteins inhibit the tumorigenic properties of human glioblastoma stem cells in this fashion. “It’s an interesting paper,” says Dirks. “It opens up the field to considering differentiation” as a therapeutic goal.

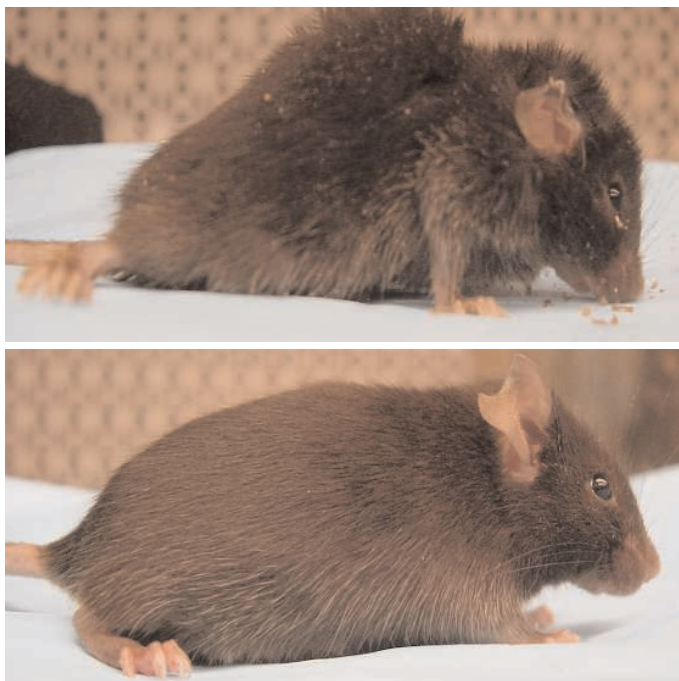
Lingering questions

Despite the outpouring of results in the past few years, fundamental questions remain about cancer stem cells. One big issue concerns the nature of the original cell that gives rise to them. “That’s a harder question than people appreciate,” Jordan says. “It doesn’t have to start with a normal stem cell.” In work done a few years ago, Irving Weissman’s team at Stanford University examined the genetic mutations in human AML cells and concluded that the original cancer-causing mutations can strike more developmentally advanced, although still immature, progenitor cells.

About a year ago, Scott Armstrong and colleagues at Children’s Hospital Boston provided further support for that idea. Many leukemia cells feature gene translocations in which two genes, previously separate from

one another, become joined and produce so-called fusion proteins. Human AML cells, for example, make a fusion protein called MLL-AF9. When the Children’s Hospital workers genetically engineered granulocyte-macrophage progenitor cells to produce MLL-AF9 and transplanted the cells into mice, they gave rise to a leukemia similar to AML. Subsequent isolation of leukemia stem cells from the mice showed that they resembled the original granulocyte-macrophage progenitors but had activated genes needed for self-renewal.

Indeed, work published by the Clarke team in the 12 June issue of the *Proceedings of the National Academy of Sciences* suggests that stem cells of a given type of cancer may arise from different cells. For these



Staying alive. The *PTEN* tumor-suppressor gene was deleted from the blood-forming cells of both mice. The mouse at top is near death due to the resulting leukemia. But treatment with the drug rapamycin, which can compensate for *PTEN* loss, kept the mouse at bottom healthy.

experiments, the researchers looked at colon cancer stem cells obtained from four different patients. There were “subtle differences” in cancer stem cell characteristics from patient to patient, says Clarke. “This suggests that the cell of origin varies from patient to patient.”

Despite the huge growth in the cancer stem cell field, skeptics remain. One potential problem is that virtually all the work has involved transplanting human cancer cells into immunodeficient mice. This has raised concerns that the experiments do not accurately reflect what happens during cancer development in humans. Indeed, Andreas

Strasser and his colleagues at the Walter and Eliza Hall Institute of Medical Research in Melbourne, Australia, have recently challenged the idea that only rare cancer stem cells can initiate tumor formation.

Instead of using human leukemia cells, these researchers worked with mice genetically engineered to develop leukemia. When they injected leukemic cells from these animals into genetically compatible healthy mice, all the recipients developed leukemia, even those injected with as few as 10 cells, Strasser’s team reported in the 20 July issue of *Science* (p. 337). That could not have happened if only a tiny minority of tumor cells had the ability to initiate tumor formation, as the stem cell hypothesis holds, Strasser says. He suggests that the mouse doesn’t provide the right environment for the growth of human cancer cells, so that only a few manage to survive and multiply, thus creating a false impression that tumor-initiating cells are rare. “Those data have been massively overinterpreted,” Strasser maintains.

Dick disagrees. He notes that the cancer stem cell hypothesis rests on the prospective isolation of distinct populations of tumor-initiating and noninitiating cells rather than just on the rarity of cancer stem cells. Dick points out that his team has re-created human leukemias in mice by injecting the animals with normal human blood-forming cells that had been engineered to carry a fusion gene known to make cells leukemic (*Science*, 27 April, p. 600).

These animals, which are more comparable to Strasser’s genetically engineered mice, developed leukemias similar to those seen in humans, and the frequency of tumor-initiating cells was relatively high—one to two in 100—rather than the one in a million seen in the early experiments. Other researchers have reported similar results. Dick suggests that different cancers will vary in cancer stem cell frequency, depending on the particular oncogenic pathways operating in the cells.

Although others share Strasser’s skepticism about cancer stem cells and many uncertainties remain, there is one sure bet. Given the possible importance of cancer stem cells as therapeutic targets, the field will continue to grow. And at least one skeptic has already been convinced. Duke University’s Rich says that he came into the research hoping to disprove the importance of cancer stem cells. Now, he says, “we are very early in studying cancer stem cells, but understanding them may impact the way we diagnose and treat patients in the near future.”

—JEAN MARX