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Stem Cells: The Real Culprits in Cancer?

A dark side of stem cells--their potential to turn malignant--is at the root of a handful of cancers and may be the cause of many more. Eliminating the disease could depend on tracking down and destroying these elusive killer cells

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After more than 30 years of declared war on cancer, a few important victories can be claimed, such as 85 percent survival rates for some childhood cancers whose diagnoses once represented a death sentence. In other malignancies, new drugs are able to at least hold the disease at bay, making it a condition with which a patient can live. In 2001, for example, Gleevec was approved for the treatment of chronic myelogenous leukemia (CML). The drug has been a huge clinical success, and many patients are now in remission following treatment with Gleevec. But evidence strongly suggests that these patients are not truly cured, because a reservoir of malignant cells responsible for maintaining the disease has not been eradicated.

Stem cells' power to self-renew already exempts them from the rules.

Conventional wisdom has long held that any tumor cell remaining in the body could potentially reignite the disease. Current treatments therefore focus on killing the greatest number of cancer cells. Successes with this approach are still very much hit-or-miss, however, and for patients with advanced cases of the most common solid tumor malignancies, the prognosis remains poor.

Moreover, in CML and a few other cancers it is now clear that only a tiny percentage of tumor cells have the power to produce new cancerous tissue and that targeting these specific cells for destruction may be a far more effective way to eliminate the disease. Because they are the engines driving the growth of new cancer cells and are very probably the origin of the malignancy itself, these cells are called cancer stem cells. But they are also quite literally believed to have once been normal stem cells or their -immature offspring that have undergone a malignant transformation.

This idea--that a small population of malignant stem cells can cause cancer--

is far from new. Stem cell research is considered to have begun in earnest with studies during the 1950s and 1960s of solid tumors and blood malignancies. Many basic principles of healthy tissue genesis and development were revealed by these observations of what happens when the normal processes derail.

Today the study of stem cells is shedding light on cancer research. Scientists have filled in considerable detail over the past 50 years about mechanisms regulating the behavior of normal stem cells and the cellular progeny to which they give rise. These fresh insights, in turn, have led to the discovery of similar hierarchies among cancer cells within a tumor, providing strong support for the theory that rogue stemlike cells are at the root of many cancers. Successfully targeting these cancer stem cells for eradication therefore requires a better understanding of how a good stem cell could go bad in the first place.

Orderly Conduct

The human body is a highly compartmentalized system made up of discrete organs and tissues, each performing a function essential to maintaining life. Individual cells that make up these tissues are often short-lived, however. The skin covering your body today is not really the same skin that you had a month ago, because its surface cells have all since sloughed off and been replaced. The lining of the gut turns over every couple of weeks, and the life span of the platelets that help to clot blood is about 10 days.

The mechanism that maintains a constant population of working cells in such tissues is consistent throughout the body and, indeed, is highly conserved among all complex species. It centers on small pools of long-lived stem cells that serve as factories for replenishing supplies of functional cells. This manufacturing process follows tightly regulated and organized steps wherein each generation of a stem cell's offspring becomes increasingly specialized.

This system is perhaps best exemplified by the hematopoietic family of blood and immune cells. All the functional cells found in the blood and lymph arise from a single common parent known as the hematopoietic stem cell (HSC), which resides in bone marrow. The HSC pool represents less than 0.01 percent of bone marrow cells in adults, yet each of these rare cells gives rise to a larger, intermediately differentiated population of progenitor cells. Those in turn divide and differentiate further through several stages into mature cells responsible for specific tasks, ranging from defending against infection to carrying oxygen to tissues. By the time a cell reaches that final functional stage, it has lost all ability to proliferate or to alter its destiny and is said to be terminally differentiated.

The stem cells themselves meanwhile remain undifferentiated, a state they maintain through their unique capacity for self-renewal: to begin producing

new tissues, a stem cell divides in two, but only one of the resulting daughter cells might proceed down a path toward increasing specificity. The other daughter may instead retain the stem cell identity. Numbers in the overall stem cell pool can thus remain constant, whereas the proliferation of intermediate progenitors allows populations of specific hematopoietic cell types to expand rapidly in response to changing needs.

The capacity of stem cells to re-create themselves through self-renewal is their most important defining property. It gives them alone the potential for unlimited life span and future proliferation. In contrast, progenitors have some ability to renew themselves during proliferation, but they are restricted by an internal counting mechanism to a finite number of cell divisions. With increasing differentiation, the ability of the progenitors' offspring to multiply declines steadily.

The practical significance of these distinctions can be observed when hematopoietic stem cells or their descendants are transplanted. After the bone marrow of a mouse is irradiated to destroy the native hematopoietic system, progenitor cells delivered into the marrow environment can proliferate and restore hematopoiesis temporarily, but after four to eight weeks those cells will die out. A single transplanted hematopoietic stem cell, on the other hand, can restore the entire blood system for the lifetime of the animal.

The hematopoietic system's organization has been well understood for more than 30 years, but similar cellular hierarchies have recently been identified in other human tissues, including brain, breast, prostate, large and small intestines, and skin. Principles of regulated stem cell behavior are also shared across these tissues, including specific mechanisms for controlling stem cell numbers and for directing decisions about the fates of individual cells. Several genes and the cascades of events triggered by their activity--known as genetic pathways--play key roles in dictating stem cells' fate and function, for example. Among these are signaling pathways headed by the *Bmi-1*, *Notch*, *Sonic hedgehog* and *Wnt* genes. Yet most of these genes were first identified not by scientists studying stem cells but by cancer researchers, because their pathways are also involved in the development of malignancies.

Many such similarities between stem cells and cancer cells have been noted. The classical definition of malignancy itself includes cancer cells' apparent capacity to survive and multiply indefinitely, their ability to invade neighboring tissues and to migrate (metastasize) to distant sites in the body. In effect, the usual constraints that tightly control cellular proliferation and identity seem to have been lifted from cancer cells.

Normal stem cells' power to self-renew already exempts them from the rules limiting life span and proliferation for most cells. Stem cells' ability to

differentiate into a broad range of cell types allows them to form all the different elements of an organ or tissue system. A hallmark of tumors, too, is the heterogeneity of cell types they contain, as though the tumor were a very disorderly version of a whole organ. Hematopoietic stem cells have been shown to migrate to distant parts of the body in response to injury signals, as have cancer cells.

In healthy stem cells, strict genetic regulation keeps their potential for unlimited growth and diversification in check. Remove those control mechanisms, and the result would be some-thing that sounds very much like malignancy. These commonalities, along with growing experimental evidence, suggest that failures in stem cell regulation are how many cancers get started, how they perpetuate themselves, and possibly how malignancies can spread.

Achilles' Heel

The presence of stem cells in certain tissues, especially those with high cell turnover such as the gut and the skin, seems to be an overly complicated and inefficient system for replacing damaged or old cells. Would it not appear to make more sense for an organism if every cell could simply proliferate as needed to supply replacements for its injured neighbors? On the surface, perhaps--but that would make every cell in the body a potential cancer cell.

Malignancies are believed to arise when an accumulation of "oncogenic" changes to key genes within a cell leads to the abnormal growth and transformation of that cell. Gene mutations typically happen through a direct insult, such as the cell being exposed to radiation or chemicals, or simply through random error when the gene is improperly copied before cell division. Because the rare stem cells are the only long-lived cells in the organs where most cancers develop, they represent a much smaller potential reservoir for cumulative genetic damage that could eventually lead to cancer. Unfortunately, because stem cells are so long-lived, they also become the most likely repository for such damage.

Indeed, stem cells' longevity would explain why many cancers develop decades after tissues are subjected to radiation--the initial injury may be only the first in a series of mutations required to transform a healthy cell into a malignant one. In addition to accumulating and preserving these oncogenic scars, a stem cell's enormous proliferative capacity makes it an ideal target for malignancy. Because nature so strictly regulates self-renewal, a cell population already possessing that ability would need fewer additional mutations for malignant transformation than would cells lacking that capacity.

Several possible paths to malignancy become apparent.

With these considerations in mind, several possible paths to malignancy become apparent. In one model, mutations occur in the stem cells themselves, and their resulting loss of control over self-renewal decisions produces a pool of stem cells predisposed to malignancy. Subsequent additional oncogenic events that trigger proliferation of the malignant cells into a tumor might happen in the stem cells or in their descendants, the committed progenitor cell population. A second model holds that oncogenic mutations initially occur in stem cells but that the final steps in transformation to cancer happen only in the committed progenitors. This scenario would require the progenitors' lost self-renewal capacity to be somehow reactivated.

Current evidence supports both models in different cancers. And at least one example exists of both processes playing a role in different stages of the same disease. Chronic myelogenous leukemia is a cancer of the white blood cells caused by the inappropriate fusion of two genes. Insertion of the resulting fused gene will transform a normal hematopoietic stem cell into a leukemia stem cell. Untreated, CML invariably progresses to an acute form known as CML blast crisis. Catriona Jamieson and Irving Weissman, both then at the Stanford University School of Medicine, demonstrated that in patients who progressed to CML blast crisis, the specific additional genetic events responsible for this more virulent version of the disease had conferred the ability to self-renew on certain progenitor cells.

Steady Pursuit

Over the past decade, evidence that stem cells could become malignant and that only certain cancer cells shared a variety of traits with stem cells strengthened the idea that the driving force underlying tumor growth might be a subpopulation of stemlike cancer cells. The theory has a longer history, but in the past the technology to prove it was lacking.

By the 1960s a few scientists were already beginning to note that groups of cells within the same tumor differed in their ability to produce new tumor tissue. In 1971 C. H. Park and his colleagues at the University of Toronto showed that within a culture of cells taken from an original, or "primary," myeloma (a cancer affecting plasma cells in bone marrow), the cells displayed significant differences in their ability to proliferate. At the time, Park's group could not interpret this phenomenon decisively, because at least two explanations were possible: all the cells might have had the ability to multiply in culture but by chance only some of them did, or else a hierarchy of cells was present in the tumor and cancer stem cells were giving rise to cells that were nontumorigenic, or incapable of proliferation.

Destroy the engine driving the disease, leaving nontumorigenic cells to die off.

Philip J. Fialkow of the University of Washington had already demonstrated in 1967 that the stem cell model was probably the correct one for leukemia. Using a cell-surface protein marker called G-6-PD, which can identify a cell's lineage, Fialkow showed that in some women with leukemia, both the tumorigenic cells as well as their more differentiated nontumorigenic progeny had all arisen from the same parent cell.

These early studies were critical in the development of the stem cell model for cancer, but they were still limited by researchers' inability to isolate and examine different cell populations within a tumor. A key event in stem cell biology, therefore, was the commercial availability, beginning in the 1970s, of an instrument called a flow cytometer, which can automatically sort different living cell populations based on the unique surface markers they bear.

A second crucial event in the evolution of cancer stem cell studies was the advent during the 1990s of conclusive tests for self-renewal. Assays to establish self-renewal in human cells did not exist until Weissman of Stanford and John E. Dick of the University of Toronto developed methods that allowed normal human stem cells to grow in mice. Using flow cytometry and this new mouse model, Dick began in 1994 to publish a series of seminal reports identifying cancer stem cells in leukemia. In 2003 Richard Jones of Johns Hopkins University identified a cancer stem cell population in multiple myeloma.

Earlier the same year our own laboratory group at the University of Michigan at Ann Arbor had published the first evidence of cancer stem cells in solid tumors. By transplanting sorted populations of cells from human breast tumors into mice, we were able to confirm that not all human breast cancer cells have the same capacity to generate new tumor tissue. Only one subpopulation of the cells was able to re-create the original tumor in the new environment. We then compared the phenotype, or physical traits, of those new tumors with that of the patient samples and found that the profile of the new tumors recapitulated the original. This finding indicated that the transplanted tumorigenic cells could both self-renew and give rise to all the different cell populations present in the original tumor, including the nontumorigenic cells.

Our study attested to the presence of a hierarchy of cells within a breast cancer similar to those identified in blood malignancies. Since then, the investigation of cancer stem cell biology has exploded, as labs across the world continue to find similar subpopulations of tumorigenic cells in other forms of cancer. In 2004, for example, the laboratory of Peter Dirks of the University of Toronto identified cells from primary human central nervous system tumors with the capacity to regenerate the entire tumor in mice. In

addition, he found a high number of the purported cancer stem cells present in one of the fastest-growing forms of human brain cancer, medulloblastoma, compared with far fewer tumorigenic cells found in less aggressive brain tumor types.

A related area of recent intensive investigation is also providing support for the cancer stem cell model. The signaling environment, or niche, in which tumors reside appears to strongly influence the initiation and maintenance of malignancy. Studies of normal body cells as well as of stem cells have already established the essential role of signals emanating from surrounding tissue and the supportive extracellular matrix in sustaining a given cell's identity and in directing its behavior. Normal cells removed from their usual context in the body and placed in a dish have a tendency to lose some of their differentiated functional characteristics, for example. Stem cells, in contrast, must be cultured on a medium that provides signals telling them to remain undifferentiated, or they will quickly begin proliferating and differentiating--seemingly as though that is their default programmed - behavior, and only the niche signals hold it in check.

In the body, stem cell niches are literal enclaves surrounded by specific cell types, such as stromal cells that form connective tissue in the bone marrow. With a few exceptions, stem cells always remain in their niche and are sometimes physically attached to it by adhesion molecules. Progenitor cells, on the other hand, move away from the niche, often under escort by guardian cells, as they become increasingly differentiated.

The importance of niche signaling in maintaining stem cells' undifferentiated state and in keeping them quiescent until they are called on to produce new cells suggests that these local environmental signals could exert similar regulatory control over cancer stem cells. Intriguing experiments have shown, for example, that when transplanted into a new niche, stem cells predisposed to malignancy because of oncogenic mutations will nonetheless fail to produce a tumor. Conversely, normal stem cells transplanted into a tissue environment that has been previously damaged by radiation do give rise to tumors.

Many of the same genetic pathways identified with signaling between stem cells and their niche have been associated with cancer, which also suggests a role for the niche in the final transition to malignancy. For example, if malignant stem cells were being held in check by the niche but the niche was somehow altered and expanded, the malignant stem cell pool would have room to grow as well. Another possibility is that certain oncogenic mutations within cancer stem cells could permit them to adapt to a different niche, again letting them increase their numbers and expand their territory. Still a third alternative is that mutations might allow the cancer stem cells to become independent of niche signals altogether, lifting environmental controls on both

self-renewal and proliferation.

Closing In

The implications of a stem cell model of cancer for the way we understand as well as treat malignancies are clear and dramatic. Current therapies take aim against all tumor cells, but our studies and others have shown that only a minor fraction of cancer cells have the ability to reconstitute and perpetuate the malignancy. If traditional therapies shrink a tumor but miss these cells, the cancer is likely to return. Treatments that specifically target the cancer stem cells could destroy the engine driving the disease, leaving any remaining nontumorigenic cells to eventually die off on their own.

Circumstantial evidence supporting this approach already exists in medical practice. Following chemotherapy for testicular cancer, for example, a patient's tumor is examined to assess the effects of treatment. If the tumor contains only mature cells, the cancer usually does not recur and no further treatment is necessary. But if a large number of immature-looking--that is, not fully differentiated--cells are present in the tumor sample, the cancer is likely to return, and standard protocol calls for further chemotherapy. Whether those immature cells are recent offspring that indicate the presence of cancer stem cells remains to be proved, but their association with the disease prognosis is compelling.

Stem cells cannot be identified based solely on their appearance, however, so developing a better understanding of the unique properties of cancer stem cells will first require improved techniques for isolating and studying these rare cells. Once we learn their distinguishing characteristics, we can use that information to target cancer stem cells with tailored treatments. If scientists were to discover the mutation or environmental cue responsible for conferring the ability to self-renew on a particular type of cancer stem cell, for instance, that would be an obvious target for disabling those tumorigenic cells.

Encouraging examples of this strategy's promise have been demonstrated by Craig T. Jordan and Monica L. Guzman of the University of Rochester. In 2002 they identified unique molecular features of malignant stem cells believed to cause acute myeloid leukemia (AML) and showed that the cancer stem cells could be preferentially targeted by specific drugs. Last year they reported their discovery that a compound derived from the feverfew plant induces AML stem cells to commit suicide while leaving normal stem cells unaffected.

Some research groups are hoping to train immune cells to recognize and go after cancer stem cells. Still others are exploring the use of existing drugs to alter niche signaling in the hope of depriving cancer stem cells of the environmental cues that help them thrive. Yet another idea under investigation is that drugs could be developed to force cancer stem cells to

differentiate, which should take away their ability to self-renew.

Most important is that cancer investigators are now on the suspects' trail. With a combination of approaches, aimed at both targeting genetic pathways unique to the maintenance of cancer stem cells and disrupting the cross talk between tumor cells and their environment, we hope to be able soon to find and arrest the real culprits in cancer.

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