9. ARE STEM CELLS INVOLVED IN CANCER?

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CANCER: IMPACT AND CHALLENGES

Data from 2007 suggest that approximately 1.4 million men and women in the U.S. population should be diagnosed with cancer, and approximately 566,000 American adults should die from cancer in 2008.[1] Based on prevalence rates from 2003–2005, it has been estimated that 40% of men and women born today will develop cancer in their lifetimes.[1] Data collected between 1996 and 2004 indicate that the overall 5-year survival rate for cancers from all sites, relative to the expected survival from a comparable set of people without cancer, is 65.3%.[1] However, survival and recurrence rates following diagnosis vary greatly as a function of cancer type and the stage of development at diagnosis. For example, in 2000, the relative survival rate five years following diagnosis of melanoma (skin cancer) was greater than 90%; that of cancers of the brain and nervous system was 35%. Once a cancer has metastasized (or spread to secondary sites via the blood or lymph system), however, the survival rate usually declines dramatically. For example, when melanoma is diagnosed at the localized stage, 99% of people will survive more than five years, compared to 65% of those diagnosed with melanoma that has metastasized regionally and 15% of those whose melanoma has spread to distant sites.[2]

The term "cancer" describes a group of diseases that are characterized by uncontrolled cellular growth, cellular invasion into adjacent tissues, and the potential to metastasize if not treated at a sufficiently early stage. These cellular aberrations arise from accumulated genetic modifications, either via changes in the underlying genetic sequence or from epigenetic alterations (e.g., modifications to gene activation- or DNA-related proteins that do not affect the genetic sequence itself).[3,4] Cancers may form tumors in solid organs, such as the lung, brain, or liver, or be present as malignancies in tissues such as the blood or lymph. Tumors and other structures that result from aberrant cell growth, contain heterogeneous cell populations with diverse biological characteristics and potentials. As such, a researcher sequencing all of the genes from tumor specimens of two individuals diagnosed with the same type of lung cancer will identify some consistencies along with many differences. In fact, cancerous tissues are sufficiently heterogeneous that the researcher will likely identify differences in the genetic profiles between several tissue samples from the same specimen. While some groupings of genes allow scientists to classify organ- or tissue-specific cancers into subcategories that may ultimately inform treatment and provide predictive information, the remarkable complexity of cancer biology continues to confound treatment efforts.

Once a cancer has been diagnosed, treatments vary according to cancer type and severity. Surgery, radiation therapy, and systemic treatments such as chemotherapy or hormonal therapy represent traditional approaches designed to remove or kill rapidly-dividing cancer cells. These methods have limitations in clinical use. For example, cancer surgeons may be unable to remove all of the tumor tissue due to its location or extent of spreading. Radiation and chemotherapy, on the other hand, are non-specific strategies – while targeting rapidly-dividing cells, these treatments often destroy healthy tissue as well. Recently, several agents that target specific proteins implicated in cancer-associated molecular pathways have been developed for clinical use. These include trastuzumab, a monoclonal antibody that targets the protein HER2 in breast cancer,[5] gefitinib and erlotnib, which target epidermal growth factor receptor (EGFR) in lung cancer,[6] imatinib, which targets the BCR-ABL tyrosine kinase in chronic myelogenous leukemia,[7] the monoclonal antibodies bevacizumab, which targets vascular endothelial growth factor in colorectal and lung cancer,[8] and cetuximab and panitumumab, which target EGFR in colorectal cancer.[8] These agents have shown that a targeted approach can be successful,

although they are effective only in patients who feature select subclasses of these respective cancers.

All of these treatments are most successful when a cancer is localized; most fail in the metastatic setting.[9] The key to treatment therefore lies in the ability to retard or halt the processes of aberrant cell division common to all cancers. To address this challenge, researchers have sought to understand how tumor cells override the signals for cell division that restrain other cells and how cancer cells can successfully create a neoplasm in a new tissue microenvironment. Based on what is known about tumoral heterogeneity and the replication process, cells that initiate tumor formation must somehow accomplish two feats: 1) generate numerous daughter cells without dying and 2) differentiate into a variety of cell types. Given that stem cells renew themselves indefinitely and have the potential to differentiate into various cell types, researchers have begun to gather evidence to support a "cancer stem cell" (CSC) hypothesis, an evolving theory that explains tumor formation as the outcome of consecutive genetic changes in a small population of stem cell-like, tumor-forming cells.[9–11] This article will discuss the CSC hypothesis and its supporting evidence and provide some perspectives on how CSCs could impact the development of future cancer therapy.

DEFINING THE "CANCER STEM CELL"

A consensus panel convened by the American Association of Cancer Research has defined a CSC as "a cell within a tumor that possesses the capacity to self-renew and to cause the heterogeneous lineages of cancer cells that comprise the tumor."[12] It should be noted that this definition does not indicate the source of these cells – these tumor-forming cells could hypothetically originate from stem, progenitor, or differentiated cells.[13] As such, the terms "tumor-initiating cell" or "cancer-initiating cell" are sometimes used instead of "cancer stem cell" to avoid confusion. Tumors originate from the transformation of normal cells through the accumulation of genetic modifications, but it has not been established unequivocally that stem cells are the origin of all CSCs. The CSC hypothesis therefore does not imply that cancer is always caused by stem cells or that the potential application of stem cells to treat conditions such as heart disease or diabetes as discussed in other chapters of this guide will result in tumor formation. Rather, tumor-initiating

cells possess stem-like characteristics to a degree sufficient to warrant the comparison with stem cells; the observed experimental and clinical behaviors of metastatic cancer cells are highly reminiscent of the classical properties of stem cells.[9]

THE CSC HYPOTHESIS AND THE SEARCH FOR CSCs

The CSC hypothesis suggests that the malignancies associated with cancer originate from a small population of stem-like, tumor-initiating cells. Although cancer researchers first isolated CSCs in 1994,[14] the concept dates to the mid-19th century. In 1855, German pathologist Rudolf Virchow proposed that cancers arise from the activation of dormant, embryonic-like cells present in mature tissue.[15] Virchow argued that cancer does not simply appear spontaneously; rather, cancerous cells, like their non-cancerous counterparts, must originate from other living cells. One hundred and fifty years after Virchow's observation, Lapidot and colleagues provided the first solid evidence to support the CSC hypothesis when they used cell-surface protein markers to identify a relatively rare population of stem-like cells in acute myeloid leukemia (AML).[14] Present in the peripheral blood of persons with leukemia at approximately 1:250,000 cells, these cells could initiate human AML when transplanted into mice with compromised immune systems. Subsequent analysis of populations of leukemia-initiating cells from various AML subtypes indicated that the cells were relatively immature in terms of differentiation.[16] In other words, the cells were "stem-like" – more closely related to primitive blood-forming (hematopoietic) stem cells than to more mature, committed blood cells.

The identification of leukemia-inducing cells has fostered an intense effort to isolate and characterize CSCs in solid tumors. Stem cell-like populations have since been characterized using cell-surface protein markers in tumors of the breast,[17] colon,[18] brain,[19] pancreas,[20,21] and prostate.[22,23] However, identifying markers that unequivocally characterize a population of CSCs remains challenging, even when there is evidence that putative CSCs exist in a given solid tumor type. For example, in hepatocellular carcinoma, cellular analysis suggests the presence of stem-like cells.[24] Definitive markers have yet to be identified to characterize these putative CSCs, although several potential candidates

have been proposed recently.[25,26] In other cancers in which CSCs have yet to be identified, researchers are beginning to link established stem-cell markers with malignant cancer cells. For instance, the proteins Nanog, nucleostemin, and musashi1, which are highly expressed in embryonic stem cells and are critical to maintaining those cells' pluripotency, are also highly expressed in malignant cervical epithelial cells.[27] While this finding does not indicate the existence of cervical cancer CSCs, it suggests that these proteins may play roles in cervical carcinogenesis and progression.

DO CSCs ARISE FROM STEM CELLS?

Given the similarities between tumor-initiating cells and stem cells, researchers have sought to determine whether CSCs arise from stem cells, progenitor cells, or differentiated cells present in adult tissue. Of course, different malignancies may present different answers to this question. The issue is currently under debate,[9,12] and this section will review several theories about the cellular precursors of cancer cells (see Fig. 9.1).
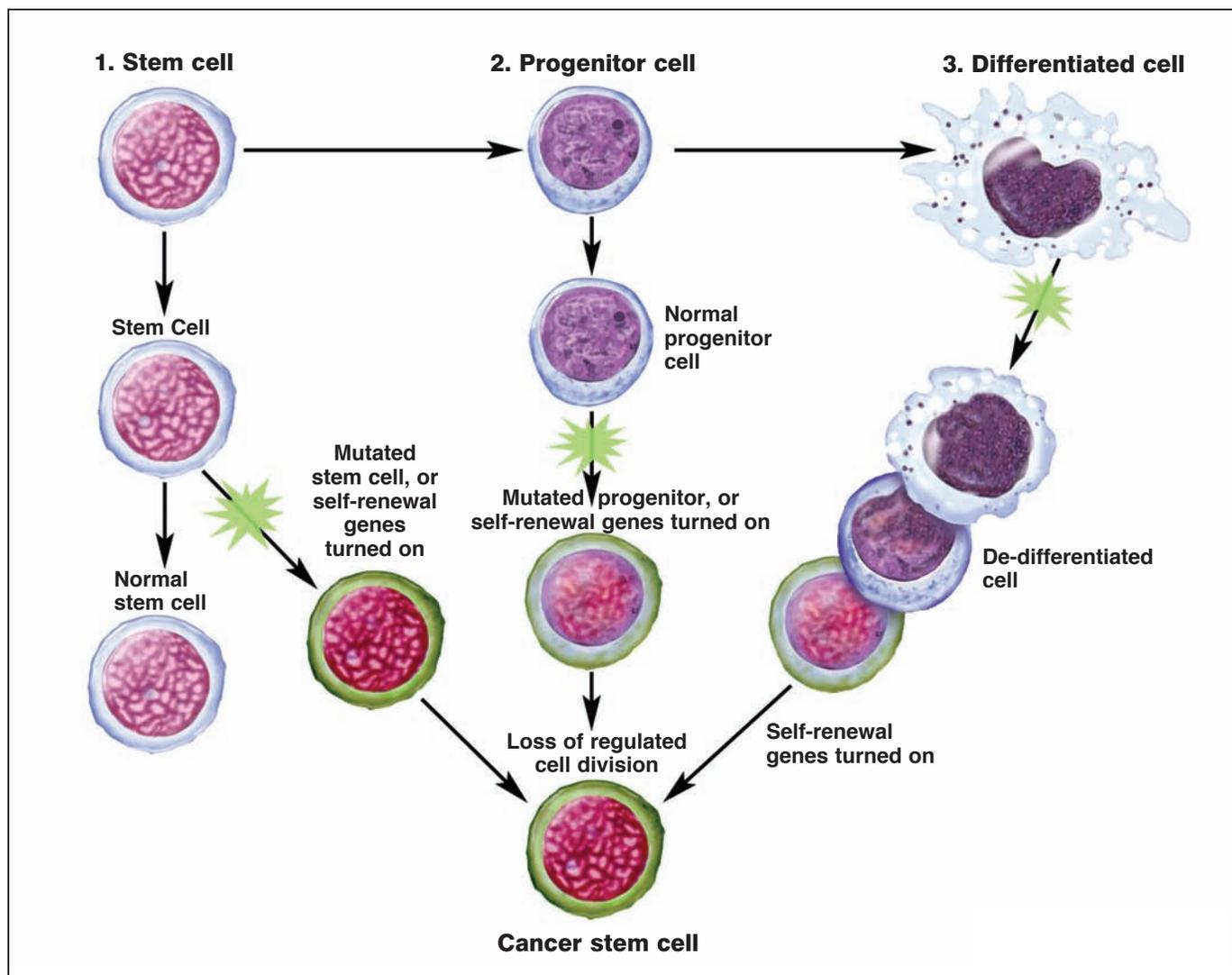

Figure 9.1. How Do Cancer Stem Cells Arise?
The molecular pathways that maintain "stem-ness" in stem cells are also active in numerous cancers. This similarity has led scientists to propose that cancers may arise when some event produces a mutation in a stem cell, robbing it of the ability to regulate cell division. This figure illustrates 3 hypotheses of how a cancer stem cell may arise: (1) A stem cell undergoes a mutation, (2) A progenitor cell undergoes two or more mutations, or (3) A fully differentiated cell undergoes several mutations that drive it back to a stem-like state. In all 3 scenarios, the resultant cancer stem cell has lost the ability to regulate its own cell division.

Hypothesis #1: Cancer Cells Arise from Stem Cells. Stem cells are distinguished from other cells by two characteristics: (1) they can divide to produce copies of themselves, or self-renew, under appropriate conditions and (2) they are pluripotent, or able to differentiate into most, if not all, mature cell types. If CSCs arise from normal stem cells present in the adult tissue, de-differentiation would not be necessary for tumor formation. In this scenario, cancer cells could simply utilize the existing stem-cell regulatory pathways to promote their self-renewal. The ability to self-renew gives stem cells long lifespans relative to those of mature, differentiated cells.[30] It has therefore been hypothesized that the limited lifespan of a mature cell makes it less likely to live long enough to undergo the multiple mutations necessary for tumor formation and metastasis.

Several characteristics of the leukemia-initiating cells support the stem-cell origin hypothesis. Recently, the CSCs associated with AML have been shown to comprise distinct, hierarchically-arranged classes (similar to those observed with hematopoietic stem cells) that dictate distinct fates.[31] To investigate whether these CSCs derive from hematopoietic stem cells, researchers have used a technique known as serial dilution to determine the CSCs' ability to self-renew. Serial dilution involves transplanting cells (usually hematopoietic stem cells, but in this case, CSCs) into a mouse during a bone-marrow transplant. Prior to the transplant, this "primary recipient" mouse's natural supply of hematopoietic stem cells is ablated. If the transplant is successful and if the cells undergo substantial self-renewal, the primary recipient can then become a successful donor for a subsequent, or serial, transplant. Following cell division within primary recipients, a subset of the AML-associated CSCs divided only rarely and underwent self-renewal instead of committing to a lineage. This heterogeneity in self-renewal potential supports the hypothesis that these CSCs derive from normal hematopoietic stem cells.[31] It should be noted, however, that the leukemia-inducing cells are the longest-studied of the known CSCs; the identification and characterization of other CSCs will allow researchers to understand more about the origin of these unique cells.

Hypothesis #2: Cancer Cells Arise from Progenitor Cells. The differentiation pathway from a stem cell to a differentiated cell usually involves one or more intermediate cell types. These intermediate cells, which are more abundant in adult tissue than are stem cells, are called progenitor or precursor cells. They are partly-differentiated cells present in fetal and adult tissues that usually divide to produce mature cells. However, they retain a partial capacity for self-renewal. This property, when considered with their abundance relative to stem cells in adult tissue, has led some researchers to postulate that progenitor cells could be a source of CSCs.[32,33]

Hypothesis #3: Cancer Cells Arise from Differentiated Cells. Some researchers have suggested that cancer cells could arise from mature, differentiated cells that somehow de-differentiate to become more stem cell-like. In this scenario, the requisite oncogenic (cancer-causing) genetic mutations would need to drive the de-differentiation process as well as the subsequent self-renewal of the proliferating cells. This model leaves open the possibility that a relatively large population of cells in the tissue could have tumorigenic potential; a small subset of these would actually initiate the tumor. Specific mechanisms to select which cells would de-differentiate have not been proposed. However, if a tissue contains a sufficient population of differentiated cells, the laws of probability indicate that a small portion of them could, in principle, undergo the sequence of events necessary for de-differentiation. Moreover, this sequence may contain surprisingly few steps; researchers have recently demonstrated that human adult somatic cells can be genetically "re-programmed" into pluripotent human stem cells by applying only four stem-cell factors (see the chapter, "Alternate Methods for Preparing Pluripotent Stem Cells" for detailed discussion of inducing pluripotent stem cells).[28,29]

HOW CANCER STEM CELLS COULD SUPPORT METASTASIS

Metastasis is a complex, multi-step process that involves a specific sequence of events; namely, cancer cells must escape from the original tumor, migrate through the blood or lymph to a new site, adhere to the new site, move from the circulation into the local tissue, form micrometastases, develop a blood supply, and grow to form macroscopic and clinically relevant metastases.[9,34,35] Perhaps not surprisingly, metastasis is highly inefficient.[9] It has been estimated that less than 2% of solitary cells that successfully migrate to a new site are able to initiate growth once there.[34,36,37] Moreover, less than 1% of cells that initiate growth

at the secondary site are able to maintain this growth sufficiently to become macroscopic metastases.[36] These observations suggest that a small, and most likely specialized, subset of cancer cells drives the spread of disease to distant organs.

Some researchers have proposed that these unique cells may be CSCs.[9,30,32,33,38] In this hypothesis, metastatic inefficiency may reflect the relative rarity of CSCs combined with the varying compatibilities of these cells with destination microenvironments. Researchers have demonstrated that stem cells and metastatic cancer cells share several properties that are essential to the metastatic process, including the requirement of a specific microenvironment (or "niche") to support growth and provide protection, the use of specific cellular pathways for migration, enhanced resistance to cell death, and an increased capacity for drug resistance.[9] There is emerging, albeit limited, evidence that these properties may also hold for CSCs.[9] Metastatic sites for a given cancer type could therefore represent those tissues that provide or promote the development of a compatible CSC niche, from which CSCs could expand through normal or dysregulated cellular signaling. Moreover, normal stem cells tend to be quiescent unless activated to divide.[39] If the CSC hypothesis holds true, then undifferentiated, dormant CSCs would be relatively resistant to chemotherapeutic agents, which act mainly on dividing cells.[10] As such, this subpopulation could form the kernel of cells responsible for metastasis and cancer recurrence following treatment and remission.

HOW THE CSC MODEL COULD AFFECT CANCER THERAPY

As noted previously, most contemporary cancer treatments have limited selectivity – systemic therapies and surgeries remove or damage normal tissue in addition to tumor tissue. These methods must therefore be employed judiciously to limit adverse effects associated with treatment. Moreover, these approaches are often only temporarily effective; cancers that appear to be successfully eliminated immediately following treatment may recur at a later time and often do so at a new site. Agents that target molecules implicated in cancer pathways have illustrated the power of a selective approach, and many researchers and drug developers are shifting toward this paradigm. If the CSC hypothesis proves to be correct, then a

strategy designed to target CSCs selectively could potentially stop the "seeds" of the tumor before they have a chance to germinate and spread.

The CSC hypothesis accounts for observed patterns of cancer recurrence and metastasis following an apparently successful therapeutic intervention. In clinical practice, however, some cancers prove quite aggressive, resisting chemotherapy or radiation even when administered at relatively early stages of tumor progression. These tumors therefore have an increased likelihood of metastasizing, confounding further treatment strategies while compromising the cancer patient's quality of life. The presence of CSC in some malignancies may account for some of these metastases. So why do some tumors succumb to therapy, while others resist it? Some scientists have suggested that the tumor aggressiveness may correlate with the proportion of CSCs within a corresponding tumor.[40–42] In this scenario, less aggressive cancers contain fewer CSCs and a greater proportion of therapy-sensitive non-CSCs.[9]

There is also some evidence to suggest that CSCs may be able to selectively resist many current cancer therapies, although this property has yet to be proven in the clinic.[9] For example, normal stem cells and metastatic cancer cells over-express several common, known drug-resistance genes.[43] As a result, breast cancer CSCs express increased levels of several membrane proteins implicated in resistance to chemotherapy.[17] These cells have also been shown to express intercellular signaling molecules such as Hedgehog and Bmi-1,[44] which are essential for promoting self-renewal and proliferation of several types of stem cells.[45] Moreover, experiments in cell lines from breast cancer[46] and glioma[40] have shown that CSCs (as identified by cell-surface markers) are more resistant to radiotherapy than their non-CSC counterparts. In the face of radiation, the CSCs appear to survive preferentially, repair their damaged DNA more efficiently, and begin the process of self-renewal.

These discoveries have led researchers to propose several avenues for treating cancer by targeting molecules involved in CSC renewal and proliferation pathways. Potential strategies include interfering with molecular pathways that increase drug resistance, targeting proteins that may sensitize CSCs to radiation, or restraining the CSCs' self-renewal capacity by modifying their cell differentiation capabilities.[9] In each case, successful development of a therapy

would require additional basic and clinical research. Researchers must characterize the CSCs associated with a given tumor type, identify relevant molecules to target, develop effective agents, and test the agents in pre-clinical models, such as animals or cell lines. However, by targeting fundamental CSC cellular signaling processes, it is possible that a given treatment could be effective against multiple tumor types.

CONCLUSION

Cancer represents a major health challenge for the 21st century. Governed by an intricate, complex interplay of molecular signals, cancers often resist systemic treatments. Yet the uncontrolled cellular growth that characterizes cancers may paradoxically hold the key to understanding the spread of disease. It has long been postulated that tumors form and proliferate from the actions of a small population of unique cells. The observation that metastatic cancer cells exhibit experimental and clinical behaviors highly reminiscent of the classical properties of stem cells has led researchers to search for and to characterize "cancer stem cells" believed to be implicated in the cancer process.

The discovery of CSCs in some tumor types has ushered in a new era of cancer research. Cancer stem cell science is an emerging field that will ultimately impact researchers' understanding of cancer processes and may identify new therapeutic strategies. However, much remains to be learned about these unique cells, which as of yet have not been identified in all tumor types. At present, evidence continues to mount to support a CSC Hypothesis – that cancers are perpetuated by a small population of tumor-initiating cells that exhibit numerous stem cell-like properties. Whether or not the Hypothesis ultimately proves true in all cases, understanding the similarities between cancer cells and stem cells will illuminate many molecular pathways that are triggered in carcinogenesis. Thus, the question, "Are stem cells involved in cancer?" has no simple answer. However, the characterization of CSCs will likely play a role in the development of novel targeted therapies designed to eradicate the most dangerous tumor cells, that may be resistant to current chemotherapy regimens, thereby providing researchers and clinicians with additional targets to alleviate the burden of cancer.

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