REVIEW ARTICLE

MECHANISMS OF DISEASE

Cancer Stem Cells

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THE DEEPENING OF OUR UNDERSTANDING OF NORMAL BIOLOGY HAS MADE it clear that stem cells have a critical role not only in the generation of complex multicellular organisms, but also in the development of tumors. Recent findings support the concept that cells with the properties of stem cells are integral to the development and perpetuation of several forms of human cancer.[1-3] Eradication of the stem-cell compartment of a tumor also may be essential to achieve stable, long-lasting remission, and even a cure, of cancer.[4,5] Advances in our knowledge of the properties of stem cells have made specific targeting and eradication of cancer stem cells a topic of considerable interest. In this article, we discuss the properties of cancer stem cells, outline initial therapeutic strategies against them, and present challenges for the future.

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BACKGROUND AND DEFINITIONS

Stem cells occur in many different somatic tissues and are important participants in their physiology (Fig. 1). Populations of cells that derive from stem cells are organized in a hierarchical fashion, with the stem cell residing at the apex of the developmental pathway (Fig. 2). Stem cells have three distinctive properties: self-renewal (i.e., at cell division, one or both daughter cells retain the same biologic properties as the parent cell), the capability to develop into multiple lineages, and the potential to proliferate extensively. The combination of these three properties makes stem cells unique. The attribute of self-renewal is especially notable, because its subversion is highly relevant to oncogenesis and malignancy.[6,7] Aberrantly increased self-renewal, in combination with the intrinsic growth potential of stem cells, may account for much of what is considered a malignant phenotype.

Many studies performed over the past 30 to 40 years, when viewed collectively, have shown that the characteristics of stem-cell systems, the specific stem-cell properties described above, or both, are relevant to some forms of human cancer.[3,4,8,9] Biologically distinct and relatively rare populations of "tumor-initiating" cells have been identified in cancers of the hematopoietic system, brain, and breast.[10-13] Cells of this type have the capacity for self-renewal, the potential to develop into any cell in the overall tumor population, and the proliferative ability to drive continued expansion of the population of malignant cells. Accordingly, the properties of tumor-initiating cells closely parallel the three features that define normal stem cells. Malignant cells with these functional properties have been termed "cancer stem cells" (Fig. 2).

Given these features, it is possible that cancer stem cells arise by mutation from normal stem cells. However, several lines of evidence indicate that cancer stem cells can also arise from mutated progenitor cells.[14-17] Such progenitors (also known as "transit-amplifying cells") can possess substantial replicative ability, but they do not usually have the self-renewal capacity of stem cells. To become a cancer stem

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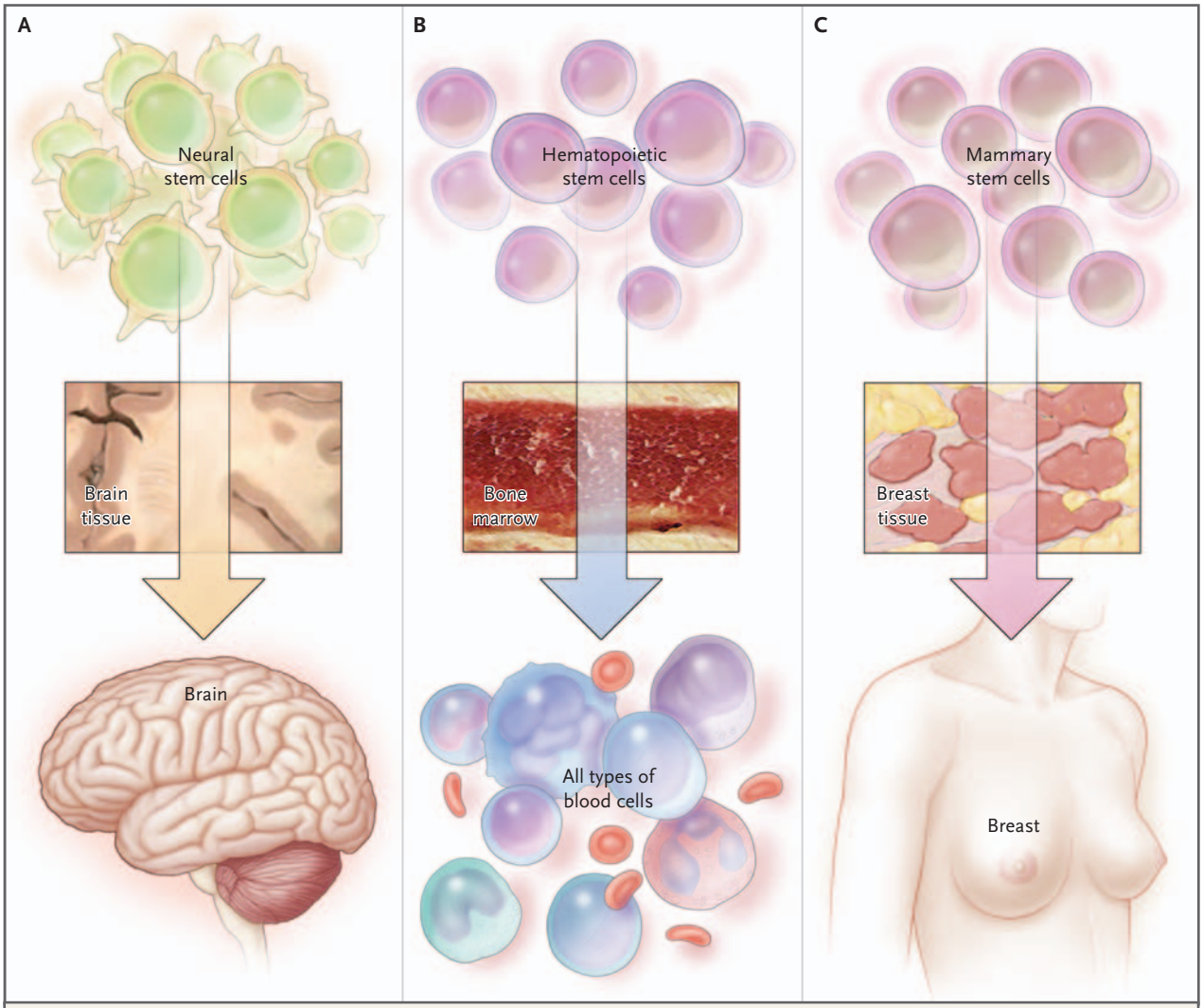

Figure 1. Examples of Stem Cells Found in Adult Somatic Tissues.

Neural stem cells generate cells in the central nervous system (Panel A). Hematopoietic stem cells generate mature blood cells (Panel B). Mammary stem cells generate breast tissue (Panel C).

cell, a progenitor cell must acquire mutations that cause it to regain the property of self-renewal. A detailed discussion of the origins of cancer stem cells is beyond the scope of this review, but it is important to acknowledge the possibility that multiple pathways and processes can give rise to cancer stem cells.

Although specific features of normal stem cells may be preserved to greater or lesser degrees in cancer stem cells, the key issue for consideration with regard to tumor biology is that a small subgroup of the cells in a tumor — the cancer stem cells — are essential for its growth. The concept of cancer stem cells can, however, vary in different contexts. For example, cancer stem cells can be the source of all the malignant cells in a primary tumor, they can compose the small reservoir of drug-resistant cells that are responsible for relapse after a chemotherapy-induced remission, or they can give rise to distant metastases (Fig. 3). The biologic features of cancer stem cells in each of these instances may differ, suggesting that the acquisition of features associated with tumor progression, such as genetic instability and drug resistance, will also be associated with cancer stem cells.

It is becoming evident that a cancer treatment that fails to eliminate cancer stem cells may allow

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regrowth of the tumor. In cases in which bulk disease is eradicated and chemotherapy is given, only to be followed by a relapse, a plausible explanation is that the cancer stem cells have not been completely destroyed (Fig. 3B). Therapeutic strategies that specifically target cancer stem cells should eradicate tumors more effectively than current treatments and reduce the risk of relapse and metastasis.

CANCER STEM CELLS IN THE HEMATOPOIETIC SYSTEM

The hematopoietic system is the best characterized somatic tissue with respect to stem-cell biology. Over the past several decades, many of the physical, biologic, and developmental features of normal hematopoietic stem cells have been defined[18,19] and useful methods for studying stem cells in almost any context have been established. Hematopoietic-cell cancers such as leukemia are clearly different from solid tumors, but certain aspects of hematopoietic stem-cell biology are relevant to our understanding of the broad principles of cancer stem-cell biology.[6] In various types of leukemia, cancer stem cells have been unequivocally identified, and several biologic properties of these stem cells have been found to have direct implications for therapy.[1,20-22]

Cancer stem cells are readily evident in chronic myelogenous leukemia (CML)[23] and acute myelogenous leukemia (AML),[10,11] and they have been implicated in acute lymphoblastic leukemia (ALL).[24-26] CML stem cells have a well-described stem-cell phenotype and a quiescent cell-cycle status. Similarly, AML stem cells are mostly quiescent,[27-30] suggesting that conventional antiproliferative cytotoxic regimens are unlikely to be effective against them. AML stem cells have surface markers, such as the interleukin-3–receptor α chain, that are not present on normal stem cells.[31] These markers may be useful for antibody-based[32] or other related therapeutic regimens.[33,34] Early efforts have demonstrated the usefulness of antibodies against the CD33 antigen in the treatment of AML,[35,36] and recent reports indicate that CD33 is expressed on some leukemia stem cells.[37] Continued development of immunotherapy against stem-cell–specific antigens is warranted.

There has been extensive research on drugs that specifically modulate pathways implicated in leukemia-cell growth (i.e., "targeted" agents).[38,39]

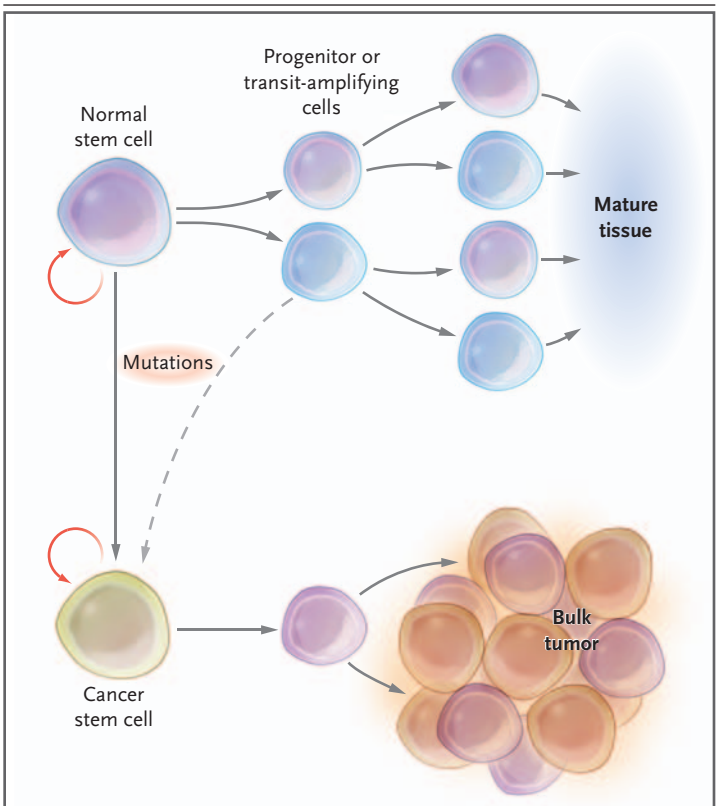

Figure 2. Stem-Cell Systems.

Normal tissues arise from a central stem cell that grows and differentiates to create progenitor and mature cell populations. Key properties of normal stem cells are the ability to self-renew (indicated by curved arrow), multilineage potential (indicated by cells of different colors), and extensive proliferative capacity. Cancer stem cells arise by means of a mutation in normal stem cells or progenitor cells, and subsequently grow and differentiate to create primary tumors (the broken arrow indicates that specific types of progenitors involved in the generation of cancer stem cells are unclear). Like normal stem cells, cancer stem cells can self-renew, give rise to heterogeneous populations of daughter cells, and proliferate extensively.

Use of the ABL kinase inhibitor imatinib mesylate (Gleevec) to treat CML has had particularly interesting results.[40] Despite the remarkable clinical responses achieved with imatinib, however, residual disease persists in many patients. In vitro studies indicate that inhibition of the CML translocation product BCR-ABL is sufficient to eradicate most or all leukemia cells, but the drug does not appear to kill CML stem cells.[41] Imatinib primarily affects the progeny of cancer stem cells, so CML usually recurs when therapy is discontinued.[42] Furthermore, although the newly approved CML agent dasatinib is effective for imatinib-resistant disease, recent data suggest that it too may fail to eradicate CML stem cells.[43]

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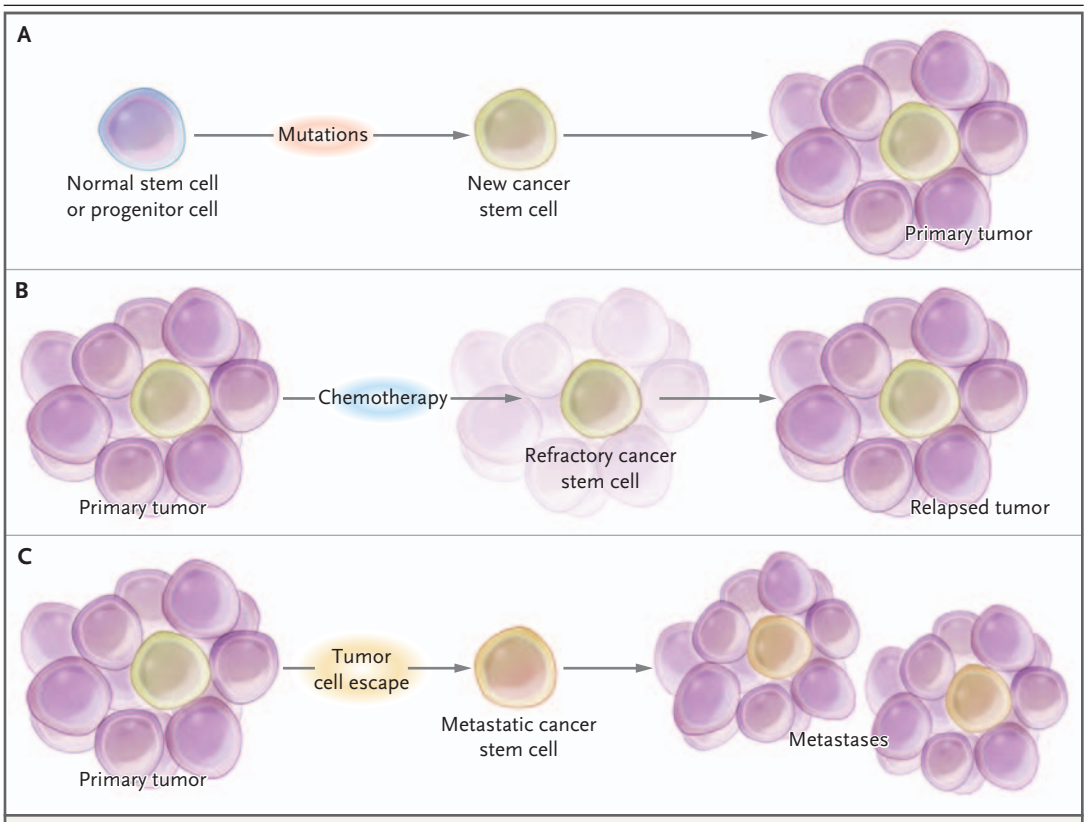

Figure 3. Scenarios Involving Cancer Stem Cells.

For tumors in which cancer stem cells play a role, at least three scenarios are possible. First, mutation of a normal stem cell or progenitor cell may create a cancer stem cell, which will then generate a primary tumor (Panel A). Second, during treatment with chemotherapy, the majority of cells in a primary tumor may be destroyed, but if the cancer stem cells are not eradicated, the tumor may regrow and cause a relapse (Panel B). Third, cancer stem cells arising from a primary tumor may emigrate to distal sites and create metastatic lesions (Panel C).

Unique molecular features of leukemia stem cells may provide opportunities for therapeutic intervention. For example, there is evidence of constitutive activation of both the nuclear factor-κB (NF-κB) and phosphatidylinositol 3′ (PI3) kinase signaling pathways in AML stem cells.[28,44] Neither NF-κB nor PI3 kinase activity is detectable in resting, normal hematopoietic stem cells, so both of these molecular factors could be tumor-specific targets. Two studies with different methods of pharmacological inhibition of NF-κB have reported specific eradication of AML stem cells in vitro, without apparent harm to normal hematopoietic stem cells.[45,46] A separate study demonstrated that inhibition of PI3 kinase reduced the growth of AML stem cells.[44] Similarly, inhibition of the downstream PI3-kinase mammalian target of rapamycin (mTOR) appears to enhance the activity of the chemotherapeutic agent etoposide against AML stem cells.[47] Inhibition of mTOR also blocks the growth of leukemia-initiating cells in a mouse model of AML.[48] Taken together, these findings indicate that leukemia stem-cell–specific therapies may be attainable.

CANCER STEM CELLS IN THE CENTRAL NERVOUS SYSTEM

Isolation of cancer stem cells of the central nervous system (CNS) has been achieved by means of antigenic markers and by exploiting in vitro culture conditions developed for normal neural stem cells. As was first observed in 1992,[49,50] CNS cells grown on nonadherent surfaces give rise to balls of cells (neurospheres) that have the capacity for self-renewal and can generate all of the principal cell types of the brain (i.e., neurons, astrocytes, and oligodendrocytes). Neurospheres in which the stem-cell compartment is maintained

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can be repeatedly split apart into single cells; a small fraction of these cells can generate a new neurosphere (Fig. 4). This capacity for repeated generation of neurospheres from single cells is generally viewed as evidence of self-renewal.[51,52] More recent studies have demonstrated that normal neural stem cells express a cell-surface protein that can be detected with an antibody against the AC133 (CD133) epitope,[53] a marker commonly found on stem cells and progenitor cells in various tissues.[54]

Application of the strategies used to generate neurospheres to specimens obtained from gliomas[55] or purification of CD133-positive cells from human gliomas[56] allows for the isolation and growth of tumor stem-cell populations. In both cases, the cancer stem-cell population is essential for establishing a tumor in vivo. Transplantation of as few as 100 CD133-positive human glioma cells into the brains of immunodeficient mice initiates the development of a glioma, whereas no tumors result from transplantation of 10^5 CD133-negative cells from the same tumors.[12]

Many studies have demonstrated that the expression of stem-cell–like properties in CNS tumor cells does not necessarily suggest that these cells originated from stem cells. In experimental systems, the expression of cooperating oncogenes in lineage-restricted progenitor cells of the CNS can yield tumors with the cytopathological characteristics of the most malignant CNS tumor (i.e, glioblastoma multiforme). For example, expression of the *ras* and *myc* oncogenes in oligodendrocyte progenitors yields cells that readily form tumors when transplanted in vivo.[57] These studies suggest that a cancer stem cell need not be derived from a bona fide tissue-specific stem cell, but instead can arise from a committed progenitor cell that acquired stem-cell–like properties when it underwent oncogenic transformation.

From a therapeutic perspective, the development of treatments directed against cancer stem cells in the brain is likely to progress substantially during the next several years. The state of knowledge of the stem cells and progenitor cells that build the CNS is sufficiently advanced to permit side-by-side analysis of these populations of cells with CNS tumor cells. Furthermore, a powerful advantage of studies of the CNS is that all of the major precursor (i.e., replicating) populations can be grown as purified populations with the capacity for extended division of stem cells and progenitor cells in vitro.[58-61] Therefore, it should be feasible to conduct high-throughput in vitro analyses to search for compounds that selectively kill cancer stem cells without killing the normal cells of the CNS.

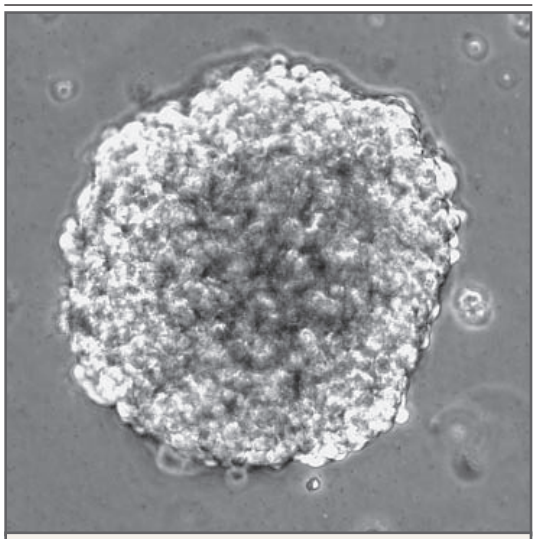

Figure 4. Primary Human Neurosphere.

CANCER STEM CELLS IN THE BREAST

In addition to cancers of the hematopoietic system and the CNS, the third major human cancer in which cancer stem cells have been definitively identified is breast cancer. Studies by Al-Hajj et al. of specimens from patients with advanced stages of metastatic breast cancer demonstrated that cells with a specific cell-surface antigen profile (CD44-positive and CD24-negative) could successfully establish themselves as tumor xenografts.[13] The experiments were conducted with immunodeficient mice, and the cells were transplanted into the mammary fat pad to provide an environment similar to that in human breast cancer. As observed for analogous studies in AML and gliomas, only the relatively rare subgroup of cancer stem cells could successfully propagate the tumor in vivo, whereas the majority of malignant cells failed to recapitulate the tumor. Furthermore, the purified CD44-positive and CD24-negative cells could differentiate and give rise to cells similar to those found in the bulk tumor population.

Definition of the characteristics of both normal cells and cancer stem cells in the breast has advanced rapidly.[62-67] Recent studies have provided detailed characterizations of normal breast

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stem cells in mice and have demonstrated the functional potential of such cells by virtue of their ability to completely regenerate a mammary gland when transplanted into a suitable host environment.[68,69] With the experimental tools developed for characterization of normal mammary stem cells, further elucidation of the biologic properties of breast-cancer stem cells should be forthcoming.

CHALLENGES FOR THERAPY TARGETED AGAINST CANCER STEM CELLS

The development of treatments that target cancer stem cells is an important objective, but the challenges are formidable. First, to design treatments that selectively eradicate cancer stem cells, it is useful to have the cognate normal stem cell or progenitor cell. This step requires the development of assays to characterize the function of normal stem cells and the means to define physical features (i.e., cell-surface antigen markers) that will permit their isolation. Without this knowledge, it is impossible to know whether a candidate drug is also cytotoxic to normal stem cells. Second, we need similar ways to describe cancer stem cells and appropriate functional assays must be validated. Third, it is critical to understand how cancer stem cells differ from normal stem cells, particularly with regard to mechanisms controlling cell survival and responses to injury. Ideally, a therapy should target pathways uniquely used by cancer stem cells to resist extrinsic insults or to maintain steady-state viability. Fourth, we must understand how therapies that effectively target the bulk of tumor cells fail to eradicate cancer stem cells. The reasons for this phenomenon may provide important clues for developing more effective and comprehensive regimens to attack both the tumor stem cells and the bulk of the disease.

An additional challenge in targeting cancer stem cells is to understand how the properties of stem cells make them particularly difficult to kill. Leukemia cancer stem cells reside in a largely quiescent state with regard to cell-cycle activity,[27,30] like their normal counterparts. Consequently, typical cytotoxic regimens that target rapidly dividing cells are unlikely to eradicate such cells. Selective targeting will therefore require regimens that kill cells independently of the cell cycle, or that selectively induce cycling of cancer stem cells. Another common feature of stem cells is expression of proteins associated with the efflux of xenobiotic toxins (e.g., multidrug-resistant proteins and related members of the ATP-binding cassette [ABC] transporter family). A variety of cancer cells, particularly during relapse, express such proteins, thus providing resistance to many chemotherapeutic agents.[70-73] The extent to which cancer stem cells can mobilize all of the measures provided by evolutionary history to protect normal stem cells is not yet known, but this information is likely to be biologically and clinically significant.

A further concern is that normal stem cells and progenitor cells may prove to be more sensitive than cancer stem cells to the effects of chemotherapy. Normal colon stem cells, for example, can inhibit DNA repair mechanisms and thereby undergo apoptosis in response to DNA damage; this mechanism avoids the accumulation of harmful mutations.[74] If, however, colon-cancer cells evade this protective mechanism, then chemotherapy could preferentially spare them. Recent studies have demonstrated that normal hematopoietic stem cells undergo premature senescence (i.e., cellular "aging") when exposed to ionizing radiation or busulfan.[75,76] This process impairs the growth and developmental potential of hematopoietic stem cells. If leukemia stem cells fail to undergo senescence, as predicted by recent studies of the genesis of cancer,[77,78] then we would expect that malignant stem cells would actually have a growth advantage after treatment with certain agents. Furthermore, it is plausible that successive cycles of chemotherapy only exacerbate the situation by increasing harm to the normal stem-cell pool (by inducing senescence) and concomitantly increasing the growth advantage of cancer stem cells, which are resistant to senescence. Clearly, a better understanding of normal and tumor stem cells is of great importance not only in designing new therapies, but also in understanding the biologic and clinical consequences of existing regimens.

If a clinical remission is achieved, the presence of residual drug-resistant cancer stem cells can initiate a relapse. Hence, we must develop better methods for detection and quantitation of cancer stem cells in patients receiving cancer therapy. Intriguing findings in leukemia indicate that the level of residual disease directly correlates with the long-term outcome[79,80]: if the number of primitive leukemia cells can be reduced below critical threshold levels, it may not be necessary

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to completely eradicate the malignant clone. Whether such residual cells are truly cancer stem cells remains to be determined, but the findings nonetheless suggest that sensitive real-time methods of cancer stem-cell detection are an important priority.

In designing specific regimens for cancer stem cells, several strategies should be considered. Given the likelihood that aberrant regulation of self-renewal is central to cancer stem-cell pathology, targeting pathways that mediate self-renewal is an attractive option. An important unknown factor is the degree to which inhibition of self-renewal mechanisms can be tolerated, because the pathways controlling self-renewal are central to a variety of biologic functions. However, even if the targeting of self-renewal pathways is feasible, we do not know whether it would kill cancer stem cells or simply suppress them. For these reasons, an alternative is to interfere with cancer stem-cell–specific survival pathways. For example, strategies that inhibit survival mechanisms or the oxidative state of the cell may be selectively cytotoxic to leukemia stem cells.[21] Antibody-based or ligand-based therapy also appears to be a promising way to destroy cancer stem cells. A small number of target antigens on cancer stem cells have been described, and with further characterization of purified populations, additional targets are likely to become available. It remains to be determined, however, whether these and other targets will distinguish cancer stem cells from normal tissues.

SUMMARY

There is now abundant evidence that stem-cell properties are highly relevant to the biology of several human cancers. However, many key questions remain. At the most fundamental level, we must determine to what extent stem-cell biology is relevant to all the major forms of human cancer. For this reason, it is premature to overstate the general role of stem cells in cancer. Nonetheless, the eradication of cancer stem cells will be necessary to improve the outcome of treatment for at least some cancers. An interesting question is whether different types of cancer stem cells have the same Achilles' heel; it should be possible to determine whether the same tumor-specific mechanisms of growth and survival are active across multiple cancer types. Because certain features of normal stem cells are conserved in different tissues,[81] determining whether there is similar conservation among cancer stem cells will be useful in the design of new therapies.

Another important issue to investigate is how existing chemotherapy agents affect the evolution of cancer stem cells during conventional treatment regimens. This question relates to both the sensitivity of normal stem cells, as compared with malignant ones, and the mechanisms by which drug resistance may arise. Do current forms of treatment provide a competitive advantage for cancer stem cells, and if so, does that selective pressure drive the emergence of drug resistance in cancer stem cells?

Finally, it will be critical to evaluate the clinical end points by which treatment success should be measured. The eradication of bulk disease is not likely to predict the efficacy of drug regimens for rare cancer cells. Therefore, the development of assays that measure the survival of cancer stem cells will be important for assessing the potential of new targeted regimens.

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