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Cancer stem cells: a model in the making

Lauren L Campbell Marotta[1] and Kornelia Polyak[1,2]

Cancer stem cells and their potential roles in tumor heterogeneity are currently subjects of intense investigation. Studies suggest that these cells may develop from any normal cell and have begun to elucidate their molecular profiles. The percentage of a tumor composed of cancer stem cells varies greatly, and researchers believe that multiple types of these cells may exist in a single neoplasm. Cancer stem cells may be formed by epithelial–mesenchymal transition and seem to be less prevalent in metastases than in corresponding primary tumors. These cells appear to have therapeutic sensitivities different from those of cancer cells with more differentiated features. Looking into the many questions that remain about the cancer stem cells model might lead to more effective cancer prevention, diagnosis, and treatment.

Addresses
[1] Department of Medical Oncology, Dana-Farber Cancer Institute, Program in Biological and Biomedical Sciences, Harvard Medical School, 44 Binney Street, D740C, Boston, MA 02115, USA
[2] Department of Medical Oncology, Dana-Farber Cancer Institute, Program in Biological and Biomedical Sciences and Department of Medicine Harvard Medical School, 44 Binney Street, D740C, Boston, MA 02115, USA

Corresponding author: Polyak, Kornelia (kornelia_polyak@dfci.harvard.edu)

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Introduction

Decades of scientific research and clinical observations have revealed much about cancer [1–3]. This disease originates when a single cell accumulates multiple mutations that together drive uncontrolled proliferation, resistance to apoptosis, and other hallmarks of malignancy. Cancer cells in resultant tumors undergo additional genetic or epigenetic changes and interact with their individual microenvironments, leading to a constantly changing variety of tumor cell types within a single neoplasm. This shifting in intra-tumoral heterogeneity, which has been proposed to also be due to the continuous selection of dominant clones [4] and the differentiation of malignant stem cells [5], underlies tumor progression and resistance to treatment and results in inter-tumoral heterogeneity. On the basis of their overall nature, tumors can be classified by organ of origin, tissue type, phenotypic subtype, and stage of progression. Knowledge of the characteristics of cancer has led to improved survival of patients, but many currently used therapies have detrimental side effects, and mortality rates are still high because of metastasis and recurrence. Therefore, a more complete understanding of cancer is needed.

Recently, the study of 'cancer stem cells' has become a popular area of cancer research, as shown by increasing numbers of articles and patents related to this field in the past few years (Table 1). By definition, cancer stem cells are a subset of cancer cells with the stem-cell-like ability to produce all cancer cell types found in a tumor, which may include cells with more differentiated features [6]. They are not necessarily derived from normal stem cells, and it is unclear whether they always have the ability to differentiate or other normal stem cell characteristics. Thus, the ''cancer stem cell'' name is more of a reflection of stem-cell-like phenotype than of true stemness. The idea that transformed stem cells are the root of cancer was proposed over a century ago [7], but cancer stem cells were not first identified until 1994 when researchers found that a purified acute myeloid leukemia cell population expressing particular cell surface markers could efficiently form tumors when injected into mice while other cell populations from the same cancer sample could not [8]. Since then, using this assay, cancer stem cells have been identified in tumors from many organs, including breast, brain, prostate, pancreas, head and neck, colon, lung, skin, liver, and ovary [9–17,18•].

Owing to their ability to initiate tumors, cancer stem cells are proposed to play roles in oncogenesis, tumor growth, metastasis, and cancer recurrence. The goal of this review is to explain why and how cancer stem cells are currently thought to be involved in each of these tumorigenic processes, focusing primarily on results from the past two years.

Cancer stem cells in oncogenesis

Cancer stem cells are likely involved in oncogenesis since the first cancer cells must give rise to all other cancer cell types, but which normal cells they are derived from as well as their exact molecular profiles are unclear (Figure 1a).

Adult stem cells, their derivative progenitor cells, or more differentiated cells may become cancer stem cells to start cancer. Adult stem cells are present in virtually all tissues

Table 1

Articles and patents related to cancer stem cells, 2001–2007, by year

Year	Articles	Patents
2001	1	0
2002	1	0
2003	7	5
2004	33	3
2005	74	9
2006	134	15
2007	257	40

The numbers of articles listed are the journal publications found by a PubMed search for 'cancer stem cells' or 'cancer stem cell,' and the numbers of patents listed are those relating to cancer stem cells according to the Delphion intellectual property network's patent search database [52].

[19], and they are long-lived, making them more likely than other cells to acquire the multiple mutations needed to become cancer. Many cancer stem cells express markers associated with adult stem cells, such as CD133 [20] and ALDH1 [21]. Cancer stem cells and normal stem cells also appear to share similar epigenetic profiles [22], gene expression profiles [23•], and activated signaling pathways, such as Notch, Hedgehog, and Wnt [24]. In acute myeloid leukemia, progenitor cells might acquire the mutations needed to become cancer stem cells, since these cells have a phenotype similar that of progenitor cells [25]. Differentiated cells that are mutated might also acquire the properties of progenitor or stem cells and be the cells from which cancer stem cells arise. It is possible that adult stem cells could acquire the first genetic or epigenetic changes and that additional mutations could accumulate in a progenitor or more differentiated daughter cell [26]. The exact cell-of-origin, acquired genetic and epigenetic alterations, and microenvironmental influences that combine to produce a cancer stem cell likely determine what markers the cancer stem cell expresses and what type of tumor will form from it.

It is not clear whether cancer stem cells retain all of their features as tumors evolve; therefore, cancer stem cells purified from human tumors may not be identical to the ones that were present earlier in tumor development. Thus, the exact molecular profiles and other characteristics of the cancer stem cells involved in oncogenesis are unclear. As far as cell surface marker proteins go, ones that have been observed on cancer stem cells from multiple tissue types, such as CD133 and CD44, are most likely to be true markers of the cancer stem cells involved in oncogenesis on account of the reproducibility of their presence on cancer stem cells. However, these markers could instead reflect the ability of certain cells to survive purification procedures or initiate tumor growth in mice. Many researchers have noted the limitations of the mouse injection assay used to identify cancer stem cells: human cancer cells are being transplanted into a particular site of a specific type of normal mouse without potentially important accessory cells and with an impaired or incompatible immune system [27–29].

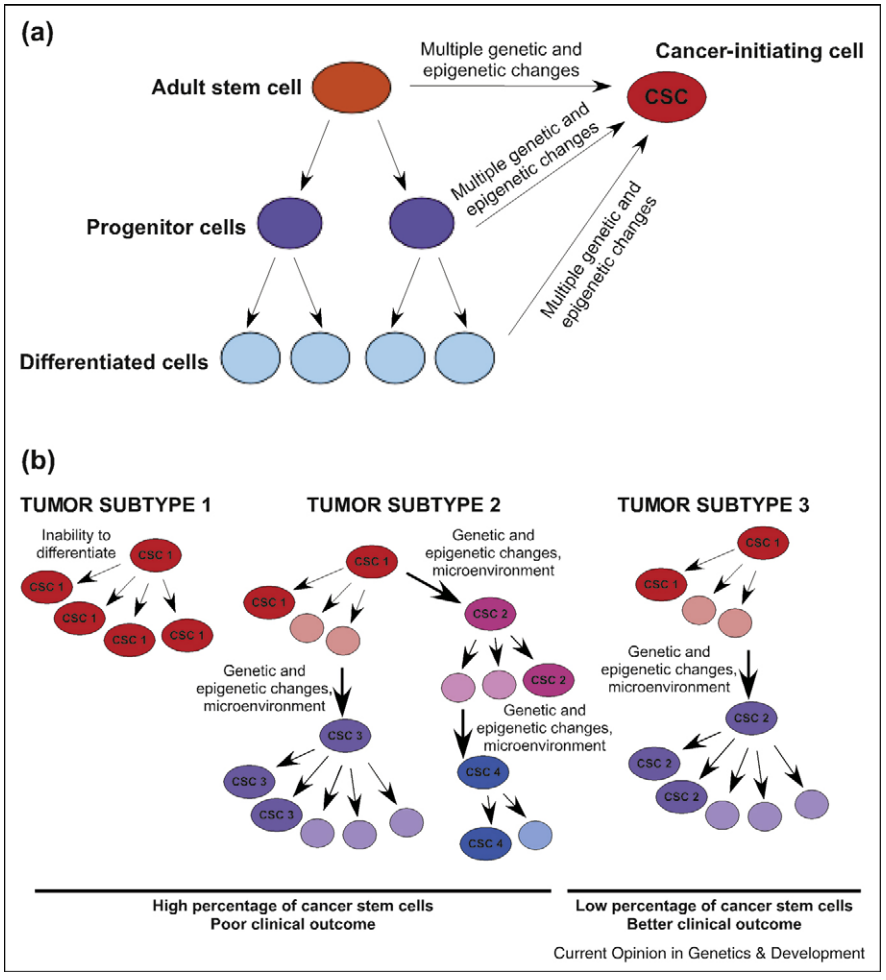
Cancer stem cells in tumor growth

Cancer stem cells are proposed to be involved in tumor growth, but the number of distinct types of cancer stem cells involved in this process and the significance of the different percentages of cancer stem cells found in tumors are unclear (Figure 1b).

More than one population of cancer stem cells are likely involved in the life of a tumor. Because cancer cells are constantly evolving via the development of new genetic and epigenetic changes and the influence of their microenvironments, any one of them can potentially become a cancer stem cell [30•]. Also, cancer stem cells themselves might acquire new alterations, as shown for leukemia cancer stem cells that underwent rearrangement of their immunoglobulin H genes [31••]. New cancer stem cells may end up being more, less, or equally as prevalent as the original cancer stem cells in the tumor in which they reside. Patterns of mutations seen within various regions in a single tumor indicate that there are multiple clonal cancer cell populations, some of which could be cancer stem cells. The use of many different markers to isolate cancer stem cells may reflect the diversity of these cells. Many populations of tumorigenic cells are likely missed because of the way in which cancer stem cells are identified, including the fact that tumor tissue samples may not be representative of the whole [32].

Tumors appear to vary in the percentage of cancer stem cells that they contain, with reported values ranging from 0.03% [25] to nearly 100% [53]. This percentage is likely determined by the particular characteristics of the cancer stem cell that initiated the tumor as well as by the microenvironment, in part by influencing how often additional cancer stem cells are created. Importantly, the way in which the mouse injection assay used to identify cancer stem cells is performed can significantly affect the estimated number of cancer stem cells in a tumor ([53]; JE Dick, unpublished). The extent to which cancer stem cell expression profiles are detectable in whole-tumor gene expression data seem to correlate with patient prognosis [23•,33], indicating that the percentage of cancer stem cells in a tumor may represent its tumor subtype or stage of progression, with more cancer stem cells, in general, corresponding to poor clinical outcome. The presence of high numbers of cancer stem cells may indicate higher proliferation rates of these cells, a more genetically unstable tumor, their lack of differentiation possibly owing to the inability to give rise to more differentiated progeny, or their selective advantage under certain microenvironmental conditions such as the presence of cancer treatment.

Figure 1



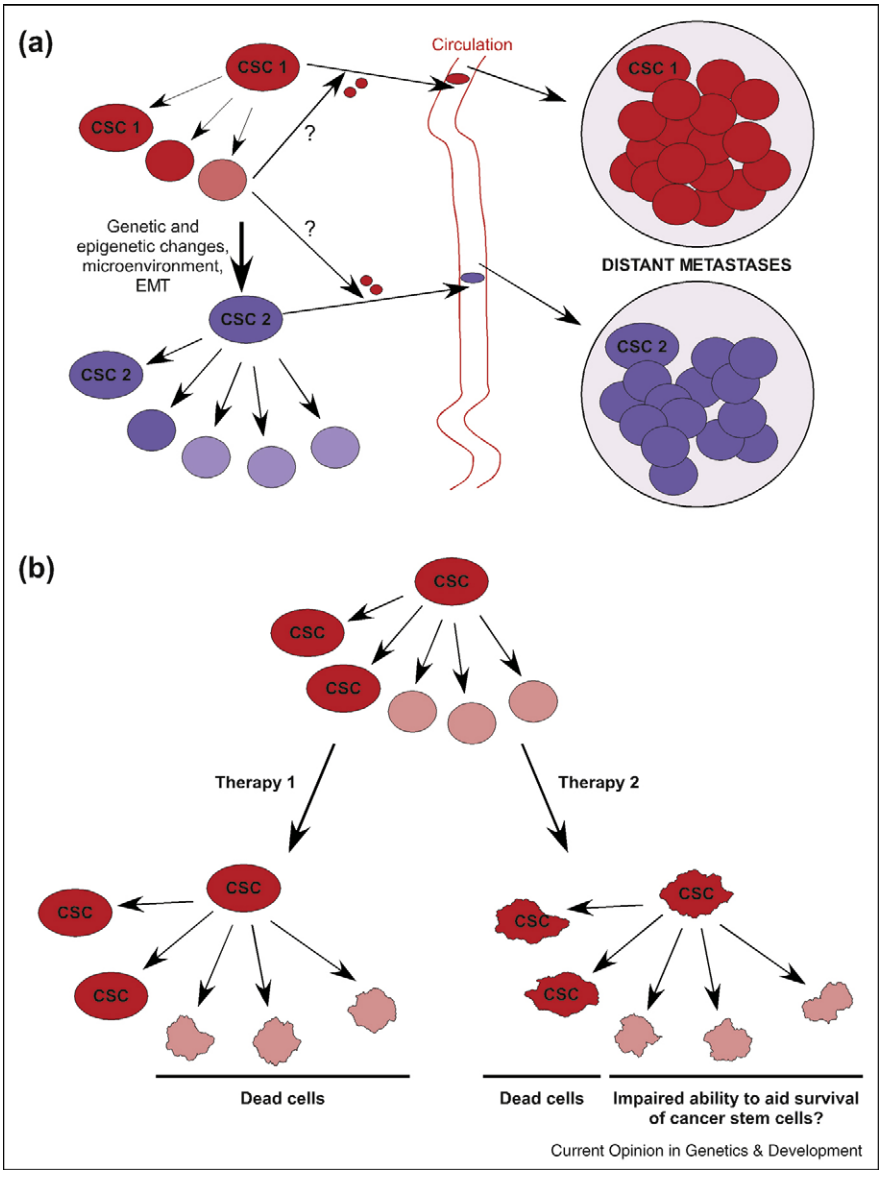
A current view of the cancer stem cells model, part 1. **(a)** Adult stem cells, progenitor cells, or differentiated cells may acquire the multiple genetic and epigenetic alterations required to become the cancer stem cell (CSC) involved in oncogenesis. This cancer-initiating cell may share some characteristics with the adult stem cells residing in the organ in which they are created, either because they originate from these cells or because they gain the properties of them. **(b)** During tumor growth, any cancer cell may develop new genetic or epigenetic alterations or be affected by the microenvironment, resulting in its change to a new type of cancer stem cell. The different cancer stem cells in a tumor will develop clonal cell populations of different sizes containing cancer stem cells and differentiated cells to varying degrees. The total percentage of a tumor that is made up of cancer stem cells may determine its subtype and associated clinical outcome.

Cancer stem cells in metastasis

Cancer stem cells are probably involved in metastasis since this involves the formation of a new tumor, but the identity of the particular cells involved and the mechanisms by which they populate metastases are unclear (Figure 2a).

There are several options for how cancer stem cells may participate in metastasis. First, the original cancer stem cells that started a primary tumor might do so, resulting in primary and metastatic tumors that evolve in parallel rather than sequential processes. Second, a new type of cancer stem cell derived from the first one or another cell in the tumor that acquires metastatic traits could be involved. Owing to additional genetic and epigenetic alterations, these cells may have a selective advantage over the original cancer stem cells or be more invasive than them and therefore more likely to metastasize. The formation of such a 'metastatic cancer stem cell' might be observed as epithelial–mesenchymal transition (EMT). EMT is often seen at the invading edge of tumors and is thought to play a role in some forms of metastasis [31••]. Furthermore, it was recently shown that EMT endows cells with properties of cancer stem cells and that putative breast cancer stem cells express EMT markers, strengthening the link between EMT and cancer stem cells [34]. In addition, a distinct subset of cancer stem cells found at the invasive edge of pancreatic carcinomas

Figure 2



A current view of the cancer stem cells model, part 2. **(a)** Metastasis might be carried out by the original cancer stem cells in a tumor or by a new cancer stem cell population, with or without additional cancer cells. The new cancer stem cells might be formed by EMT. In metastases, microenvironmental influences shift the cell population toward a more differentiated phenotype. **(b)** Cancer stem cells in particular are resistant to some types of therapy, such as radiation, resulting in cancer recurrence. However, these cells are sensitive to other types of therapy, such as the drug lapatinib. Such treatments may kill cancer stem cells directly or through indirect effects on other cells that support their survival.

was found to be essential for metastasis [35••]. Finally, a cancer stem cell may metastasize together with another type of cancer cell.

Once cancer stem cells have metastasized, they must contribute to neoplastic growth in the new location. It appears that distant metastases contain a smaller percentage of cancer stem cells and a higher percentage of more differentiated cancer cells than do their corresponding primary tumors [22,23•]. Therefore, the cell or cells that metastasize and grow in the location of a metastasis are likely influenced by the new microenvironment, which may include cancer treatment, to either acquire or maintain the more differentiated cell phenotype or to differentiate.

Cancer stem cells in cancer recurrence

Cancer stem cells are thought to be involved in cancer recurrence owing to their tumorigenic properties and

supposed resistance to many conventional therapies, and recent data showing that cancer stem cells in particular seem to be resistant to some treatments yet sensitive to others support this notion (Figure 2b).

Numerous studies have lately indicated that cancer stem cells are resistant to some treatments, supporting the idea that they may evade traditional therapies and cause cancer recurrence. Breast cancer stem cells grown in culture were resistant to chemotherapeutic agents [36], and cancer stem cells from leukemia were found to be resistant to the chemotherapy drugs, daunorubicin and Ara-C [37]. Pancreas and colon cancer stem cells are also resistant to chemotherapy [35••,38], and some cancer stem cells have been shown to be resistant to radiation [39,40]. Additionally, breast cancer patients were found to have higher percentages of cells with breast cancer stem cell properties after chemotherapy treatment [41], suggesting that therapy may have been less effective at killing cancer stem cells than at eliminating other cancer cells. However, this study relied on the measurement of cell surface proteins in patient biopsies, so it is unclear whether the measured proteins were actually present on clonally related cancer cell populations, whether the pattern of their expression was changed by treatment, and whether cells were differentially sensitive to treatment in patients who demonstrated complete response to therapy. The mechanisms of drug resistance in cancer stem cells are not well-understood, but possible explanations include the overexpression of proteins that pump drugs out of cells, enzymes that metabolize drugs, or antiapoptotic proteins [42].

There have been many recent reports of drugs that seem to specifically target cancer stem cells, suggesting that these cells may be eliminated in patients. For instance, parthenolide and rapamycin appear to kill cancer stem cells from acute myeloid leukemia but not normal hematopoietic stem cells [43,44]. Also, temozolomide preferentially eliminates cancer stem cells in glioblastoma [45], and brain cancer stem cells treated with bevacizumab have decreased tumorigenicity [46]. In several cases, particular cancer-specific genetic alterations have been inhibited or introduced to successfully kill cancer stem cells, demonstrating that the targeting of a mutation present in all tumor cells can be an effective therapeutic approach and that both cancer stem cells and more differentiated cells may be involved in cancer recurrence. In mice, pharmacological inhibition of promyelocytic leukemia protein, which results from a translocation, targets cancer stem cells in leukemia [47]. Also in mice, removal of beta-catenin from skin tumors results in the loss of cancer stem cells [48]. In these studies, the loss of cancer stem cells was able to lead to the elimination of cancer recurrence in mice, essentially a complete cure. But perhaps the most striking example of targeting a particular cancer-specific genetic alteration to eliminate cancer stem cells is the use of the HER1/HER2 inhibitor lapatinib in HER2-positive breast cancer, which involves the amplification of the HER2 gene. In human patients, cells with a breast cancer stem cell phenotype increased after chemotherapy treatment but decreased after lapatinib treatment, and some patients had no signs of any remaining tumor after follow-up chemotherapy [49••]. Although the decrease observed was not statistically

Table 2

Summary of questions remaining about cancer stem cells

Unanswered questions	Possible approaches
What are the defining characteristics of cancer stem cells?	Search for more accurate markers using varied and humanized mouse systems and combinations of proteins, molecular profiling, identification of pathways required for phenotype
Are cancer stem cells derived from adult stem cells?	Identification and characterization of adult stem cell populations, comparison of adult stem cells to cancer stem cells, clonality studies
How do cancer stem cells evolve during cancer?	Molecular and functional analyses of tumor cell populations in patients at different stages of disease, *in vivo* imaging using cell surface markers, research into the normal cell hierarchy
What is the role of the microenvironment in determining cancer stem cell properties?	Coinjection of tumor cell populations with stromal cells into mice, high-resolution imaging with markers of cancer stem cells and stromal cells, studying the effects of inhibition of stromal factors on cancer stem cells
What are the best drugs for targeting cancer stem cells?	Cell culture drug screens, tumor profiling before and after treatment in patients and animal models, early-stage clinical trials

For each question, several suggestions for how to approach future research are listed.

significant, and it is unclear whether lapatinib actually selectively killed cancer stem cells because of the aforementioned caveats associated with studies of cell surface marker proteins in patient biopsies, the results of this study are promising.

Conclusions

As with all cancer research, the purpose of studying cancer stem cells is to learn something that will allow for the development of more effective cancer prevention, diagnosis, and treatment. The currently proposed roles of cancer stem cells in oncogenesis, tumor growth, metastasis, and cancer recurrence suggest some strategies that should be kept in mind when treating cancer. First of all, signaling pathways and cell surface markers uniquely utilized by cancer stem cells could be targeted to block cancer progression at each stage by killing these cells or by changing their phenotype. Second, cancer stem cells appear similar to normal stem cells, so great care must be taken when targeting the cancer cells not to kill the normal ones. Finally, since any cell can become a cancer stem cell and since metastases result in patient death, all cancer cells must be ablated by treatment, and this result will likely require a cocktail of drugs [50,51].

The known roles of cancer stem cells also bring to mind many unanswered questions about the model (Table 2). These may be answered in the future using a variety of new and improved experimental approaches. In particular, the continued purification and study of individual cell populations at molecular and functional levels as well as *in vivo* imaging will be extremely important in sorting out the exact relationships of the various cell types within a tumor to one another. This should be accompanied by careful analyses of cancer patients and clinical trials designed to test hypotheses formulated based on data obtained in experimental systems.

The cancer stem cells model clearly has a place in cancer research, especially in explaining tumor heterogeneity. However, it needs to continue to be refined with every new relevant discovery. This will hopefully allow researchers to efficiently tackle the most important remaining questions in the field, leading to additional insights into cancer and hopefully someday to curative cancer treatments.

Potential conflicts of interest

KP receives research support from and is a consultant to Novartis Pharmaceuticals, Inc. KP also receives research support from Biogen Idec, Inc. and is a consultant to and stock shareholder of Aveo Pharmaceuticals, Inc.

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