Cancer Stem Cells

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LEARNING OB JECTIVES:

1. Define the *cancer stem cell* model of tumorigenesis.
2. Describe the characteristics of cancer stem cells (CSCs).
3. Relate the biologic characteristics of CSCs to their impact on cancer therapeutics.
4. Identify novel targets for therapeutic approaches directed at CSCs.

ABSTRACT: The cancer stem cell (CSC) hypothesis has had a major effect on the fields of cancer cell biology and clinical oncology. CSCs were originally described in hematologic malignancies, and subsequently in a variety of solid tumors. Their unique biological characteristics, including self-renewal capability, stem cell signaling pathways, relative quiescence and resistance to standard chemotherapy and radiotherapy are providing researchers and clinicians with new challenges. One important outcome of this new perspective on tumors is the recognition that effective treatment approaches will need to target both the rapidly proliferating bulk tumor cells, and the quiescent CSCs, which contain the ability to re-establish the malignancy when treatment is withdrawn. The clinical laboratory will undoubtedly see an influx of new molecular and histopathological tests to augment initial diagnosis, treatment decisions, and prognostic monitoring of cancer patients related to identifying and quantifying these as CSCs.

INDEX TERMS: Cancer stem cells, leukemia stem cells, cancer therapeutics, stem cell signaling pathways

Clin Lab Sci 2012;25(1):50

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INTRODUCTION

It has been a widely held belief that malignancy resulted from the uncontrolled proliferation of cancer cells. It is generally accepted that tumors originate from the transformation and expansion of a clone of abnormal malignant cells derived from a single mutated cell. In the past, cancer has been thought of as more or less a homogeneous mass of rapidly proliferating cells.

It is clear, however, that most tumor masses carry significant heterogeneity, both morphologically and functionally. Pathologists and morphologists have noted for a hundred years that not all tumor cells looked alike. Furthermore, some tumors displayed morphologically recognizable patterns of differentiation. A number of studies have noted a functional heterogeneity within tumors as well.

Studies in mice from as early as the 1930s described subpopulations of cells within a tumor with distinct proliferative and differentiative capabilities.[1,2] The first report supporting the functional heterogeneity in human cancer was the work of Southam and Brunschwig in 1961. They performed autologous transplantation of malignant cells from patients with a variety of carcinomas into subcutaneous tissue.[3] Tumor reinitiation was rare, and the smallest quantity of transferred cancer cells capable of inducing a tumor was ~10^6. This suggests that large numbers of cells were necessary to initiate tumor growth, likely because only subpopulations of cells were consistently capable of engrafting. Other investigators injected acute leukemia patients with tritiated thymidine to estimate the proliferation rate of the leukemic blasts. They found that the vast majority of circulating blasts were post-mitotic; less than 10% were actively cycling and could remain dormant for weeks or months.[4,5]

Models of Tumorigenesis

There are two general models for tumor initiation and heterogeneity that have been debated for decades: the "stochastic model" and the "stem cell model".

The stochastic model proposes that a cell becomes tumorigenic after an initial mutation, followed by additional subsequent mutations resulting in both proliferative and survival advantages of selected cell clones. Predominant clones would produce identical cancer cells. It assumes that most, if not all, tumor cells can proliferate and propagate the tumor (all cells within a tumor mass would have a similar capacity for self-renewal).[6]

The cancer stem cell (CSC) model maintains that only some cells can indefinitely self-renew and sustain tumor growth, and that cancer likely originates in tissue stem or progenitor cells. This model suggests that many (if not most) tumors are hierarchically organized into *tumor initiating cells* (TIC) or CSCs and more differentiated progeny (non-TICs), which can be phenotypically differentiated. Tumors would thus have a CSC at the apex, with the characteristics of a cell that retains or acquires both the properties of self-renewal and (limited) differentiation. These would be the only cells within the tumor responsible for tumor initiation, both *in vitro* (clonogenic assays) and *in vivo* (tumor propagation, metastasis, and transplantation to new sites). The other cells within the tumor would be thought of as "transit amplifying" cells or "mature" cells, with limited or no ability to initiate or maintain the tumor.[6,7]

In reality, most malignancies are characterized by a relatively low clonogenic capacity, meaning the ability to proliferate in vitro, producing colonies of cells in a cell culture assay.[8,9] The clonogenic cells within acute myelogenous leukemia (AML) patients are distinct from the bulk of the leukemic blasts.[10,11,12] Also not all cells within a tumor have an equal capacity to induce new tumor formation in vivo. Only a fraction of cells appear to be tumorigenic,[11] so that a large number of cells are generally needed to transfer the tumor.[3]

If distinct subpopulations of cells exist within a tumor, organized in a hierarchy with more primitive CSCs at the apex (CSC model), it should be possible to sort tumor cells into fractions with tumorigenic or clonogenic activity, and those without. If all cells within a tumor clone have an equal capacity for clonogenic or tumorigenic activity (the stochastic model), such a separation would not be possible, and clonogenic or tumorigenic cells would be found in various phenotypically distinct subpopulations.[7]

The Cancer Stem Cell Model

Several investigators in the nineteenth century proposed that cancer arises from a rare population of cells with stem cell characteristics.[13,14,15] Till and McCullough in the twentieth century supported the stem cell origin of leukemias.[16,17] However formal proof of the theory required the ability to prospectively isolate identifiable subpopulations of cells from the heterogeneous tumor mass, and to assay their ability to recapitulate the tumor growth in a recipient animal.[7]

Significant advances between the mid-1970s and mid-1990s including the identification of distinct phenotypic surface markers for stem and progenitor cells, the development of technologies allowing the sorting of subpopulations of cells labeled with monoclonal antibodies to these markers (e.g. fluorescence activated cell sorters/FACS) and the development of human xenograft assays enabling the transplantation of human cells into a nonhuman host resulted in the seminal studies in the late 1990's which led to the current CSC model.

Lapidot and coworkers from John Dick's laboratory in Toronto isolated a phenotypically distinct subpopulation of human AML cells that were capable of giving rise to leukemia when transplanted into immunecompromised SCID (severe-combined immunodeficient) mice.[18] They found that the $CD34^+CD38^-$ population of cells from AML patients were capable of initiating disease upon transplantation into SCID mice whereas the phenotypically more mature $CD34^+CD38^+$ and $CD34^-CD38^+$ populations were not able to transmit the disease. Thus cells with the phenotypic profile of hematopoietic stem cells (HSC) appeared to be functioning as "leukemic stem cells" (LSC). Bonnet and Dick identified LSCs in most types of AML, and although the frequency of LSC varied significantly among patients, they were generally found at a low frequency, with an average of ~1 x 10^{-6}.[19] LSCs were subsequently described for other leukemias, including chronic myelogenous leukemia (CML),[20] acute lymphoblastic leukemia (ALL),[21] and acute promyelocytic leukemia (APL).[22]

Since the original reports of CSCs identified in patients with leukemias, CSCs have been identified in a wide variety of human solid tumors, based on specific cell surface antigen profiles and/or functional characteristics. These include breast,[23] brain,[24,25] multiple myeloma,[26]

melanoma,[27] prostate,[28] head and neck,[29] colon,[30,31] pancreas,[32] colorectal,[33] lung,[34] and liver.[35] The frequency of CSCs in solid tumors appears to be generally higher than that in leukemia (from <1% to ~25%). However, there is significant variation between tumors of the same type.[36]

Cancer Stem Cells

CSCs are a sub-population of the total tumor cells that can be identified by the expression of specific cell surface molecules such as CD markers, high expression of cell-adhesion molecules, cytoprotective enzymes, and drug-efflux pumps.[37] They can thus be identified and isolated using flow cytometry and/or FACS.[38]

In the cancer stem cell model, the "CSC" does not refer to the cellular origin of the cancer, but rather to the mechanisms by which the cancers propagate themselves. The cell of origin refers to the cell that received the first oncogenic "hit(s)". The CSCs would be the cells that maintain the ability to self-renew as well as to differentiate and give rise to the diverse cells within the tumor. CSCs may originate from either normal stem cells, or from normal progenitor cells (or even mature cells).[7] A lineage-committed progenitor cell could function as a CSC if it were to reacquire the property of self-renewal. Tumors often develop and progress from deregulated self-renewal pathways.[39] Importantly, the cellular source of CSCs may change as the disease evolves. The CSCs in chronic phase CML are generally believed to be leukemic counterparts of hematopoietic stem cells.[20] However, CML in blast crisis is pathologically different from CML chronic phase. The CSCs for blast crisis CML are more differentiated progenitor cells, likely the granulocyte macrophage progenitor cells (GMP).[40,41]

Both putative CSCs and adult somatic stem cells are thought to share the properties of long term persistence and self-renewal.[39] Similar pathways regulate the biology of both.[42] There are differences, however. During the process of self-renewal, normal tissue stem cells undergo an asymmetric division in which one daughter cell retains stem cell capabilities, while the other becomes a transit-amplifying cell, which terminally differentiates. In CSC, however, the self-renewal pathway is aberrant, and the transit-amplifying cells fail to differentiate normally (e.g. they undergo maturation arrest) and as a result, accumulate. This aberrant regulation of self-renewal pathways leads to uncontrolled cancer growth.[19]

Both normal SCs and CSCs share many of the same growth-regulating signaling pathways. Also like normal SCs, the CSCs have been shown to be relatively quiescent.[43] Initial reports suggested that CSCs were rare cells. However, subsequent studies indicate that CSC may exist at much higher frequencies in some malignancies than previously believed.[44,45,46]

Controversies Surrounding the CSC Model

Several reports have been published that question whether the CSC model is universally applicable to all cancers.[47,48] One controversy has been the relative number of CSCs. Although the original work of Dick and co-workers indicated that CSCs were a relatively rare subpopulation of the total tumor cells in AML (on average ~1 in 10^6), later studies identified significant differences in frequencies of self-renewing tumor initiating cells in different forms of cancer. As many as 25% of the cancer cells in certain tumors appear to have the properties of CSCs.[45,49] Interestingly, the cell surface phenotype as well as the frequency of CSCs can vary considerably among different patients for a given type of cancer.[50] CSC frequencies may also differ depending on the stage of malignant progression of a particular tumor.[51,52] Additionally CSC-like properties may not only be a function of the cell type of origin and the stage of malignant progression, but also signals from the stromal microenvironment.[48] Thus lack of an appropriate microenvironment may be responsible for the low number of human cancer cells that grow in mouse transplantation models, accounting for the low frequency of CSCs in early studies.[53]

The dispute concerning whether the ability to propagate human cancers is a property of a small subset of the tumor cells (the CSCs) or whether most/every tumor cell is capable of this function is ongoing. Many, however, now believe that both perspectives may be correct, depending on the unique circumstances of the tumor cells themselves, and the assay conditions used to evaluate the presence and function of the CSC.[44,54]

Implications of the CSC Model for Therapy

In spite of considerable progress in the therapeutic treatment of malignancies over the past several decades, many tumors remain refractory to treatment or relapse following initial remission. Conventional cancer therapies (chemotherapy and radiotherapy) primarily target the most rapidly proliferating cells, which represent the bulk of the tumor cell population. While

this may produce significant initial results in terms of reduction of tumor mass, it often fails to result in long-term remissions. One possible reason for this may be the existence of CSCs, which are generally not actively proliferating (i.e. they are largely quiescent). Thus treatment failure may reflect the relative inability of most current agents to target CSCs which generally are intrinsically more resistant to antineoplastic agents.[43,55]

CSCs in a number of human malignancies have been shown to be more resistant to therapy than other cells in these cancers. Bao and coworkers reported that glioblastoma cells were resistant to ionizing radiation due to an enhanced ability to repair damaged DNA.[56] Several reports have shown breast cancer CSCs to be resistant to various forms of therapy, including both chemotherapy and radiotherapy.[57,58] Even the molecularly-targeted therapies used in hematologic malignancies may be of limited usefulness against CSCs. Currently available therapies targeting molecular markers of diseases such as bcr-abl inhibitors (for CML) or Jak-2 inhibitors (for other myeloproliferative disorders) appear to have minimal effects on CSCs.[59,60]

If only a defined subpopulation of cells within the tumor can initiate and maintain malignant growth, they are the cells that must be effectively targeted to achieve a definitive, long-term "cure". Thus novel therapeutic approaches will be needed to eradicate these CSCs.[61] The optimum approach to treating various malignancies will likely include therapy aimed at reducing the tumor mass (aimed at rapidly proliferating cells) combined with targeted elimination of CSCs (avoiding the risk of relapse).[43] The current approach for APL provides an informative example. All-trans retinoic acid (ATRA), in combination with chemotherapy using arsenic trioxide, appears to possibly be effective in targeting the CSC. While ATRA promotes differentiation of the malignant promyelocytes, arsenic trioxide is able to inhibit growth, clonogenity and stemness features of the subpopulation of leukemic cells carrying stem cell markers.[62]

There are several potential approaches to targeting CSCs, including immunologic approaches and therapies targeting the unique phenotypic and functional characteristics of the stem cells themselves. Monoclonal antibodies have proven to be effective targeted therapies for the treatment of a number of human malignancies. Unlike traditional chemotherapy, antibody treatment is not neutralized by drug transporter mechanisms or the quiescent status of the CSC.[63] Monoclonal antibodies directed against specific epitopes expressed on CSCs but not normal tissue stem cells are of particular interest. CD123, the interleukin-3 receptor alpha (IL-3R) chain is expressed on AML LSC, but not on normal HSC, and overexpression of CD123 is associated with AML proliferation and poor prognosis.[64,65] Pre-clinical trials have shown that monoclonal antibody-mediated targeting of CD123 can eliminate human AML LSC in a mouse model.[66] In recent years, a number of antigens have been identified that are preferentially expressed on AML LSC compared with normal HSC, and for several, monoclonal antibodies targeting the antigen are in preclinical and/or early clinical trials.[63]

Another interesting approach is harnessing the body's own immune system to attack and destroy the malignancy. The possible development of vaccines targeting specific CSCs is being explored. Studies have centered on inducing the adaptive immune system to target phenotypic properties of CSCs, as well as targeting stemness-associated signaling pathways. Preliminary data suggest that vaccines targeting CSCs may be both feasible and possibly superior to chemotherapeutic approaches targeting primarily the bulk tumor cells.[67]

Many tumors are characterized by an exponential increase in both CSC and non-CSC populations due to enhanced symmetric stem cell divisions. Normal SCs typically undergo asymmetric cell divisions, in which one daughter cell remains a stem cell and the other daughter cell initiates terminal differentiation. In symmetric cell divisions, both daughter cells retain the essential SC characteristic of self-renewal. Thus another potential therapeutic approach would be to develop a systemic therapy designed to control or eliminate symmetrical CSC cell division in tumors while minimally affecting normal SC.[68]

The inhibition of signaling pathways related to the "stem cell" nature of CSC are obvious targets for the development of novel therapeutics. However, there may be significant unwanted effects against healthy tissue SC. Because CSC have the unique ability of self-renewal, signaling pathways that play a role in this process are particularly attractive therapeutic targets. The Wnt, Notch and Hedgehog (Hh) pathways are critical pathways in the development of many diverse tissues, and are commonly activated in many types of

cancer.[69]

The Wnt-signaling pathway plays essential roles in the self-renewal and maintenance of SCs of a variety of tissues, including skin, blood, intestine and brain. Abnormal functioning of the Wnt pathway results in neoplastic proliferation of these same tissues, presumably through regulation of CSCs. Drugs that target aberrant activation of the Wnt pathway are likely to have therapeutic potential, and several compounds are in early preclinical and clinical trials.[70,71] While deregulated Notch signaling has been found in many malignancies, it is particularly associated with T-cell lymphoblastic leukemia/lymphoma. Gain-of-function mutations occur in ~60% of primary human T-LLs.[72] Drugs targeting the Notch signaling pathway (e.g. □-secretase inhibitors) are in clinical trials.

The Hedgehog (Hh) pathway is also implicated in the maintenance of stem cells in a variety of cancers. Studies have shown it to be particularly important in myeloid leukemias (CML), where it is required for maintenance of the LSC. Pre-clinical studies have shown that CML stem cells can be depleted when Hh signaling is inhibited. Imatinib, the standard therapy for CML, is effective at controlling CML in most patients, but does not appear to cure the disease, as LSCs evade treatment.[73] Interestingly, cells that are resistant to imatinib are depleted using Hh pathway inhibitors.[74]

Additional molecular targets for which therapeutic drugs are being investigated include microRNA-based therapeutics, designed to target the specific microRNA expression profile of CSCs,[75] and epigenetic manipulations to reduce stem cell numbers, including histone deacetylase (HDAC) inhibitors, and demethylating agents (to reverse the action of DNA methyltransferases).[37]

Like their normal stem cell counterparts, CSCs appear to occupy specialized microenvironments in many organs. These CSC niches are thought to be important for maintaining quiescence, as well as control of self-renewal, cellular survival, proliferation and differentiation for those cells.[76] Targeting the microenvironment of CSC is another new therapeutic approach, and drugs that target the niche are currently in clinical trials.[77]

In spite of recent advances, the development of highly selective, targeted therapeutic agents for cancer will require further investigations of individual tumors at the molecular level, and the development of better biomarkers (biological indicators) that reflect the unique characteristics of CSCs.[78] Just as the fields of cancer cell biology and clinical oncology have recognized the significant impact of the CSC model of tumorigenesis on the pathophysiology of human neoplasms and therapeutic approaches, the clinical diagnostic field will need to reevaluate diagnostic and prognostic approaches to cancer patients. New clinical methodologies and paradigms that will allow evaluation of the fate of CSCs will need to be developed.[79]

CSC and Patient Prognosis

Patients with tumors that have a high proportion of CSCs have a poorer prognosis than patients with a lower proportion of CSCs. Also, the most poorly differentiated tumors have the highest burden of CSCs.[80]

In AML a high LSC gene expression profile was associated with poor outcomes (poor overall survival, event-free survival, and relapse-free survival) independently of age, presence of *FLT3* or *NPM1* mutations, and cytogenetic risk group.[81,82] An enriched CSC profile correlated with adverse patient outcomes for breast cancer, lung cancer, ALL, colorectal cancer, medulloblastoma and oral squamous cell carcinoma.[84-87] Furthermore, several studies have suggested that tumor grade and metastasis incidence is proportional to the frequency of stem cell populations.[80,83,88]

CSC and Metastasis

Cancer is known to be a progressive disease, with descriptions of its onset as a localized growth and evolution to a pervasive disease over a thousand years ago.[89] Despite fairly significant advances in the treatment of certain malignancies over the past two decades, survival rates for patients with metastatic diseases remain poor.

The cellular origin of metastasis has been an issue intriguing scientists for years. Not every cell in a tumor has the ability to metastasize to other organs. In 1977 studies demonstrated that subpopulations of cells could be isolated from a population of tumor cells that had varying degrees of metastatic competence.[90] More recently it has been proposed that CSCs may be the source of metastasis.[39,91] Presumably the capacity of

CSCs to self-renew was required not only during primary tumorigenesis, but also for the re-initiation of growth by disseminated (metastatic) cancer cells. However, it is unlikely that all CSCs are equally competent for metastasis. It has been suggested that a given tumor's CSC compartment may include subpopulations of tumorigenic stem cells with varying degrees of metastatic competence.[92]

CONCLUSION

The identification of cancer stem cells and their unique properties is reshaping our concept of malignancy and our therapeutic and diagnostic approaches. Because CSCs are a small subpopulation of the total tumor mass (in many cancers) and are inherently resistant to many of the traditional therapies, new approaches are needed. Therapies targeting these quiescent cells with their unique capacity of self-renewal, and new tools allowing the assessment of CSC to evaluate therapeutic effectiveness are being developed. This rapidly changing field will produce a battery of new assays for the clinical diagnostic laboratory.

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