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Cancer Stem Cells: A Guide for Skeptics

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ABSTRACT

Cancer stem cells (CSC) were postulated to exist many years ago as cells within a tumor that regenerate the tumor following treatment. A stochastic clonal evolution model was used to explain observed tumor heterogeneity. Recently, xenotransplantation studies have demonstrated that prospectively identifiable subpopulations from human cancers can initiate tumors in immune deficient mice, and these results along with recent advances in stem cell biology have generated much excitement in the cancer field. The modern CSC theory posits a hierarchy of cells analogous to normal stem cell development. Some controversy remains, however, as to whether these tumor initiating cells truly represent CSC, and whether the modern CSC field can live up to the promise of providing improved cancer treatments based on a novel model of cancer biology. Recent data from CSC investigators are discussed critically. J. Cell. Biochem. 106: 745–749, 2009.

KEY WORDS: CANCER; STEM CELLS; MOUSE MODELS

The insight that cancers contain a minority population of tumor initiating cells, or cancer stem cells (CSC), that resist treatment and give rise to the bulk of more differentiated tumor cells has generated considerable enthusiasm among researchers and lay public alike. The central character of a popular medical television drama, a brilliant clinician, recently exclaimed, ''Cancer stem cells are real. They explain everything.'' (Fox Television, House, M.D., Season 5, episode ''Not Cancer''). On the other end of the media spectrum, the British news magazine, The Economist, recently devoted a cover story to the excitement surrounding the CSC field [Economist, 2008].

A functional hierarchy of tumor cells, and the existence of treatment-resistant tumor stem cells was postulated years ago to explain the kinetics of disease relapse in mice and humans following chemotherapy [Skipper, 1986], and recent experimental data continues to validate this model. The modern CSC theory represents a subtle shift from the more stochastic model of clonal evolution, and supports a strictly hierarchical model of cancer cell development.

Several recent scientific reviews have enthusiastically described the state-of-the-art of the CSC field [Pardal et al., 2003; Jordan et al., 2006; Cho and Clarke, 2008]. Here, the intention is not to dampen enthusiasm for promising avenues of research, but to temper expectations with a critical appraisal of the recently rediscovered CSC theory so that biomedical researchers and cancer clinicians can work together efficiently toward their shared goals. After a brief introduction to the simple tenets of the CSC model, we will highlight concerns with the model, including methodological weaknesses and clinical examples that complicate the model, and will conclude by identifying bottlenecks that may limit the fulfillment of the clinical promises of the CSC concept.

WHAT ARE CANCER STEM CELLS?

The cellular heterogeneity of cancers has been appreciated by clinical pathologists for over a century [Virchow, 1860], but the fact that immature malignant cells can differentiate in vivo to form more mature appearing progeny was not clearly established until the techniques of molecular biology were applied [Fearon et al., 1986]. Considerable excitement was generated decades ago when the first bioassays for tumor stem cells were developed, that involved the growth of tumor-derived colonies in soft agar [Hamburger and Salmon, 1977]. The hope then, as now, was that more effective cancer therapies could be chosen based on the sensitivity of tumor stem cells to chemotherapy [Salmon et al., 1978]. The concept of treatment-resistant CSC provides an explanation for why cancer therapies recur after initially successful treatments, and also provides a strategy to potentially cure more cancers: develop treatments targeting the CSC.

The field was reinvigorated in 1997 by the demonstration that a small subpopulation of acute myeloid leukemia (AML) cells with an immature immunophenotype possess the ability to colonize immune deficient NOD/SCID mice, to give rise to more differentiated leukemia cells and to recapitulate the heterogeneous phenotype of the bulk tumor [Bonnet and Dick, 1997]. The majority of

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immunophenotypically more mature leukemia cells, on the other hand, fail to engraft in mice, therefore establishing a prospectively identifiable tumor cell hierarchy. These cells are most accurately referred to as NOD/SCID repopulating cells, or tumor-initiating cells, and most investigators are careful to use this terminology, but the conflagration of a NOD/SCID tumor initiating cell as a CSC is problematic. Many investigators in the field have accepted a purified subpopulation of cells with an immature phenotype that recapitulates a diversity of more differentiated tumor cells in NOD/SCID mice as evidence of a CSC, but the lack of a consensus definition has constrained broad acceptance of the CSC concept.

The potential implications of the hierarchical CSC model to older models of cancer growth deserve mention. Many years of work in murine tumor models supported a clonal evolution model that described tumor cell populations as continually diversifying in multiple dimensions, for example, to facilitate growth in metastatic locations or to resist chemotherapy [Fidler and Hart, 1982]. The clonal evolution model explains why cancers can be so difficult to eliminate in patients, but the hierarchical CSC model provides the hope that effective therapies may be developed by targeting specific, prospectively identifiable CSC populations. The degree to which the CSC and clonal evolution models are mutually exclusive, and whether a single CSC hierarchy exists in patients with cancer remain open questions.

PATHWAYS INVOLVED IN SELF-RENEWAL

The recent improvements to our basic understanding of stem cell biology and the parallels of stem cell biology to cancer biology are real. Stem cells, both normal and neoplastic, by definition have the ability during cell division either to generate more differentiated daughter cells or to undergo self-renewal. The Wnt, sonic hedgehog (SHH), PTEN, and BMI1-INK4a pathways have all been implicated in normal and neoplastic self-renewal [Cho and Clarke, 2008]. A discussion of the cellular pathways governing self-renewal are beyond the scope of this review, but here we will highlight recent findings that have illuminated a distinction between physiologic Notch signaling and ectopic activation of self-renewal pathways.

The Notch signaling pathway controls cell fate decisions in numerous cell types, including T-lymphocytes, and is essential for the development of adult HSC. Based on Notch's role in HSC development, and the increased self-renewal seen in gain-of-function experiments, it was widely hypothesized that Notch activation would play a significant role in normal hematopoietic stem cell self-renewal. Activation of the Notch pathway using the Notch ligand Delta-1 can increase the self-renewal property of hematopoietic stem cells and facilitates the repopulating ability of hematopoietic progenitor cells grown ex vivo [Ohishi et al., 2002]. Surprisingly however, genetic experiments designed to test the role of Notch signaling in adult hematopoiesis revealed that HSC do not require the canonical Notch pathway for self-renewal [Maillard et al., 2008]. Transduction of HSC with GFP fused to an N-terminal fragment of mastermind-like 1 (MAML) functioned as a clean dominant negative MAML, specifically interfered with Notch transciption factor complexes, and yet had negligible impact on HSC self-renewal activity. Maillard and colleagues confirmed these findings using a conditional Rbpj knock-out mouse in which this DNA-binding factor required for Notch signaling also failed to demonstrate a role for Notch signaling in HSC development or function in adult animals [Maillard et al., 2008]. Although the relevance to physiologic Notch signaling in HSC has been called into question, the Notch pathway reporter mouse appears to be useful for studying the effects of specific oncogenes on symmetric cell division [Wu et al., 2007].

SUCCESSFUL TARGETING OF LEUKEMIA CSC WITHOUT PROSPECTIVE IDENTIFICATION

The hierarchy model is attractive because of the Achilles heel it represents for potential therapies. The CSC model has been put forth as an important step toward achieving improved clinical outcomes, but cursory and dismissive discussion of modern cancer care tacitly encourages frothy expectations, and an overestimation of the likely contributions of the CSC field to the translational research agenda. For example, in a recent review it was stated that, ''chemotherapies that cause primary tumour regression rarely prevent metastases,'' [Pardal et al., 2003], but neo-adjuvant treatment of locally advanced breast cancer not only shrinks the primary tumor, but does improve long-term survival [Fisher et al., 2002].

Also, the dramatic success of imatinib mesylate (imatinib, Gleevec) in the treatment of patients with chronic myelogenous leukemia (CML) provides an informative example of the gap between the current CSC mania and the treatment of cancer patients. Before imatinib was introduced in 2001, the treatment of choice for newly diagnosed patients with CML was allogeneic bone marrow (stem cell) transplantation (SCT). Currently, allogenic SCT remains the only curative option for patients with CML—in other words, on the basis of long-term disease-free survival, this treatment can be said to eradicate CML stem cells quite effectively. Of course, the significant toxicity of allogeneic SCT, along with the fact that only a fraction of eligible patients have an HLA-matched sibling donor available has limited the success of allo-SCT treatment. So, in the case of CML, clinicians have abandoned the CML stem cell-ablating therapy (allo SCT) in favor of a drug that may have little effect on the CML stem cell population. In contrast to the significant toxicities of allo-SCT such as graft-versus-host disease, imatinib is a well-tolerated oral drug with manageable side effects that often diminish with long-term treatment.

Accelerated phase and blast phase CML patients frequently develop imatinib resistance, but the majority of patients present in chronic phase, and respond significantly to imatinib monotherapy. Given the current emphasis of the cancer research community on the importance of self-renewal pathways, one might expect that the tremendous success of imatinib to indicate a targeted effect on CSCs, but the opposite may be true. Cessation of imatinib therapy is often associated with the rapid increase in detectable CML cells, suggesting that although mature progeny are eliminated, CML stem cells are not eliminated by inhibitor treatment.

In patients with CML, quantitative PCR measurements are useful clinical test that is a nearly perfect tumor marker. The quantitative

measurement of BCR-ABL transcripts in patients with CML being treated with imatinib allowed the development of a mathematical model of cell population dynamics [Michor et al., 2005]. Such a CSC model has the benefit of being independent of xenotranplantation studies, and useful for guiding the design of patient clinical trials. Rarely, CML patients who have discontinued imatinib therapy have remained free of disease for extended periods. Intriguingly, some apparently cured patients received interferon alpha (IFN) prior to receiving treatment with imatinib, suggesting that IFN may target the CML stem cell [Krause and Van Etten, 2008]. Efforts are underway to modify the existing regimen based on these data, which, if could provide a disease cure, i.e., successful targeting of the CSC stem cell, without having prospectively isolated one.

The dramatic successes in clinical oncology made in recent decades are often ignored by CSC enthusiasts. Imatinib has transformed the way we treat patients with CML, and for the majority of patients has turned this disease into manageable condition that does not interfere with their ability to have children and productive careers. Although more progress must be made, we have a number of treatments that result in long-term remissions and cures in the hematopoietic malignancies, demonstrating success at targeting the CSC in their respective diseases. Other examples of leukemias for which we have highly successful therapies include acute promyelocytic leukemia (treated with all-trans retinoic acid (ATRA) and arsenic trioxide), hairy cell leukemia (2-CDA), Hodgkin's disease treated with radiation or chemotherapy, and AML with good risk cytogenetics (high dose ARA-C). Certainly, current therapies are insufficient, but significant cancer treatment milestones should cause some circumspection regarding the potential significance of CSC isolation tools to cancer medicine.

MARKERS OF SOLID TUMOR CSC OFFER PROMISE

It was the identification of markers of a breast cancer CSC that really began the current excitement [Al-Hajj et al., 2003], and the identification of CSC in different cancer types using candidate surface markers is an area of active research. CSCs have also been identified in squamous head and neck cancers [Prince et al., 2007], in malignant melanoma [Schatton et al., 2008], and in colon cancer [Dalerba et al., 2007; O'Brien et al., 2007], among others. Primary human breast cancer cells are immunophenotypically heterogeneous and CD44+ subpopulations are tumorigenic in NOD/SCID mice bearing estrogen pellets [Al-Hajj et al., 2003]. The CD44 and CD133 markers have emerged as markers of immature epithelial cells useful for isolating CSC in several tissue types, but again, uniform definitions remain elusive. Colorectal cancer cells expressing CD133 were identified as colon cancer initiating cells (CC-IC), but another group identified colon CSC (Co-CSC) as (EpCAM) high/CD44+, and noted that some tumors are CD133 negative. The precise differences in these populations are unclear. Both groups carefully examined the resulting xenografts and found that isolated subpopulations of immature cells gave rise in vivo to glandular structures with colon-specific maturation markers. Neither group reported histologic features that would conclusively establish the malignant nature of the xenografts, namely tumor invasion or metastasis.

Another example of a CSC marker is the ABCB5 multi-drug resistance transporter recently identified in melanoma. ABCB5 is associated with disease progression in melanoma disease, and marks more primitive sub-population of melanoma cells with the capability of initiating tumors in mice [Schatton et al., 2008]. The frequency of the ABCB5+ cells within bulk tumor populations is 1.6–20%. The variable frequency of tumor initiating cells in some models has been raised as an objection to the CSC theory [Kelly et al., 2007], but in response to this criticism, the Dick group has emphasized that it is "the prospective purification of cells with tumor-initiating capacity, irrespective of frequency" that is the core of the CSC theory [Kennedy et al., 2007]. While tumor cell populations may be enriched for CSC using surface markers to identify immature cells, a definitive CSC immunophenotype has yet to be conclusively demonstrated and may prove illusory.

THE STROMAL COMPONENT

An essential component of the traditional clonal evolution model of tumor cell development is the heterogeneous microenvironments encountered by tumor cell metastases [Fidler and Hart, 1982]. A hierarchical model of tumor cell dynamics has been demonstrated by xenotransplantation, but it has been argued that such models underestimate the contribution of the tumor stroma to cancer cell growth [Kelly et al., 2007]. A single CSC isolated from rat mammary adenocarcinoma cell line can cause tumors in NOD/SCID mice [Zucchi et al., 2007]. The xenotranplantation assay for CSC provides a significantly constricted growth environment by virtue of the large number of growth factors and cellular adhesion factors known to support cell growth and survival that do not cross species barriers. It is likely that a larger subset of tumor cells can survive to reinitiate cancer in the patient's autologous stroma than in the relatively barren milieu provided by the context of murine tissue stroma. A partial list of cytokines that display species specificity would include: the IFNs [Glasky et al., 1964], IL-6 [van Dam et al., 1993], stem cell factor [Lev et al., 1993], tumor necrosis factor [Lewis et al., 1991], oncostatin M [Lindberg et al., 1998], and GM-CSF [Shanafelt et al., 1991].

Recently, modifications of the xenotransplantation procedure—use of Matrigel and profoundly immunodeficient NOD/SCID/interleukin-2 gamma receptor null ($Il2rg^{-/-}$) mice—were shown to substantially increase the detected frequency of CSC in human melanoma samples [Quintana et al., 2008]. Transplantation of single human melanoma cells with diverse surface imunophenotypes can induce tumors in this new system, demonstrating the importance of the stromal environment in xenotransplantation studies, and dealing a serious blow to the hypothesis that human tumors universally harbor a prospectively identifiable tumor cell hierarchy [Quintana et al., 2008]. In some murine cancer systems, populations of tumor cells provide paracrine growth stimuli that has led to a model of dynamic cancer cell "societies" [Heppner, 1993]. The modern CSC field is still too new to exclude the possibility of

cancer cell cross-talk in human tumors. It appears likely that specific cancer subtypes will need to be carefully reanalyzed to characterize the roles of diverse microenvironments in tumor growth and progression [Bissell et al., 2005; Shipitsin and Polyak, 2008].

BOTTLENECKS TO IMPROVING CANCER CARE

At the end of the day, precise elucidation of CSC biology may be less relevant to improving the clinical utility of cancer therapeutics than a rethinking of the drug development process. The biomedical research community has provided cancer patients and their physicians a steady diet of "novel paradigms" advertised to transform cancer care (the cell cycle, apoptosis, signal transduction, angiogenesis, etc.), and yet the development of effective biotechnology products has been disappointing [Pisano, 2006].

Many CSC researchers, sincerely believing that stem cell biology tools have tremendous potential value to the healthcare industry, have filed patents to legally define their intellectual property, and therefore have given the practical issues of drug development at least some consideration. There is currently only one US patent with a "CSC" claim, but 28 pending applications. There are nine issued US patents with "notch" and "cancer" in their claims, 15 issued US patents for CD133, and 13 issued US patents for CD44 and cancer (http://www.uspto.gov/). The ABCB5 gene has been patented ("...human P-glycoprotein homologue on chromosome 7p15-21 and uses thereof"), and its use in identifying CSCs is the subject of two pending applications (http://www.uspto.gov/). While a detailed discussion of patent law is beyond the scope of this review, it should be noted that while the biomedical research community is focused on the cutting edge of molecular and cell biology, our efforts may be inadvertently contributing to drug development bottlenecks by adhering to antiquated models of intellectual property [Van Overwalle et al., 2006]. For example, pharmaceutical companies may decline to pursue promising research leads if a compound is subject to "reach through" claims of patents on multiple biochemical targets and pathways [Heller, 2008].

Recently, a number of examples have been identified, including the area of pharmaceutical development, where overly narrow private property rights have thwarted resource utilization, resulting in a kind of innovation gridlock [Heller, 2008]. Realization of the promise of CSC research, and biotechnology efforts generally, may require creative changes to the biotechnology status quo.

CONCLUSION

For students of cancer medicine, this is an exciting era, with dramatic technical advances and an increasingly complete understanding of cellular biology providing great potential for significant advances in treatment. The CSC theory has identified novel targets for potential pharmaceutical intervention, and has provided a useful paradigm to discuss the complex dynamics of cancer growth. The elucidation of self-renewal pathways has identified novel pharmaceutical targets, which are, as always, desperately needed. Novel compounds are being developed with pre-clinical activity and high hopes for clinical utility [Guzman et al., 2007].

The current CSC fad has thankfully drawn the attention of cancer biologists to more realistic models of malignancy, long appreciated by clinical pathologists, involving tumors that are characterized by heterogeneous cell populations. The transformed cell lines used for decades in cancer research are typically monomorphic, highly mutated populations with behavior that often fails to recapitulate that of primary cancer cells. The CSC field has shown the importance of using primary cells in cancer research. Even if the divide between a clonal evolution model and a hierarchical CSC model proves to be a false dichotomy, xenotransplantion using prospectively identified candidate CSC is an important new technology. The development of a novel and successful cancer therapy using these techniques will be an event welcomed by skeptics and enthusiasts alike.

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