Concise Review: Emerging Concepts in Clinical Targeting of Cancer Stem Cells

Zeshann A. Rasheed, *Johns Hopkins University*
Jeanne Kowalski, *Emory University*
B. Douglas Smith, *Johns Hopkins University*
William Matsui, *Johns Hopkins University*

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Zeshaan A. Rasheed[1], **Jeanne Kowalski**[2], **B. Douglas Smith**[1], and **William Matsui**[1,#]

[1]The Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, Maryland 21287

[2]Winship Cancer Institute, Emory University, Atlanta, Georgia 30322

Abstract

Cancer stem cells (CSCs) are functionally defined by their ability to self-renew and recapitulate tumors in the ectopic setting. They have been identified in a growing number of human malignancies and their association with poor clinical outcomes has suggested that they are a major factor in dictating clinical outcomes. Moreover, recent studies have demonstrated that CSCs may display other functional attributes, such as drug resistance and invasion and migration, that implicates a broad role in clinical oncology spanning initial tumor formation, relapse following treatment, and disease progression. Although our knowledge regarding the basic biology of CSCs continues to improve, a major issue remains proof that they are clinically relevant, and translation of the CSC hypothesis from the lab to the clinic is of paramount importance. We will review current evidence supporting the role of CSCs in clinical oncology and discuss potential barriers and strategies in designing trials examining CSC-targeting agents.

Keywords

Cancer stem cells; Metastasis; Drug resistance; Antineoplastic agents

Introduction

The cancer stem cell (CSC) concept was initially proposed several decades ago to explain two recurring observations. The first is that most cancers consist of phenotypically heterogeneous tumor cells that may resemble distinct stages of normal tissue development. The other is that only a fraction of cells from both hematologic and solid malignancies are tumorigenic (1–3). Studies in the early 1990's of human chronic (CML) and acute myeloid leukemias (AML) using *in vitro* long-term cultures or severe combined immunodeficient (SCID) mice demonstrated that phenotypically distinct cells resembling primitive hematopoietic cells were capable of giving rise to leukemia (4, 5). Importantly, these studies established that tumorigenic potential was not equally shared by all cells within an individual tumor but inherently linked and restricted to phenotypically distinct subsets. Since then cells with enhanced tumor initiating potential have been prospectively identified

in a wide variety of human malignancies based on specific cell surface antigen expression as well as functional characteristics, such as aldehyde dehydrogenase (ALDH) activity and increased drug efflux potential (6–10). Moreover, these cell populations can give rise to the full spectrum of cell types that recapitulates the original tumor as well as maintain tumorigenic potential during serial transplantation. Therefore, the definition for CSCs has been expanded to include the capacity for differentiation and self-renewal (11).

Clinical implications of the CSC hypothesis

Given their unique functional properties, the CSC hypothesis has provided a potential explanation for several phenomena in clinical oncology. One such phenomenon is the noted discordance between early measures of clinical response and long-term outcomes for many cancers. For example, the efficacy of anti-tumor therapy is usually determined by serially evaluating tumor burden, and it is generally assumed that improvements in these parameters result in clinical benefit. Although disease control can alleviate symptoms in individual patients, the achievement of objective responses (i.e., tumor reduction) across a population may not be associated with improvements in long-term outcomes, such as the duration of overall survival (12). In two large randomized clinical trials comparing initial therapies for newly diagnosed patients with AML, the administration of increased doses of chemotherapy significantly improved the proportion of patients achieving complete remissions (13, 14). However, despite improved disease control, no differences were observed in overall survival between treatment groups in either trial. In the plasma cell malignancy multiple myeloma, the relationship between the depth of response and overall survival has been examined in a variety of large randomized trials utilizing high-dose chemotherapy and autologous stem cell transplantation (15). In many of these studies, the achievement of a complete response and total eradication of all macroscopic disease has not been associated with increased overall survival compared to patients achieving only partial remissions. These findings are also noted in solid tumors. For example, a number of large randomized studies utilizing combination chemotherapy in pancreatic adenocarcinoma have demonstrated improved rates of tumor response or progression free survival, but these demonstrated only negligible or no improvements in overall survival (16, 17). Overall, these studies demonstrate that response and survival rates may be independent entities in many diseases and suggest that each reflects treatment effects against distinct cancer cell types.

It is possible that the rarity of CSCs may explain the discrepancy between clinical response and survival in many malignancies since disease response primarily reflects short-term changes in bulk tumor cells, whereas long-term outcomes, such as disease relapse and progression, are dictated by rare CSCs. Initial studies using syngeneic transplants of murine cancer cells demonstrated that the frequency of tumor initiating cells are rare in multiple myeloma and lymphoma (1, 3), and similar findings have been observed through xenografting studies of primary human tumors in a wide variety of diseases (6, 9, 10, 18). However, a recent study in melanoma questioned the rarity of CSCs by demonstrating that modifications to the xenotransplantation protocol, including the use of more immunodeficient NOD/SCID/IL2γc^{null} (NSG) mice, co-injection of tumor cells with matrigel, and waiting at least 20 weeks to assess tumor formation, dramatically increased the frequency of tumor initiating cells and eliminated their restriction to any specific phenotype (19). These results may be specific to melanoma, since these experimental modifications have failed to significantly increase the frequency of clonogenic tumor cells in pancreatic, lung, and ovarian carcinomas (20). Therefore, the relationship between disease response and survival may depend on the frequency of CSCs within a specific disease.

Tumor regrowth following treatment implies that CSCs persist and are relatively drug resistant compared to bulk tumor cells, and the mechanisms involved in this process have

begun to be elucidated. CSC drug resistance can be broadly categorized as the ability to reduce intracellular levels of cytotoxic agents or the enhanced capacity to repair cytotoxic injury. In multiple myeloma, CSCs have been found to express several intrinsic properties that promote the resistance of normal stem cells to toxic injury (21). These include increased expression of membrane-bound drug transporters and intracellular detoxification enzymes that mediate drug efflux and metabolism. CSCs in mantle cell non-Hodgkin's lymphoma (NHL) and CML have also been found to be relatively quiescent, and this property may promote drug resistance to cytotoxic agents that are dependent on cell cycle progression for their activity or by decreasing the expression of proteins or pathways inhibited by targeted therapies (22, 23).

In contrast, stem cells in glioblastoma and breast cancer have been found to be relatively radioresistant compared to the bulk tumor population based on enhanced repair mechanisms. Glioblastoma CSCs have increased activation of the DNA damage checkpoint response, while breast CSCs are able to minimize DNA damage via enhanced handling of reactive oxygen species (24, 25). Moreover, two studies using mouse xenograft models of colorectal and pancreatic cancer showed that tumors are enriched in CSCs following conventional chemotherapy, suggesting that they are also relatively drug resistant *in vivo*(26, 27). Finally, in a clinical study of patients with breast cancer, both the frequency of CSCs and the clonogenic growth potential of tumors were increased after treatment with conventional chemotherapy (28). Therefore, CSCs have been found to be relatively resistant compared to bulk tumor cells *in vitro*, *in vivo*, and in the clinical setting.

Associating CSCs with clinical outcomes

Despite the identification of CSCs in many diseases and their potential to explain the failure of many anti-cancer therapies to extend overall survival, little data actually exists that they are clinically relevant. However, if CSCs truly drive the natural history of tumors, including disease relapse or progression, then their frequency or functional potential should correlate with clinical outcomes. A number of reports have examined whether CSCs can serve as predictive biomarkers and have included studies examining the association between the frequency of phenotypic CSCs, the expression of CSC-specific gene signatures, and the quantification of functional CSC properties with clinical outcomes.

The potential relationship between patient outcomes and phenotypic CSCs has been examined in a number of malignancies. The frequency of CD133^{+}CSCs assessed by immunohistochemistry (IHC) in glioblastoma has been associated with worse progression free and overall survival (29). IHC analysis of ALDH has also been performed in a number of malignances and found to be associated with worse clinical outcomes in patients with breast and pancreatic adenocarcinoma (7, 30). Furthermore, primary tumors displaying relatively high percentages of CD44^{+}CD24^{−/low}breast CSCs by IHC have been associated with higher rates of distant metastases, suggesting that CSCs may also be responsible for tumor dissemination (31).

The initial study examining the relationship between gene expression of isolated CSCs and patient outcomes was carried out in breast cancer (32). Here, a comparison of the gene expression profiles of CD44^{+}CD24^{−/low}breast CSCs and normal breast epithelium reveled a 186-gene "invasiveness" gene signature (IGS) that was significantly associated with worse overall and metastasis-free survival in patients with localized disease. Interestingly, the IGS was also associated with worse prognosis in patients with medulloblastoma, lung cancer, and prostate cancer suggesting that CSCs from different diseases may utilize similar regulatory pathways. Another study using gene expression microarrays found that primary breast tumors with expression patterns similar to isolated CD44^{+}CD24^{−/low}cells from

normal and malignant breast tissues were associated with worse clinical outcomes (33). Together these studies indicate that specific gene expression by CSCs may be able to predict clinical outcomes, either because CSCs are increased in these tumors or the tumors as a whole take on CSC characteristics.

Functional CSC activity has also been found to correlate with disease prognosis. The engraftment potential of primary AML specimens in immunocompromised mice has been found to be associated with poor overall survival (34). Similarly, the *in vitro* quantification of neurosphere formation and *in vivo* tumor formation in mice has been found to correlate with outcomes in patients with glioblastoma (35). Therefore, a range of phenotypic, genetic, and functional assays based on CSCs may serve as useful predictors of clinical outcomes.

Development of CSC targeting strategies

Evidence that the inhibition of CSCs leads to improvements in long-term outcomes may provide the most definitive proof that CSCs are clinically relevant. However, it has been challenging to identify specific cellular process that can serve as CSC targets because of the inability to isolate pure populations of CSCs for molecular analysis. One alternative approach has been to examine cellular pathways that are required for the regulation of normal stem cells. Evidence has suggested that CSCs may arise from both normal stem cells or progenitors and share regulatory mechanisms, such as signaling pathways active during normal development (11). Thus, a major focus has been on developmental signaling pathways, including Hedgehog, Notch, and Wnt, and novel agents inhibiting these pathways have been found to target CSCs in multiple diseases (36–39). Other cellular process important for normal stem cell maintenance, such as telomerase, have also been targeted in CSCs (40, 41). Clinical trials utilizing inhibitors of Hedgehog pathway (GDC-0449, PF-04449913, BMS-833923, IPI-926, TAK-441), Notch pathway (RO4929097, BMS-906024, MK0752), Wnt pathway (PRI-724, and telomerase (GRN163L), have begun to emerge (42, 43, http://clinicaltrials.gov), but their efficacy against CSC function remains to be determined.

High throughput screens to identify CSC-targeting compounds have been hampered by the rarity of CSCs as well as the complex nature of the clonogenic assays used to assess their function. However, a breast cell line engineered to induce properties of EMT has been used to screen compounds with anti-CSC activity (44, 45). Although these cells were found to be resistant to conventional chemotherapy, the novel compound salinomycin was found to selectively inhibit CSCs by inducing epithelial cell differentiation. Therefore, similar approaches that expand CSCs may allow high throughput screens to be carried out. Furthermore, self-renewal potential normally correlates inversely with the degree of cellular maturation, and strategies to induce terminal differentiation may be able to modulate CSC function in the clinical setting (46).

Designing and interpreting clinical trials to evaluate CSC targeting therapies

Several trials investigating potential CSC targeting agents have been initiated, but a major challenge has been to develop accurate methods evaluating their efficacy in the clinical setting. In general, the clinical endpoint most commonly associated with anti-tumor activity of an agent is response rate. Unfortunately, the rare nature and functional properties exhibited by CSCs suggest that this endpoint is not likely to measure a true change in the quantity or quality of the remaining CSCs. Conversely, therapies that successfully eliminate or minimize CSCs may better be judged by improved durability of response or even enhanced survival. Yet, these trials are often not feasible due to the large sample sizes and

long periods of follow-up that are required to show such differences. Studying clinical examples of therapies that impact survival without obvious early changes in the patients' tumor burden may provide insights into the patterns of response that may be observed in CSC targeting trials. In CML, inhibitors of BCR-ABL kinase activity, such as imatinib, have emerged as the standard of care because of their relative safety and ability to produce rapid responses (47). Prior to the advent of these agents, most CML patients were treated with alpha-interferon. Although fewer responses are seen with interferon and those that do occur may take several years to manifest, a significant proportion of patients achieving complete remissions remain disease-free when the drug is stopped (48). In contrast, virtually all patients treated with imatinib will relapse following its discontinuation (49). These observations may be explained by findings that interferon can eradicate CML stem cells *in vitro,* unlike imatinib that is primarily active against differentiated tumor cells and progenitors (50, 51). It is possible that therapies, or combinations of agents, directed against both CSCs and differentiated cells can lead to long-term remissions as suggested by a recent study in which molecular remissions were maintained for several years after imatinib withdrawal in CML patients who received induction with imatinib and interferon (52). In breast cancer, HER2/*neu* has been found to play a role in CSC self-renewal (53), and lapatinib, an inhibitor of HER2/*neu* signaling, has been studied in a randomized trial in combination with the standard cytotoxic agent capecitabine (54). Compared to patients treated with capecitabine alone, the combination of capecitabine and lapatinib did not improve response rates, but the use of both agents significantly increased overall survival. Therefore, response rates are unlikely to adequately serve as indicators of activity against CSCs that dictate overall survival.

In addition to overall survival, it is possible that other clinical endpoints can be used to evaluate anti-CSC activity within the proper context. For example, initial tumor debulking with conventional therapy may allow the effects of a CSC-targeting agent on relapse-free survival to be examined (Figure). Since several functional properties have been attributed to CSCs in addition to their tumorigenic potential, other outcome measures may also be considered. If the primary function of a particular CSC is to mediate metastasis, then the optimal clinical scenario to test a targeting agent might be when a patient presents with localized (non-metastatic) disease, following induction therapy with conventional treatment, or as adjuvant therapy. In these settings, it would be most appropriate to measure relapse or metastasis-free survival in addition to overall survival. Alternatively, if the predominant property of a particular CSC population is therapeutic resistance, then a novel anti-CSC therapy might be studied in combination with a known cytoreductive therapy. In this setting tumor response rates maybe appropriate primary endpoints along with progression free survival.

Another important consideration in developing clinical trials is to identify surrogate endpoints capable of detecting the inhibition or reduction of CSCs early in the course of treatment, since these may be informative in early phase trials inadequately powered to evaluate survival. Several novel functional assays have been developed during the efforts to identify and characterize CSCs, and it is possible that these assays can act as surrogates within CSC targeting clinical trials. We have found that the serial quantification of *in vitro* clonogenic multiple myeloma growth is associated with progression-free survival and may predict clinical relapse (55). Therefore, the development of biomarker strategies that quantify CSCs in a serial fashion may provide novel endpoints to monitor CSC based clinical trials.

Conclusions

The growing number of reports regarding CSCs has significantly increased the complexity of our understanding of their basic and clinical biology. A number of properties, such as drug resistance and migratory and invasive potential, are now attributed to CSCs in addition to their defining characteristics of tumorigenicity and self-renewal that suggests a major role in disease relapse and progression. Although these findings have served as a foundation to develop novel therapies, proof that CSCs are clinically relevant is still lacking. Improved overall survival resulting from the documented inhibition of CSCs will provide the most definitive evidence for their importance, but challenges exist in identifying the proper endpoints to clinically assess therapies targeting these cells. Therefore, the development of novel clinical trial designs and biomarker strategies are likely to be equally important as the development of CSC-targeting drugs themselves.

Acknowledgments

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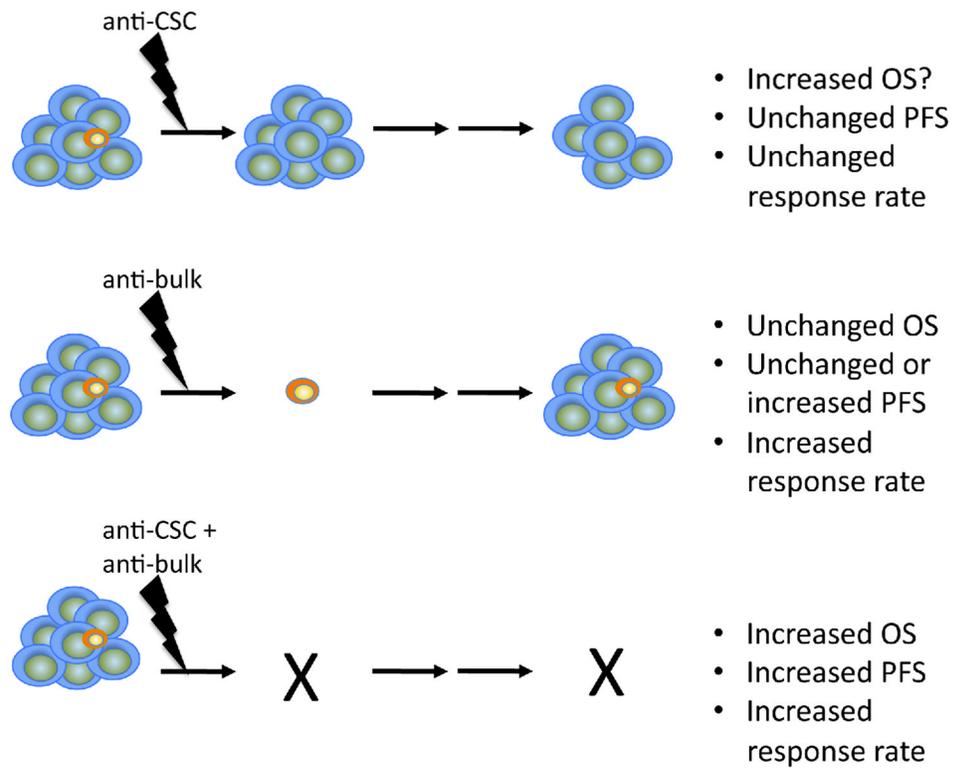
Figure.
Anticipated clinical outcomes with agents targeting the cancer stem cell population (top), bulk tumor population (middle), or both (bottom). OS -overall survival, PFS -progression free survival.