Molecular Pathways

Cancer Stem Cells and Chemosensitivity

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Abstract

Cancer lethality is mainly due to the onset of distant metastases and refractoriness to chemotherapy. Thus, the development of molecular targeted agents that can restore or increase chemosensitivity will provide valuable therapeutic options for cancer patients. Growing evidence indicates that a cellular subpopulation with stem cell–like features, commonly referred to as cancer stem cells (CSCs), is critical for tumor generation and maintenance. Recent advances in stem cell biology are revealing that this cellular fraction shares many properties with normal adult stem cells and is able to propagate the parental tumor in animal models. CSCs seem to be protected against widely used chemotherapeutic agents by means of different mechanisms, such as a marked proficiency in DNA damage repair, high expression of ATP-binding cassette drug transporters, and activation of PI3K/AKT and Wnt pathways. Moreover, microenvironmental stimuli such as those involved in the epithelial-mesenchymal transition and hypoxia indirectly contribute to chemoresistance by inducing in cancer cells a stem-like phenotype. Understanding how CSCs overcome chemotherapy-induced death stimuli, and integrating such knowledge into clinical research methodology, has become a priority in the process of identifying innovative therapeutic strategies aimed at improving the outcome of cancer patients. Clin Cancer Res; 17(15); 4942–7. ©2011 AACR.

Background

Adult stem cells are a rare and long-living cellular fraction that ensures tissue homeostasis by replacing senescent or damaged cells (1). The stem cell fate is regulated within specialized microarchitectonic structures, or niches, that respond to both local and systemic conditions (2). When required, stem cells divide asymmetrically, generating a slow-cycling daughter cell that retains the biological properties of the mother, and a more active daughter cell that produces a progeny of more specialized cells undergoing terminal differentiation.

Recent advances in cancer biology support the critical role of an uncommon cellular population with stem-like features in tumor generation and propagation (3–6). This population, commonly referred to as cancer stem cells (CSCs), displays three characteristics: (1) expression of a repertoire of markers common to stem and progenitor cells, (2) unlimited growth in vitro using media optimized for normal stem cell cultures, and (3) the ability to reproduce the parental tumor upon injection into immunocompromised mice.

The concept that a transformed stem cell is the progenitor of an entire tumor population implies that cancers are organized in a stringent hierarchy with a CSC at the apex of the pyramid (the hierarchical model), in a distortion of the functional architecture of a normal tissue. Consistent with this hypothesis, increasing evidence suggests that CSCs aberrantly exploit molecules and pathways governing the self-renewal program. This is confirmed by the marked asymmetry in the distribution of self-renewal components between CSCs and their differentiated progeny (7, 8), and by the antitumor activity displayed by inhibitors of the self-renewal pathway in many preclinical models (9, 10).

It also appears that CSCs successfully secure appropriate microenvironmental stimuli by displacing normal stem cells from their niches. The interaction between CSCs and the microenvironment is bidirectional, as indicated by the trans-differentiation process employed by CSCs to generate vascular precursors (11, 12). Like their normal counterpart, CSCs exhibit multifaceted defensive machinery that protects them from the effects of antiblastic compounds (13). In addition, the CSC fraction is probably enriched after chemotherapy, as demonstrated by the increased expression of stemness markers in patients with early breast cancer who are receiving primary systemic therapy (14).

The mechanisms underlying chemoresistance can be schematically subdivided into two groups: CSC-intrinsic and CSC-extrinsic (or indirect). The first group includes...
proficient DNA repair machinery, high expression of ATP-binding cassette (ABC) drug transporters, and altered cell cycle kinetics. The latter group includes microenvironmental influences that indirectly contribute to chemoresistance (Fig. 1).

**CSC-intrinsic mechanisms of chemoresistance**

Preservation of the genetic code from exogenous or endogenous injuries is critical for maintaining normal cellular function. After cells sense DNA damage, they begin repair activities that restore the original sequence of the genome. Alternatively, severe lesions lead to the elimination of irreversibly damaged cells by triggering programmed cell death. Several partly overlapping repair signals are involved in the maintenance of genome integrity (15). Each pathway corrects a specific form of genetic lesion that in turn reflects the type of damage induced by the causal agents. Considerable evidence indicates that embryonic (16) and adult (17) stem cells have a greater ability to repair their genetic code than their offspring. However, aged stem cells tend to accumulate genetic/epigenetic mutations as a consequence of a reduced ability to correct genetic lesions (18), which may account for the increased cancer incidence with aging. Although DNA damage repair is crucial for preventing malignant transformation, transformed cancer cells take advantage of improperly activated repair pathways, exploiting them to overcome chemotherapy-induced cell death.

Glioblastoma stem-like cells (GBM-SCs) are resistant to chemotherapy, independently of their ability to extrude cytotoxic drugs (19). After exposure to ionizing radiation, GBM-SCs activate ATM and Chk1 undergoing cell cycle arrest, and repair their DNA more readily than do non-CSCs (20). Likewise, lung CSCs (LCSCs) exposed to genotoxic stress activate Chk1 and Chk2, whereas differentiated lung cancer cells are vulnerable to chemotherapy (Bartucci M. et al., unpublished data). When chemotherapy is combined with Chk1 inhibitors, LCSCs undergo cell death through mitotic catastrophe. Hyperactivation of the ATR/Chk1 pathway also protects colon CSCs (CCSCs) from platinum derivatives, whereas this chemoresistant phenotype is reverted by the inhibition of ATR/Chk1 signaling (21).

The phosphatidylinositol-3 kinase PI3K/AKT pathway is often deregulated in high-grade primary brain tumors and mediates different protumorigenic activities (22). Moreover, an intricate connection links PI3K/AKT and DNA repair machinery (23). In glioblastoma cells, inhibition of PI3K or AKT prolongs ionizing radiation-induced DNA damage, as demonstrated by the delayed clearance of...
γ-H2AX foci (24). Accordingly, Akt inhibitors efficiently target GBM-SCs, determining a reduction of viable cells and abrogating neurosphere formation (25).

DNA sensor and repair pathways act in concert with apoptotic signaling to decide cell fate. Thus, the imbalance of the apoptotic machinery toward an antiapoptotic state favors cancer cell survival (26). Interleukin-4 (IL-4) is known to amplify the expression of antiapoptotic mediators in different epithelial cancers (27). The chemotherapy-resistant phenotype of CSCs seems to be sustained, at least in part, by the release of IL-4 in a process that is abrogated by either a neutralizing antibody or a mutant form of IL-4 (28). Because IL-4 can be produced in a number of tumors (29, 30), it is likely that other CSC types can exploit IL-4 to counteract the cytototoxic activity of chemotherapeutic drugs.

The multidrug resistance (MDR) phenotype is a further critical hurdle for chemotherapy efficacy. ABC drug transporters are the main players in this phenomenon, because they actively extrude from cancer cells a variety of structurally and functionally unrelated drugs of natural origin (31). Both normal stem cells and their malignant counterparts express high levels of ABC pumps (32). In fact, the ability of CSCs to actively exclude the HOECHST 33342 dye has been exploited to facilitate their isolation and purification. These CSCs, defined as the side population (SP) by the above-indicated assay, have been studied in different malignancies, including acute myeloid leukemia (AML) and neuroblastoma. The AML SP is characterized by a greater efficiency in extruding daunorubicin and mitoxantrone compared with the non-SP (33), and a similar pattern has been documented for neuroblastoma stem-like cells (34). Moreover, the doxorubicin-selected breast cancer cell line MCF-7/ADR acquires stem-like properties and a molecular portrait dominated by epithelial-to-mesenchymal transition (EMT)-related and self-renewal–related genes (35). The gain of this stem-like state is coupled with the overexpression of both MDR-linked genes and the cyclophosphamide-metabolizing enzyme aldehyde dehydrogenase 1.

A further mechanism involved in CSCs’ resistance to chemotherapy is cell quiescence. In normal stem cells, a prolonged exit from the cell cycle ensures the longevity of adult tissues by ensuring that stem cells do not exhaust their proliferative potential (36). Quiescent stem cells efficiently repair DNA damage and reenter the cell cycle to reconstitute the damaged tissue after exposure to cytotoxic injury (37). In a malignant context, quiescent CSCs are mostly spared by chemotherapy-induced cytotoxicity and are therefore capable of reconstituting the original tumor. Initial evidence connecting quiescence to CSC chemoresistance comes from label-retaining approaches, indicating that pancreatic adenocarcinoma label-retaining cells (LRCs) encompass the operative criteria of CSCs and survive 5-fluorouracil treatment, unlike their non-LRC counterpart (38). Similarly, putative ovarian CSCs display lower proliferative activity, enhanced tumorigenicity in xenograft models, and increased resistance to cisplatin compared with the non-CSC fraction (39).

**Indirect mechanisms of chemoresistance**

The interplay between CSCs and the microenvironment is a dynamic process that leads to the continuous remodeling of both compartments. Experimental evidence confirms the critical role of the EMT in the development of cancer metastases and chemoresistance. Recent findings have demonstrated that EMT is induced by the activation of a transcriptional complex influenced by different paracrine-acting signals, including the self-renewal–associated pathways Hedgehog (40), Notch (41), and Wnt (42). This complex leads to radical cytoskeletal rearrangements culminating in a switch toward a mesenchymal-like phenotype. Cells undergoing these morphofunctional changes are typically located at the tumor-stroma interface, where they gain prometastatic traits coupled with increased clone diversity and enrichment in stem cell-associated markers (43).

In addition to the EMT, hypoxia is also emerging as a critical regulator of the CSC pool. Hypoxia derives from different cooperating factors, such as the chaotic and dysfunctional vasculature that supplies malignant tumors, and poor oxygen diffusion within rapidly expanding neoplasms. Low oxygen tension activates the family of hypoxia-inducible factors (HIFs), which trigger adaptive changes at multiple levels, including the generation of new blood vessels in the attempt to ensure sufficient oxygen and nutrients (44). However, the abnormal architecture of newly formed vessels limits drugs perfusion, leading to a suboptimal concentration of chemotherapeutic agents within the tumor. Besides this mechanistic hypoxia-mediated drug resistance, direct evidence connects HIF factors and CSCs. Cancer cells cultured under low oxygen conditions or low pH express higher levels of stemness markers, acquire a stem-like phenotype, and overexpress stemness-related genes (45–47). Furthermore, based on functional similarities between adult stem cells and their malignant counterparts, it has been proposed that hypoxic areas within a tumor act as niches for CSCs (48).

**Clinical-Translational Advances**

It is possible to speculate that different mechanisms of chemoresistance are preferentially, if not exclusively, responsible for distinct phases of cancer relapse and progression. The temporal pattern of disease recurrence that is characteristic of many solid tumors suggests that the slow replication kinetics of CSCs could account for the limited efficacy of adjuvant chemotherapy in eradicating microscopic residual disease. Conversely, altered mechanisms of DNA repair, overexpression of ABC transporters, and improper activation of antiapoptotic signaling may be predominant during metastatic progression, when differentiated tumor cells are killed by chemotherapy and resistant CSCs are forced to reenter the cell cycle to numerically restore the tumor population.

When chemotherapy-enhancing therapeutic approaches are considered, attention often turns to agents that interfere with DNA repair. Poly-ADP ribose polymerase (PARP)

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**Clinical Cancer Research**

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inhibitors are the prototype drugs of this class. The logic behind the development of PARP inhibitors relies on the concept of synthetic lethality, defined as the cooccurrence of two genetic events that lead to cell death. To exploit this concept, cancer cells that are defective for a specific DNA repair pathway are exposed to compounds that inhibit a different signaling avenue that partially overlaps the defective one. The combined (genetic and pharmacological) abrogation of two redundant DNA repair pathways results in an increased sensitivity of cancer cells to specific DNA-damaging agents. Different PARP inhibitors have demonstrated encouraging activity against tumors with inherent defects in DNA repair, such as breast (49) and ovarian (50) carcinomas harboring BRCA1 or BRCA2 germline mutations. Chk1 inhibitors have also recently entered clinical development in combination with gemcitabine, irinotecan, and cytarabine. In the case of Chk1 inhibitors, the principle of synthetic lethality involves the p53 tumor suppressor: p53-defective cells are unable to undergo G1 arrest, and as a result depend on Chk1 to activate cell cycle checkpoints in response to DNA-damaging agents (51).

In p53-defective CSCs, a synthetic lethality-driven regimen should include a Chk1 inhibitor and a DNA-damaging agent, even though the ability of Chk1 inhibitors to preferentially kill p53-deficient cells is still debated (52). Given the close relationship between DNA repair and apoptosis, compounds that target antiapoptotic proteins, such as Bcl-2 family member inhibitors, may be useful for p53 wild-type tumors. With this approach, one can selectively block sequential oncogenic activities by taking into account the temporal and functional connections between different therapeutic targets.

In addition to inhibiting various components of the DNA repair pathway to potentiate the activity of alkylating agents, investigators have developed inhibitors/modulators of ABC drug transporters as chemosensitizers to increase the intracellular levels of ABC pump substrates, including taxanes, anthracyclines, and vinca alkaloids. After the failure of first- and second-generation ABC inhibitors, more-potent and specific third-generation antagonists have been synthesized and are currently undergoing clinical development (32). Although direct proof of the antitumor activity of such compounds is still lacking, ABC inhibitors offer the possibility to block pumps distributed in different body sites, such as the blood-brain barrier, thus improving drug biodistribution within sanctuary sites.

A further strategy for eliminating CSCs entails the use of differentiation-inducing agents that enhance chemosensitivity while depleting the CSC pool. The prodifferentiation activity of the bone morphogenetic protein 4 (BMP4) on GBM-SCs may be exploited for the treatment of high-grade gliomas (53). Likewise, BMP4 was recently shown to promote apoptosis, differentiation, and chemosensitization of colon CSCs through the inhibition of PI3K/AKT (54). Of note, the combined use of BMP4, 5-fluorouracil, and oxaliplatin can induce tumor eradication in a CSC-based model of colon cancer. Because the sequential use of differentiating agents and chemotherapy has shown considerable efficacy in acute promyelocytic leukemia (55), it is likely that the increasing research on CSCs will promote the use of similar strategies in solid cancers. In this context, it is conceivable that protective signals coming from the microenvironment could counterbalance the activity of differentiation-inducing agents. Thus, we envision that cotargeting intrinsic and extrinsic mechanisms associated with CSC maintenance by combining differentiation-inducing agents with antiangiogenic compounds or inhibitors of EMT/hypoxia-associated effectors may lead to a greater depletion of the CSC pool.

The ability to easily expand in vitro CSCs from several solid tumors has radically modified the preclinical models of human cancer based on cancer cell delivery in immunocompromised mice. Many standard cancer cell lines generate tumors whose phenotype appears extremely different from human tumors. It is likely that the orthotopic transplantation of CSCs in the appropriate murine background will allow more reliable testing of anticancer agents by taking into account the specific molecular settings of the tumor-initiating cells of each human malignancy (56, 57). Such models are very flexible and may allow investigators to test potential approaches for both adjuvant and metastatic therapies.

In this regard, the discovery of CSCs has also brought into question the general approach that is presently employed to validate novel pharmacological compounds. Currently, anticancer drugs are initially tested in metastatic patients and, if found effective, are then moved to the adjuvant setting. However, all evidence concerning CSCs suggests that this approach may be conceptually wrong, and that employing parameters of rapid tumor response, evaluated in metastatic disease, may underestimate the benefit of multiple drugs. On the one hand, it is likely that inhibitors of paracrine-acting pathways could offer considerable opportunities as adjuvant therapies targeting minimal residual disease. On the other hand, compounds producing rapid tumor shrinkage may be more suitable for the metastatic or neoadjuvant setting. The lack of benefit from bevacizumab (58) and cetuximab (59) as adjuvant therapy in colorectal cancer patients, despite their pivotal role in the management of metastatic disease, corroborates this hypothesis. Likewise, a phase II study comparing FOLFOX or FOLFIRI plus bevacizumab with or without the Smoothened inhibitor GDC-0449 in patients with metastatic colorectal cancer failed to reach its primary endpoint (60), despite the encouraging activity of GDC-0449 in tumors with activating mutations of the Hedgehog pathway (61). Similar unsatisfactory results were observed in a randomized placebo-controlled phase II trial with GDC-0449 as maintenance therapy in advanced ovarian cancer (62).

Finally, it is worth noting that efforts to develop chemotherapy-enhancing agents aimed at eliminating CSCs must take safety issues into account. To this end, research efforts should be oriented toward a deeper characterization of adult stem cells in order to avoid, or at least minimize, the inhibition of crucial mechanisms for normal stem...
cell maintenance. This aspect is even more relevant when anti-CSC drugs are considered for the treatment of pediatric patients and young adults.

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No potential conflicts of interest were disclosed.

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