Cancer Stem Cells in Radiation Resistance
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Abstract
Highly tumorigenic subpopulations of several solid cancers share characteristics with somatic stem cells. We showed recently that cancer stem cells, or tumor-initiating cells, derived from human glioblastoma surgical specimens and xenografts display resistance to radiation due to increased activation of the DNA damage checkpoint. We additionally showed that these same tumor subpopulations promote tumor angiogenesis through increased expression of vascular endothelial growth factor. These studies and subsequent reports from other researchers support critical roles for cancer stem cells in determining tumor response to therapy. Hypoxia and stem cell maintenance pathways may provide therapeutic targets to sensitize cancer stem cells to cytotoxic therapies to improve cancer patient treatments. [Cancer Res 2007;67(19):8980–4]

Despite the recent advances in molecularly targeted cancer therapies, much of the increase in cancer survival in recent decades has been due to improvements in early tumor detection and decreased toxicities from therapy. Advanced solid malignancies remain therapeutic challenges despite maximal therapy, in part, due to the development of resistance to radiation and chemotherapy, prompting the aggressive study of the molecular mechanisms underlying cell survival and DNA damage in cancer cells. Many of these studies have used established cancer cell lines in cell culture assays. Although great insights have been made using these models, established cancer cell lines poorly represent the cellular heterogeneity in tumors. Solid tumors are essentially aberrant organ systems with a diversity of neoplastic cells as well as differentiated cells undergoing “dedifferentiation” may initiate tumors in vivo. Regardless of origin, cancer stem cells may share developmental programs with normal stem cells that endow these cells with key traits in carcinogenesis—maintained proliferation, activation of survival responses, promotion of blood vessel formation, and enhanced motility. In addition, normal stem cells face greater requirements for management of genotoxic stresses as genetic changes may be transmitted to potentially large daughter populations. Our laboratory and others are beginning to show that cancer stem cells may have direct translational importance for cancer therapy.

Glioblastomas are among the most lethal of cancers with current therapies offering only palliation. Standard-of-care for glioblastoma consists of surgical resection, ionizing external beam irradiation, and chemotherapy. Radiotherapy has been the most effective nonsurgical treatment modality yet recurrence is essentially universal. Therefore, the determination of the mechanisms of radioresistance may provide novel therapeutic approaches to sensitize cancer cells to radiation and improve patient outcome. Due to the capacity for cancer stem cells to generate tumors at very small numbers, cancer stem cells have been hypothesized to be involved with tumor regrowth after treatment yet evidence supporting this role has been lacking (4). As cancer cure requires effective control of 100% of neoplastic cells, the presence of a small fraction of cancer cells highly resistant to treatment would explain clinical observations of occasional tumor responses in patients followed by tumor recurrence.

To investigate the possibility that cancer stem cells may represent the source of the resistant subpopulation, we interrogated the effect of ionizing radiation on human glioma models. The choice of model may be considered a minor issue, but the role of cancer stem cells in different models has yet to be fully determined. The optimal source for human cancer is from patient surgical specimens, but the absolute number of cancer stem cells derived from a single specimen may be limited requiring longer term culture that may dramatically skew the tumor cell subpopulations. Established human cancer cell lines have served the cancer biology community as workhorses and some groups have determined that cancer cell lines contain subpopulations with stem-like characteristics (5), but culture conditions have been widely recognized to induce cellular alterations and a recent work suggests that serum-containing medium alters the gene expression and chromosomal integrity to a much greater degree than stem cell medium (6). In contrast, xenografts may more faithfully reflect specific characteristics consistent with the original tumor from which they were derived if maintained in vivo (7). Thus, xenografts and short-term cultures derived from them may be reliable sources of large numbers of cancer stem cells. Based on these concerns, we used both patient surgical specimens and human glioma xenografts maintained in immunocompromised mice. In every model system that we tested and both in short-term culture and whole tumors, we found that the tumor cells became enriched in cells expressing
the cell surface stem cell marker CD133 (prominin-1; ref. 8). Although the direct contributions of the CD133 protein to stem cell biology remain unclear (9), CD133+ cells purified from CNS tumors (gliomas and medulloblastomas; refs. 10–14) and colorectal carcinomas (15, 16) are highly enriched for cancer stem cells (also called cancer/tumor-initiating cells), albeit with some variability. On the administration of ionizing radiation, the percentage of CD133+ cancer cells increase 2- to 4-fold depending on the model (8). The change in absolute number of CD133+ cells seemed modest as the original tumors contained <5% CD133+ cells, but tumor formation assays using increased fractions of CD133+ cells showed a dramatic decrease in tumor latency and increase in tumor size and vascularity with a 4-fold change in CD133+ cellular fraction. The data suggested that the tumor cells surviving radiation may have a greater relative number of cancer stem cells and that this change may alter tumor growth patterns.

To dissect the responses of cancer stem cells to radiation, we prospectively enriched for cancer stem cells using the CD133 marker. CD133+ cells showed the characteristics attributed to cancer stem cells, including potent efficiency to form neurospheres (a three-dimensional structure formed by neural stem cells) that can self-renew, expression of multiple stem cell markers, capacity for multilineage differentiation, and, most importantly, formation of tumors on transplantation with small cell numbers (300–1,000; ref. 8). In our studies, CD133+ glioblastoma cells preferentially survived radiation treatment at increased rates relative to the majority of cells (which were CD133−) and were able to repopulate both in cell culture and in vivo. Further, the CD133+ cells generated tumors with nearly the same efficiency as nonirradiated cells.

To link the resistance of the cancer stem cells to a specific mechanism, we investigated the DNA damage responses in matched CD133+ and CD133− cells derived from gliomas. Radiation consistently induced DNA damage to similar degrees in CD133+ and CD133− cells, but CD133− cells repaired the DNA damage more efficiently and underwent less apoptosis. dsDNA breaks caused by ionizing radiation and some chemotherapies activate the DNA damage checkpoint responses that include several kinases, including ataxia telangiectasia mutated and the checkpoint kinases, CHK1 and CHK2. CD133+ cells activated these kinases to a greater degree on irradiation relative to CD133− cells, although which checkpoint protein that was preferentially activated and to what degree varied between the CD133+ cells. Notably, CD133+ cells often showed basal activation of one component in the DNA damage checkpoint, rad17, suggesting that cancer stem cells are primed to respond to genotoxic stresses. These results suggest that a single, unified mechanism of checkpoint activation in cancer stem cells may not be present but that activation of the checkpoint is critical in these tumor cells. Thus, the development or maintenance of cancer stem cell may select for an activated checkpoint rather their activation in response to DNA damage in cancer stem cells may provide insights into the underlying mechanisms of treatment failure, but the cause of the preferential activation of the DNA damage checkpoint in cancer stem cells remains an area of active investigation.

We have not seen overexpression of checkpoint proteins but rather their activation in response to DNA damage in cancer stem cells, suggesting that other molecular mechanisms may regulate the checkpoint activity. Common genetic lesions that occur in human glioblastomas regulate cellular responses to DNA damage, including p53, PTEN, and p16INK4A. In our studies, we used surgical specimens and xenografts with different genetic backgrounds, and we did not detect any differences in the activation of the DNA damage checkpoint in CD133+ cells derived from tumors regardless of status in the p53, PTEN, and p16INK4A loci as determined by fluorescence in situ hybridization, suggesting that these pathways were not essential in the resistance of the CD133+ cells (data not shown). An enticing set of pathways that may underlie cancer stem cell radioresistance are key stem cell pathways that include Wnt/β-catenin, Notch, sonic hedgehog, PTEN, epidermal growth factor receptor (EGFR), and Bmi-1.

Recent studies have suggested that Wnt and β-catenin signaling may contribute to radioresistance of cancer stem cells (20, 21). Wnts and β-catenin have clearly defined roles in normal stem cell development and cancer (reviewed in ref. 22), but the role of the pathway in cancer stem cell biology is less clear. Several links between β-catenin and DNA damage responses have been made. Ku70 and poly(ADP-ribose) polymerase-1 (PARP-1) compete with β-catenin for binding to the T-cell factor-4 (Tcf-4) transcription factor (23) that mediates many of the effects of Wnt pathway signaling. PARP-1 normally augments Tcf-4 transcriptional activity (24) but on DNA damage, PARP-1 is modified preventing binding to Tcf-4. Instead, Ku70 binds to Tcf-4 preventing creation of an activating transcriptional complex that includes β-catenin (23). With this model, the preferential stabilization or expression of β-catenin may overcome the transcriptional effects of DNA damage mediated through the Lef/Tcf transcription factors. Notably, aberrant Wnt and β-catenin signaling has been linked to chromosomal instability through regulation of the mitotic spindle (25–27). Wnt/β-catenin regulates conductin, which localizes to the mitotic spindle and binds to polo-like kinase 1, to promote chromosomal instability in cancer cells (25). Further, a member of the Wnt/β-catenin pathway, adenomatous polyposis coli, localizes to the kinetochores in complex with Bub1 and Bub3 to prevent mitotic defects and induction of apoptosis (26). These reports would suggest that Wnt/β-catenin signaling may lead to tolerance of DNA damage even if repair is not successful. In fact, Wnt signaling activates a DNA damage response (28) and genomic instability may drive the malignant transformation of nontumorogenic stem cells to glioblastoma stem cells (29). One transcriptional target of β-catenin is survivin, which promotes cellular survival in response to apoptotic stimuli (30). Survivin expression correlates with stem cells in solid cancers are resistant to conventional cytotoxic therapies and linked this resistance to a specific molecular mechanism amenable to molecular targeted therapies (Fig. 1).

Our study focused on glioblastomas and radiation, but we expected that these studies may be more broadly applicable to other cancer types and other treatment modalities as checkpoint activation is an early event in some cancers. Indeed, elegant confirmatory studies for radioresistance of cancer stem cells in breast cancers and mammary progenitor cells from other groups have been published recently (19–21). Thus, the study of cancer stem cells may provide insights into the underlying mechanisms of treatment failure, but the cause of the preferential activation of the DNA damage checkpoint in cancer stem cells remains an area of active investigation.
Cancer stem cells display enhanced resistance to radiation due to preferential activation of the DNA damage checkpoint. A, radiation treatment enriches for cancer stem cells. Tumors treated with ionizing radiation show a significant increase in cancer stem cells due to lower rates of apoptosis in the cancer stem cell compartment after radiation. Surviving cancer stem cells have the capacity to repopulate the tumor. B, cancer stem cells activate the DNA damage checkpoint on irradiation to a greater degree than the majority of cancer cells (non–cancer stem cells). Activation of the checkpoint enables cells to determine if recovery is possible through DNA repair or induce apoptosis/senescence. The outcome of radiation-induced DNA damage is skewed toward survival and repair in cancer stem cells, whereas non–stem cancer cells are more likely to undergo apoptosis. Checkpoint kinase inhibitors may be able to reverse the resistance mechanisms in cancer stem cells.

Figure 1. Cancer stem cells display enhanced resistance to radiation due to preferential activation of the DNA damage checkpoint. A, radiation treatment enriches for cancer stem cells. Tumors treated with ionizing radiation show a significant increase in cancer stem cells due to lower rates of apoptosis in the cancer stem cell compartment after radiation. Surviving cancer stem cells have the capacity to repopulate the tumor. B, cancer stem cells activate the DNA damage checkpoint on irradiation to a greater degree than the majority of cancer cells (non–cancer stem cells). Activation of the checkpoint enables cells to determine if recovery is possible through DNA repair or induce apoptosis/senescence. The outcome of radiation-induced DNA damage is skewed toward survival and repair in cancer stem cells, whereas non–stem cancer cells are more likely to undergo apoptosis. Checkpoint kinase inhibitors may be able to reverse the resistance mechanisms in cancer stem cells.
with tumor grade and prognosis in gliomas (31) as with other cancer types providing an additional link between processes regulated by Wnt/β-catenin and glioma biology. The variety of mechanisms by which Wnt/β-catenin may control cellular responses to DNA damage decreases the likelihood of a single mechanism through which Wnt/β-catenin functions in cancer stem cell radioresistance, but it will be imperative to determine if the effects of Wnt in regulating cancer stem cell radioresistance occur due to regulation of the "stemness" of these cells or by direct activation of resistance mechanisms. Many of the effects of Wnt/β-catenin signaling occur through transcriptional regulation. As we have not seen the differential expression of checkpoint proteins, but rather their activation, it is possible that Wnt/β-catenin signaling regulates an upstream target in the DNA damage response cascade or a key regulator of checkpoint activation. Although many parallels can be drawn between cancer stem cells from solid cancers of different origins, the roles of the DNA damage checkpoint and Wnt/β-catenin signaling across cancer types in cancer stem cell radioresistance remain unclear.

In addition to Wnt/β-catenin, it is probable that other stem cell pathways promote resistance to DNA damage in cancer stem cells. Phillips et al. (19) found that Notch activity increased in response to radiation in mammospheres in established breast cancer cell lines. Targeting Notch protein cleavage has proved to be an effective antitumor therapy in preclinical studies (32). Hedgehog and Gli signaling also can be inhibited to attenuate cancer stem cell renewal and tumor growth (33). Loss of PTEN function alters CHK1 localization and function to initiate genetic instability (34) and confers radiation resistance in glioblastoma cell lines (35). EGF is a key growth factor used in culturing and maintaining cancer stem cells (6) and EGFR mediates radioresistance in glioma models that can be disrupted by EGFR tyrosine kinase inhibitors (36, 37). Thus, inhibitors of stem cell pathways may sensitize cancer stem cells to the effects of ionizing radiation providing a broad therapeutic paradigm.

Cancer stem cell therapeutic responses cannot be considered in isolation from the tumor microenvironment. Recently, cancer stem cells in brain tumor models have displayed a potential dependence on the perivascular compartment (38). Further, we have noted that intratumoral necrosis is an exclusive feature of tumors derived from CD133+ glioma cells in contrast to rare tumors from CD133- cells (data not shown). Brain cancer stem cells may be enriched in areas of hypoxia (39) and the fraction of CD133+ medulloblastoma cells is increased under hypoxia in vitro (40). As hypoxia promotes stem cell maintenance and blocks differentiation (41, 42), the critical role of hypoxia in defining cellular sensitivity to radiation may link additional processes of stem cell maintenance and therapeutic resistance. Recent studies have showed that hypoxia-inducible factor-1 (HIF-1) regulates tumor radioresponses through multiple mechanisms (43): HIF-1 sensitizes tumor cells to radiation through induction of ATP metabolism, proliferation, and p53 activation but HIF-1 also permits increased survival of endothelial cells (43). Thus, HIF-1 may function in opposing fashions to determine the net tumor response to radiation under hypoxia. We have interrogated the cellular response of cancer stem cells to hypoxia and have found striking molecular differences relative to the bulk of tumor cells further strengthening the ties between cancer stem cell biology and hypoxia. A recent report suggests that HIF-1 and β-catenin compete for Tcf-4 binding (44), implicating an additional regulatory element for radioresistance in cancer stem cells under hypoxia. The interaction between cancer stem cell pathways and hypoxic responses is not fully defined but both Notch and Oct4, a regulator of embryonic stem cells through formation of a transcriptional complex with Sox2 and Nanog, are regulated by hypoxia (reviewed in ref. 42). Tumor response to hypoxia and radiation is critically dependent on tissue vascularity. During our studies of radiation resistance, we noted that tumors formed by cancer stem cells were markedly vascularized relative to the rare tumors formed by non–cancer stem cells. Tumor angiogenesis is an important aspect of glioblastomas [in fact, the WHO includes vascular proliferation in its histopathologic grading system as an indicator of the glioblastoma diagnosis (grade IV glioma)]. Tumor angiogenesis has been further associated with radioresistance, suggesting that angiogenic responses may be involved in radiation responses. We therefore investigated a potential role of cancer stem cells in tumor angiogenesis. We found that glioblastoma cancer stem cells promote tumor angiogenesis through elevated expression of vascular endothelial growth factor (VEGF) relative to the non–cancer stem cells (45). Further, the VEGF neutralizing antibody bevacizumab (Avastin) specifically inhibits cancer stem cell angiogenesis with marginal efficacy against non–stem cancer cells. Thus, targeting VEGF in cancer therapy may function as an anticancer stem cell therapy. We have reported recently that bevacizumab in combination with a topoisomerase inhibitor, irinotecan, shows a high level of activity in patients with recurrent glioblastoma (46). These results suggest that antiangiogenic therapies may additionally improve targeting of cancer stem cells and improve activity of radiation in solid cancers.

Oncology is witnessing an explosion in novel therapies, but the steps forward have been tenuous. Notably, the most effective of targeted therapies may have activity against cancer stem cells or the stem cell niche. Essential in the optimal development stem cell–targeted therapies will be the ability to disrupt cancer stem cells without affecting normal somatic stem cells. Identifying regulators of cancer stem cells that are less critical in normal stem cell biology will be important in achieving greater success in control of cancer stem cell growth. Although molecular therapies targeting cancer stem cells have promise, it seems that most targeted therapies may be most effective when used in combination with cytotoxic therapies or with other targeted therapies. Thus, the apparent resistance of cancer stem cells to conventional therapies does not mean that these therapies will not remain the mainstays of cancer treatment. Rather, it is likely that the greatest improvement in tumor control will initially be through the identification of resistance mechanisms within cancer stem cells that may be disrupted to augment durability of patient response.

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1 Z. Li, S. Bao, Q. Wu, and J. Rich, unpublished observations.
References


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