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# Radiation Responses of Cancer Stem Cells

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## Abstract

Recent experimental evidence indicates that many solid cancers have a hierarchical organization structure with a subpopulation of cancer stem cells (CSCs). The ability to identify CSCs prospectively now allows for testing the responses of CSCs to treatment modalities like radiation therapy. Initial studies have found CSCs in glioma and breast cancer relatively resistant to ionizing radiation and possible mechanisms behind this resistance have been explored. This review summarizes the landmark publications in this young field with an emphasis on the radiation responses of CSCs. The existence of CSCs in solid cancers place restrictions on the interpretation of many radiobiological observations, while explaining others. The fact that these cells may be a relatively quiescent subpopulation that are metabolically distinct from the other cells in the tumor has implications for both imaging and therapy of cancer. This is particularly true for biological targeting of cancer for enhanced radiotherapeutic benefit, which must consider whether the unique properties of this subpopulation allow it to avoid such therapies.

### Keywords

cancer stem cells; cancer initiating cells; radiation biology

## INTRODUCTION

Cancer cells in solid carcinomas display considerable heterogeneity in many aspects of their malignant phenotype and a single tumor can harbor cells with a wide range of radiosensitivity [Suwinski et al., 1999] and tumorgenicity [Hill and Milas, 1989]. One possible interpretation of this observation is that, like normal tissues, malignant tumors are organized hierarchically and contain a relatively rare and radioresistant subpopulation of cells that have an increased ability to initiate tumor growth and display accelerated regrowth after a sublethal treatment [Reya et al., 2001]. In a consensus publication that prospectively identified cells with increased tumorgenicity, this subpopulation was termed “Cancer Stem Cells” (CSCs) [Clarke et al., 2006].

This concept of CSCs has been and still is being rejected by some radiobiologists [Hill, 2006; Hill and Perris, 2007] because for some time the existence of such a CSC subpopulation could only be demonstrated retrospectively using a functional test, which left room for the interpretation that every cell in a tumor could gain a CSC phenotype if it had enough time. However, recent technical progress supports the presence of a hierarchical organization for



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breast cancer [Al-Hajj et al., 2003], brain tumors [Hemmati et al., 2003; Singh et al., 2003], prostate cancer [Collins et al., 2005], colon cancer [Ricci-Vitiani et al., 2007], head and neck cancer [Prince, 2007], lung cancer [Eramo et al., 2007], and melanoma [Schatton et al., 2008], which seems to replicate the hierarchical organization of the corresponding normal tissue of origin.

This review will summarize current data describing the radiation response of CSCs.

## THE ORIGIN OF CANCER STEM CELLS

Normal tissue stem cells are defined by their ability to self-renew and their multi-lineage potency. Together with increased tumorgenicity the same features define CSCs [Clarke et al., 2006]. This definition led to considerable confusion as it was inferred that normal stem cells were the origin of CSCs. Even without data on the origin of CSCs, this controversy over semantics is despite the point. The reality is that a subpopulation of cancer cells exist that can be identified prospectively, that have characteristics of "stemness," and that are important if we want to improve cancer treatment. The field should acknowledge their importance and study them rather than fighting over terminology [Jordan, 2009].

Four recent publications addressed the origin of CSCs using elegant mouse models. Reports from two independent groups reported that oncogene expression in intestinal stem cells but not in committed progenitor or differentiated cells led to the formation of intestinal tumors [Barker et al., 2009; Zhu et al., 2009]. Comparable results were reported for neuronal stem cells. Only oncogene expression in cells of the subventricular zone caused astrocytomas to form [Alcantara Llaguno et al., 2009]. Additionally, Perez-Caro et al. [2009] demonstrated that bcr-abl oncogene expression in sca-1-positive bone marrow cells was sufficient to induce leukemia and that elimination of CSCs cured the disease while STI571 application did not alter its course. These reports indicated that at least in murine tumor models CSCs arise from normal tissue stem cells. It remains to be shown if this is also the case for human cancers.

## IDENTIFICATION AND PROPAGATION OF CANCER STEM CELL

Assays to propagate stem cells and precursor cells developed in the neural stem cell field [Smukler et al., 2006] and were essential for the development of ways to propagate CSCs and identify them phenotypically. If performed accurately [Singec et al., 2006], when tumor cells are seeded at clonal densities of <1,000 cells/ml in serum-free conditions only CSCs and early progenitor cells survive and form non-adherent spheres cultures, consisting of up to a few hundred cells. Addition of a limited number of growth factors, mainly bFGF and EGF, stimulates growth and helps maintaining the stem cell phenotype. These conditions are clearly distinct from spheroids that had previously been commonly grown. These were cultured in the presence of serum, usually consisted of thousands of cells, and with no effort to make them clonal. Interestingly, spheroids were often appeared to be a better model of tumors than were monolayer cultures, which could be because of enrichment for CSCs in the spheroid central region. Reinvestigation of spheroids with an emphasis on CSC content might give a better understanding of radiobiological data obtained with these systems in the past.

Initially, CSCs were prospectively identified using combinations of antibodies against cell surface proteins. In a landmark publication, Al-Hajj et al. [2003] identified a subset of $CD24^{-/low}/CD44^{high}/ESA^{+}$ cells from hormone receptor-positive breast cancer specimens that exhibited increased tumorgenicity and multi-lineage potency. These cells were enriched if cells were cultured as mammospheres. A breast CSC population was also found in murine breast cancer models. However, in this case the cells were defined by a CD29+/CD49+ expression profile, which was also later used to identify normal mammary stem cells in mice. Interestingly, $CD24^{-/low}/CD44^{high}/ESA^{+}$ cells seem to be the earliest cells found in breast cancer metastases

suggesting that these cells initiate meta-static disease, although the number of $CD24^{-/low}/CD44^{high}/ESA^{+}$ cells in the primary tumor section did not predict for outcome in another study. However, using the gene expression profile of $CD24^{-/low}/CD44^{high}/ESA^{+}$ cells, Michael Clarke's group defined a gene expression signature that was highly predictive for clinical outcome indicating the clinical significance of breast CSCs [Cho et al., 2008].

More recently Gabriela Dontu's laboratory reported expression and activity of aldehyde dehydroxygenase 1 (ALDH1), an enzyme already known to be overexpressed in hematopoietic stem cells, as an even better marker for breast CSCs. In this study, $ALDH1^{+}$ breast CSCs partially overlapped with $CD24^{-/low}/CD44^{high}/ESA^{+}$ cells in human breast cancers, indicating heterogeneity of $CD24^{-/low}/CD44^{high}/ESA^{+}$ cells [Ginestier et al., 2007].

Two publications, one from the laboratory of Harley Kornblum [Hemmati et al., 2003] and a second by Singh et al. [2003] reported comparable data for a subset of $CD133^{+}$ cells in brain tumors. In both cases, this subpopulation not only exhibited increased tumorgenicity but the xenografts also reassembled the histopatho-logical phenotype of the original tumor. In an additional study, the presence of high numbers of CD133+ cells in gliomas was shown to be a valuable predictor of clinical outcome [Pallini et al., 2008].

Since 2003, several groups have identified CSCs in a variety of solid carcinomas (Table I). However, all require dissociation of the tumor to identify CSCs by marker expression and were thus not suitable for in vivo investigations. The first study addressing this problem expressed GFP under the control of the regulatory elements of BMI-1 [Hosen et al., 2007]. BMI-1 is a E3-ubiquitin ligase, which is upregulated in some normal tissue stem cells and CSCs. BMI-1 itself is degraded by the 26S proteasome [Cao et al., 2005]. More recently, we reported that CSCs in breast cancers and gliomas have low proteasome activity and we utilized this feature to identify, track, and target CSCs in vivo [Vlashi et al., 2009]. Using this system we were able to show that, as in leukemia [Perez-Caro et al., 2009], elimination of CSCs was sufficient to cause regression of solid cancers [Vlashi et al., 2009]. This system provides a unique opportunity to investigate the effect of cancer therapies on CSCs in vitro and in vivo.

## RADIATION RESPONSES OF CANCER STEM CELLS

Currently, localized solid cancers can only be cured by surgery or radiation treatment and solid tumors that have metastasized are by definition incurable. If tumor growth and regrowth after therapy is a property of CSCs, the response of these cells to radiation is a critical parameter for curability. Again, the first studies addressing the radiation response of CSCs were performed in glioma and breast cancer. Bao and coworkers reported radiation resistance of CD133+ cells in glioma. This resistance was attributed to constitutive activation of the DNA repair checkpoint and inhibition of the corresponding kinase radiosensitized CD133+ cells [Bao, 2006]. We reported radioresistance of breast CSCs but, contrary to glioma, CSCs breast CSCs produced less reactive oxygen species in response to radiation indicating a high level of expression of free-radical scavengers [Phillips et al., 2006].

Since then, radiation resistance of CSCs has been confirmed by several independent groups [Woodward et al., 2007; Chiou et al., 2008; Hambardzumyan et al., 2008; Diehn et al., 2009; Lu et al., 2009; Chang et al., 2009]. Interestingly, survival curves of CSCs isolated from the MCF-7 breast cancer lines showed a clear shoulder. While this could be interpreted as enhanced DNA repair, they failed to phosphorylate H2AX in response radiation suggesting diminished damage or alternative mechanisms might operate [Phillips et al., 2006]. Our data on breast CSCS was confirmed by Diehn et al. [2009] who were able to show a strong radical scavenger gene expression signature using single cell RT-PCR. Interestingly, radiation activated the Notch signaling pathways in breast CSCs in a PI3K-dependent fashion through upregulation of Notch receptor ligands. This pathway is involved in stem cell maintenance in breast cancer

and its activation by radiation increased the number of CSCs [Phillips et al., 2006]. Activation of the Notch pathway by radiation was recently confirmed in endothelial cells [Scharpfenecker et al., 2009] indicating that this pathway may contribute to the radiation response of normal and malignant tissues.

Oxygen has long been known to be one of the most potent radiosensitizing agents. Tumors contain areas of low oxygen tension and cells residing in these areas were considered to be relatively protected from radiation. Consequently, considerable effort has been made to overcome tumor hypoxia to improve radiation treatment results. Surprisingly, CSCs were reported to reside in a perivascular niche [Calabrese et al., 2007; Vlashi et al., 2009] and are therefore unlikely to be protected from radiation by hypoxia. However, this observation offers an attractive explanation for the efficiency of anti-angiogenic therapies combined with radiation as they may target the CSC niche rather than tumor cells in general. Anti-angiogenesis combined with radiation, as a concept, is counterintuitive because one would expect the proportion of hypoxic cells and hence radioresistance to increase under such a treatment. However, it supports the importance killing CSCs over the bulk of the tumor because the effects of anti-angiogenic therapies on the CSC niche seem to render therapy-induced tumor hypoxia irrelevant. Those in the radiation field have of course always known that partial responses to therapy are relatively meaningless in terms of patient outcome and that what is most important is killing the last surviving tumor clonogen, which may now be termed a CSC.

## CONCLUDING REMARKS

Over the last 114 years, radiation therapy techniques have evolved to a degree of precision that far exceeds the need in most daily standard cancer treatments. At the same time, progress in cancer cure has advanced at a much slower pace and for many cancers like glioma, pancreatic cancer, and lung cancer the success rate of state-of-the-art treatments is still unacceptable and has remained unchanged for decades. This indicates that the cost of future improvements in the technical aspects of radiation delivery is unlikely to be justified by improved treatment outcomes and that cure, for example, of a glioma patient will only occur if we radically change the way we approach the disease.

The existence of CSCs in solid cancer has been advocated by radiobiologists for decades [Withers et al., 1988; Trott, 1994]. However, until recently this concept was only hypothetical. Novel marker signatures and culture systems now allow the unique features of CSCs to be studied and novel therapies tested for their efficiency in killing these cells. The fact that radiation cures cancer patients already implies that this therapy modality is effective against CSCs. Unlike many chemotherapeutic treatments for which anti-cancer efficacy is judged only by temporary partial tumor responses that may not involve CSCs, radiation therapy can undergo biological refinement by combination with agents that increase its efficacy against this critically important CSC subpopulation. Thus, targeting CSCs with radiation holds enormous potential for eventual cure for many of our cancer patients and it should encourage opponents of the CSC concept to stop fighting over terminology and to return to the bedsides and benches.

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**TABLE I**

Surface Markers of Solid Cancer Stem Cells

| Tumor type | Stem cell marker | Refs. |
|---|---|---|
| Breast cancer | $CD44^{high}/CD24^{-/low}/lineage^{-}$ | Al-Hajj et al. [2003] |
| Brain tumors | $CD133^{+}$ | Hemmati et al. [2003], Singh et al. [2003] |
| Prostate cancer | $CD44^{+} / \alpha_2\beta_1^{high} / CD133^{+}$ | Collins et al. [2005] |
| HNSCC | $CD44^{+}$ | Prince et al. [2007] |
| Colon cancer | $CD133^{+}$ | Ricci-Vitiani et al. [2007] |
| Lung cancer | $CD133^{+}$ | Eramo et al. [2007] |
| Pancreatic cancer | $CD44^{+}/CD24^{+}/ESA^{+}$ | Li et al. [2007] |
| Melanoma | $ABCB5^{+}$ | Schatton et al. [2008] |