Breast Cancer RESEARCH

EDITORIAL

Radiation resistance in breast cancer: are CD44$^+$/CD24$^-$/proteosome^{low}/PKH26$^+$ cells to blame?

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See related research by Lagadec *et al.*, http://breast-cancer-research.com/content/12/1/R13

Abstract

Identification and characterization of cancer-initiating cells (CICs) enriched for stem cell-like functions and the establishment of a link between CICs and tumor recurrence, chemotherapy resistance and radiation resistance, and metastasis have been the focus of cancer research for the last eight years. Although this field has its share of controversies, it is becoming apparent that cells isolated from recurrent or residual tumors or both are enriched for cancer cells that have a specific phenotype compared with heterogeneous cells in the primary tumor. Enrichment of CICs in tumors subjected to radiation therapy could be due in part to the delivery of sublethal doses of treatment and the efficient radical scavenging system within CICs. Sublethal doses of radiation are sufficient to induce senescence of non-CICs while forcing CICs to gain several new properties related to cell cycle progression in addition to maintaining or enhancing stem cell characteristics of pre-treatment CICs. Characterizing pathways responsible for the increase in CICs after therapy and exploiting the unique characteristics of therapy-resistant CICs for developing targeted therapies are becoming a central focus of research in the rapidly evolving field of CICs.

In the previous issue of *Breast Cancer Research*, Lagadec and colleagues [1] used breast cancer cell line models to examine the self-renewal and cell cycle properties of cancer cells that survive during the course of fractionated sublethal radiation. Previous studies from the same group and others have demonstrated enrichment or resistance of cancer-initiating cells (CICs) during the course of fractionated radiation [2,3]. The current report demonstrates that the enriched CICs are capable of enhanced self-renewal for three generations compared with CICs from non-irradiated cells. Contrary to non-irradiated CICs, which are non-proliferating and are predominantly in the G_0 phase of the cell cycle, radiation mobilizes CICs from a quiescent to a proliferative state (G_2 phase). Whether CICs undergo similar cell cycle changes *in vivo* upon radiation and whether cycling CICs become susceptible to drugs targeting cells moving through G_2 phase remain to be determined.

Cancer cells expressing the cell surface marker CD44 but not CD24 (CD44$^+$/CD24$^{-/low}$) were the first described breast cancer CICs [4]. Subsequent studies have identified additional phenotypic or functional markers or both to characterize CICs: elevated levels of aldehyde dehydrogenase (ALDH1$^+$) [5], lower levels of proteosome activity [6], enhanced PKH26 dye-retaining capacity [7], CD24^{high}/CD49F^{high}/Delta-notch-like EGF (epidermal growth factor) repeat-containing transmembrane (DNER)^{high} [7], CD24^{high}/CD49F^{high}/Delta-like 1 (DLL1)^{high} [7], CD49F$^+$/DLL1^{high}/DNER^{high}, and expression of a mammosphere signature, including low expression of claudin [8]. CD44$^+$/CD24$^-$ CICs and non-CD44$^+$/CD24$^-$ cells expressing different levels of these markers have been tested in *in vitro* (mammosphere) and *in vivo* (tumorigenicity in NOD/SCID [non-obese diabetic/severe combined immunodeficiency disease] mice) assays. CD44$^+$/CD24$^-$/ALDH1$^+$ cells display the highest level of CIC activity in tumorigenicity assays compared with CD44$^+$/CD24$^-$ or ALDH1$^+$ cells [5]. Lagadec and colleagues [1] observed a subpopulation of CD44$^+$/CD24$^{-/low}$ cells with low proteosome activity, although this specific subpopulation was not characterized further for stemness. Although these studies are beginning to refine CICs into distinct subgroups, the remaining question is which of the phenotypically distinct CICs are responsible for chemotherapy resistance and radiation resistance and organ-specific metastasis. Also, it is

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unclear whether the above markers can identify CICs for all intrinsic subtypes of breast cancer [9] or whether these markers favor characterization of CICs derived from the basal subtype or the $HER2^+$ subtype of breast cancer [10,11].

$CD44^+/CD24^{-/low}$ cells are generally enriched in basal subtype breast cancers as well as in cell lines that have undergone epithelial-to-mesenchymal transition [10,12]. In contrast, luminal type breast cancers that express estrogen receptor-alpha (ERα) contain less than 1% $CD44^+/CD24^{-/low}$ cells [10]. Lagadec and colleagues [1] were able to demonstrate enrichment of $CD44^+/CD24^-$ cells upon fractionated radiation of $ER\alpha^+$ breast cancer cell lines MCF-7 and T47-D. A significant number of enriched cells retained PKH26 dye and contained low proteosome activity compared with non-irradiated cells or cells with high proteosome activity. Thus, radiation-resistant cells display three characteristics of CICs: $CD44^+/CD24^{-/low}$, low proteosome, and $PKH26^+$. Interestingly, enriched cells from T47-D cells, but not from MCF-7 cells, displayed enhanced self-renewal capacity for three generations, suggesting differences in CICs between ERα breast cancer cell lines. Both MCF-7 and T47-D cells represent luminal type A breast cancer, which generally belongs to a favorable prognostic subgroup [13]. However, the two cell lines differ in their p53 mutation status; T47-D cells contain mutant p53, whereas p53 is wild-type in the MCF-7 cells [14]. Based on the previously described role of p53 in cancer stem cells in mouse models [15], it is tempting to speculate that p53 status is one of the factors that determine the self-renewal capacity of cancer cells that survive a sublethal dose of radiation. It is important to emphasize that the enrichment of CICs was observed only upon exposure to a sublethal dose of radiation. Therefore, similar enrichment of CICs may not occur in the clinical setting unless a significant fraction of cancer cells receive a sublethal dose or contain a p53 mutation or both. In this respect, recent clinical trials have demonstrated significant survival benefits of radiation therapy for breast cancer patients [16]. Further optimization of radiation delivery to avoid exposure to sublethal doses may lead to increased benefits of radiation therapy.

Abbreviations

ALDH1, aldehyde dehydrogenase; CIC, cancer-initiating cell; DLL1, Delta-like 1; DNER, Delta-notch-like EGF (epidermal growth factor) repeat-containing transmembrane; ERα, estrogen receptor-alpha.

Competing interests

The author declares that he has no competing interests.

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