Targeting breast cancer stem cells

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Introduction

Although a relationship between carcinogenesis and stem cell biology has been postulated for a long time, it is only recently that advances in stem cells biology have enabled direct testing of this hypothesis. In traditional or “stochastic” models of carcinogenesis, any cell may become transformed and all cells within a tumor potentially have equally malignant potential. The cancer stem cell hypothesis is a fundamentally different way of describing carcinogenesis. According to this model, cancers arise in cells that display dysregulated self-renewal. This may involve dysregulation of normal stem cell self-renewal or the acquisition of self-renewal potential by other cell populations. As a result of this, tumors are organized in a hierarchical structure in which self-renewing cancer stem cells drive tumorigenesis. This highlights the importance of understanding the processes which regulate normal mammary stem cell self-renewal.

Our laboratory has developed a number of in vitro and mouse models to elucidate self-renewal pathways in normal and malignant mammary stem cells. We demonstrated that normal mammary stem and progenitor cells could be enriched through cultivation in anchorage independent conditions to form “mammospheres”. These mammospheres are enriched in both stem and progenitor cells capable of multilineage differentiation. Furthermore, we have recently described the development of humanized NOD/SCID mouse models that facilitate the growth of human mammary stem cells. In these systems which represent a modification of those first described by Kupperwasser and Weinberg.1 Irradiated mammary fibroblasts are used to condition the cleared mammary fat pads of NOD/SCID mice. Mammosphere initiating cells, when placed into this environment, form ductal alveolar structures (Fig. 1).

The mammosphere and mouse systems have proved valuable in identifying putative stem and progenitor cell markers. One of the most valuable markers is Aldehyde dehydrogenase. Previously, increased activity of Aldehyde dehydrogenase was described in hematopoietic and neuronal stem and progenitor cells. Activity of aldehyde dehydrogenase is readily assayed by the commercially available Aldefluor assay. We have determined that the ALDH-A1 isoform is the one primarily expressed in normal and malignant mammary cells and can be used for immunodetection. We have previously demonstrated that normal mammary stem cells capable of mammosphere formation and regeneration of ductal alveolar structures are exclusively contained within Aldefluor-positive populations.5 We have utilized these systems to analyze self-renewal pathways in normal and malignant mammary stem cells which include Notch,6 Hedgehog2 and Akt pathways.7 Furthermore, we have utilized these systems to demonstrate that normal human mammary stem cells do not express estrogen receptor but give rise to estrogen receptor expressing proliferative progenitor cells that, in turn, give rise to terminally differentiated estrogen receptor-positive cells (Fig. 2). Together these systems and studies provide a background for understanding the role of stem cells in breast cancer development.

As indicated above, the cancer stem cell model of carcinogenesis is fundamentally different from the classic “stochastic” model. In the stochastic model tumors result from random mutation and clonal selection. In the cancer stem cell model self-renewing cancer stem cells drive tumorigenesis as well as generating non-renewing cells that form the bulk of tumors. The first evidence for cancer stem cells was provided in human leukemias in pioneering studies by John Dick’s laboratory.9 Utilizing similar methodologies our group, together with Michael Clarke, first described the prospective isolation of tumor initiating populations from human mammary carcinomas. These cells were characterized as ESA+/CD44+/CD24−. As few as 100 cells bearing this phenotype were able to produce tumors in NOD/SCID mice whereas over 100-fold greater cells that did not bear this phenotype was non-tumorigenic. Furthermore, the “cancer stem cells” produced tumors which recapitulated the phenotypic heterogeneity of the initial tumor.10 Subsequently we demonstrated that the Aldefluor assay can also be used to enrich for tumor initiation cells. Furthermore, we demonstrated overlap between the CD44+/CD24− and Aldefluor-positive population such
Fig. 1. In vitro and mouse models to study human mammary stem/progenitor cells. Stem/progenitor cells can be propagated as floating spherical colonies termed "mammospheres" (A). Cells from mammospheres can be induced to undergo lineage specific differentiation when they are cultured on collagen coated dishes (B). Mammosphere derived cells undergo morphogenetic differentiation when cultured in a three-dimensional matrix of basement membrane material (C). Mammosphere forming cells form ductal alveolar structures in the humanized cleared fat pads of NOD/SCID mice (D). References: Dontu & Wicha 2003, Kuperwasser & Weinberg 2004, Liu & Wicha 2006, Weaver & Bissell 1999.

Fig. 2. Normal mammary development and estrogen receptor expression. In the human mammary gland stem cells are ER-negative but differentiate into highly proliferative ER-positive progenitor cells. Under the action of estrogen and progesterone, these progenitor cells may interact with stem cells via paracrine loops, involving Wnt and growth hormone. Proliferating ER-positive progenitor cells give rise to quiescent ER-positive and -negative terminally differentiated cells in the adult gland.

that as few as 20 CD44+/CD24−/ALDH+ cells are capable of forming tumors. Immunochemistry can be utilized to detect ALDH1 expression in human breast carcinoma tissue arrays. In a series of 477 tumors, we found that ALDHA1 expression is an independent prognostic factor in multivariant analysis and predictor of poor outcome. Interestingly ALDH expression shows correlation with tumor grade ER and PR negativity and HER2 overexpression.

In addition to primary tumors and xenografts, we have also demonstrated that many breast cancer cell lines also contain functional cancer stem cells that can be isolated by virtue of their Aldehyde dehydrogenase expression. Interestingly, all basal breast carcinoma cell lines contain an Aldefluor-positive population whereas many luminal cell lines do not. This is consistent with our clinical findings that basal carcinomas and some luminal type B tumors contain ALDH and ALDHA1 expressing cells whereas many luminal A do not. This has led us to postulate that different molecular subtypes of human breast cancer may originate from different stem/progenitor cells. We hypothesized that the triple-negative for basal cancers arise from the ER-negative stem cells. Luminal type B also arises from the similar cell population. However, the mutations in these cells allow some cells to express estrogen receptor. These tumors, which are ER-positive, are driven by an ER-negative stem cell. In contrast, the luminal type A tumors largely arise from transformation of ER-positive progenitor cells. These tumors are either ALDH-negative or contain ALDH-positive cells that are also ER-positive. We are currently testing the clinical implications of this model.

Stem cells and metastasis

We have utilized ALDH containing breast cancer cell lines to examine the relationship between the stem cell phenotype and tumor invasion and metastasis. We demonstrated that ALDH-
positive cells have far greater invasive and metastatic capacity than ALDH-negative cells when these cells are introduced into the left ventricle of NOD/SCID mice.11 This is also consistent with our findings that cells expressing stem cell markers are found on the leading or invasive front of breast carcinomas. This may explain the high local recurrence rate which occurs in following incomplete resections that have histologically positive margins. These studies also have implications for an understanding of metastatic patterns of human breast cancer. Clinical studies have indicated that up to 1/3 of women with localized breast cancer display bone marrow micrometastasis at the time of diagnosis. However, clinical follow-up reveals that only about 50% of these develop overt metastasis over 10 years.13 Current studies are elucidating whether recurrence can be predicted by the presence of breast cancer stem cells at metastatic sites. In addition, the issue of tumor dormancy may relate to stem cells at a distant site which remain dormant for extended periods. This fits the profile of displaying a low level of cell proliferation but may be activated by hormonal or inflammatory signals.

Implications of the cancer stem cell hypothesis for treatment

The cancer stem cell hypothesis has important implications for cancer therapeutics. Current agents have largely been selected for their ability to cause tumor shrinkage in preclinical models and clinical trials. The so-called RECIST criteria relying on tumor measurements are an example. However, the cancer stem cell model suggests that tumor initiating cells may comprise only a small fraction of tumors and thus may not be reflective of changes in tumor bulk. Consistent with this, recent studies have suggested that breast cancer stem cells are relatively resistant to both radiation14 and chemotherapy. Furthermore, in a recent neoadjuvant clinical trial15 the percentage of CD44+/CD24− and mammosphere forming cells increased following neoadjuvant chemotherapy. Interestingly, a separate group of patients with HER2 amplification were treated with the HER2 and EGFR inhibitor Lapatinib in addition to chemotherapy. These patients’ tumors initially contained a higher percentage of cancer stem cells which were reduced following HER2 inhibition. This may be explained by our recent observation that HER2 overexpression increases the stem cell content of tumors. HER2 targeting agents such as trastuzumab are able to reduce the cancer stem cell population which drives tumorigenesis and metastasis.16

Interactions between Notch and HER2 signaling

Our group and others have previously shown that the Notch pathway plays a role in normal and malignant mammary stem cell self-renewal. Interestingly, Magnifico et al.17 recently showed that Notch signaling may also regulate HER2 overexpression. In breast cells with activated Notch signaling they demonstrated that the cancer stem cell populations displayed increased HER2 expression. These cells were inhibited by trastuzumab. These findings have important clinical implications since they suggest that trastuzumab may benefit some patients whose tumors do not display HER2 amplification but may nevertheless, overexpress HER2 in the cancer stem cell compartment. This may provide an explanation for the recent report that a group of patients whose tumors did not display HER2 amplification upon central review, nevertheless, benefited from adjuvant trastuzumab.18,10 Furthermore, this suggests that at the level of the cancer stem cell strategies that combine Notch inhibition with HER2 inhibition may be a rational approach to increase the efficacy of HER2 blockade. Since agents such as gamma secretase inhibitors and Notch DLL ligand inhibitor are currently entering clinical trials allowing for a direct test of the hypothesis. In addition, other strategies aimed at targeting breast cancer stem cells are currently in development and are entering clinical trials. These studies should directly test the hypothesis that the targeting and elimination of breast cancer stem cells can improve therapeutic outcome.


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References