Commentary

Recent translational research: stem cells as the roots of breast cancer

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Abstract

Common phenotypes of cancer and stem cells suggest that breast cancers arise from stem cells. Breast epithelial cells with stem cell phenotypes have been shown to be more susceptible to immortalization and neoplastic transformation. Breast tumor stem cells with $CD44^+/CD24^{-/low}Lineage^-$ markers have been isolated. The role of these cells in tumor progression and clinical outcome is not clear. The relationship between breast stem cell and tumor stem cell may be elucidated by further studies of carcinogenesis of nonadherent mammosphere cells with stem cell features and by derivation of $CD44^+/CD24^{-/low}$ cells from an adherent breast epithelial stem cell type.

Introduction

As summarized by Hanahan and Weinberg [1], cancer cells possess several characteristics that may be considered hallmarks of cancer. However, some major characteristics of cancer cells were not included in that list, namely common phenotypes of cancer and stem cells such as deficiency in gap junctional intercellular communication (GJIC) [2] and expression of genes that are involved in stem cell specific function and self-renewal (e.g. Oct-4 [3] and other pathways [4]). These common phenotypes suggest that cancers arose from stem cells.

In human breast cancer, two lines of observations have been cited as evidence in favor of the stem cell theory of carcinogenesis. First, pregnancy may reduce the risk of breast cancer possibly by inducing the differentiation of mammary gland and reducing the number of breast stem cells [5]. Second, in a Japanese study of the effects of atomic bomb detonation [6], evaluation of the radiation effect revealed that young women were more susceptible to radiation-induced breast cancer. Breast epithelial cells with stem cell features have been isolated [7,8]. Characterization of these cells provides more direct evidence for the stem cell hypothesis of breast tumorigenesis. Recently, several different human cancers, including breast cancer, were shown to contain tumor-initiating stem cells [9-11]. These cells are believed to sustain breast tumor growth and to be targets for cancer treatment. However, the role of these cells in breast tumor progression and the prevalence of these cells in clinical outcome are not yet clear [12].

Common phenotypes of human breast epithelial stem cells and tumor cells

Two types of normal human breast epithelial cells (HBECs) derived from reduction mammoplasty have been isolated and characterized [7]. Type I HBECs express estrogen receptor (ER)-α and luminal epithelial cell markers (i.e. epithelial membrane antigen and cytokeratin [CK]18). These cells also exhibit stem cell characteristics, including deficiency in GJIC [7], capacity for anchorage-independent growth (AIG) [13], ability to differentiate into basal (type II HBECs) and luminal (acini-forming) epithelial cells [7,13], expression of the embryonic stem cell marker Oct-4 [3], and the ability to form budding/ductal structures on Matrigel [13,14]. In contrast to type II HBECs, which have basal epithelial characteristics [7,13], type I HBECs share many common phenotypes with breast carcinoma cells such as MCF-7, including deficiency in GJIC and expression of ER-α, epithelial membrane antigen, CK18, and Oct-4 [3,7,13-16].

Breast epithelial stem cells as major target cells for carcinogenesis

Type I HBECs have been shown to be more susceptible to telomerase activation and immortalization following SV40 (simian virus 40) large T-antigen transfection [14]. These immortalized type I HBECs can then be converted to weakly tumorigenic and highly tumorigenic cells by X-ray irradiation (the best known breast carcinogen) and c-erbB2/neu (a well known breast oncogene) [17]. Expression of Oct-4 [3] and

AIG = anchorage independent growth; CK = cytokeratin; ER = estrogen receptor; GJIC = gap junctional intercellular communication; HBEC = human breast epithelial cell.

lack of expression of the protease inhibitor maspin [16] were identified in these cells at all stages of neoplastic transformation, providing strong evidence that type I HBECs are major target cells for carcinogenesis. It should be noted that, in contrast to type II HBECs, type I HBECs do not express three genes (connexin 26, α_6 integrin, and maspin) that are considered to be tumor suppressor genes [18-20]. Furthermore, type I HBECs were capable of AIG at low and high frequencies before and after overcoming senescence, respectively [7,13]. In the literature many SV40 immortalized HBECs have been reported, but none of them was capable of AIG (for references, see the report by Kao and coworkers [7]). Many of the tumor phenotypes possessed by type I HBECs may contribute to the high susceptibility of these cells to neoplastic transformation.

A different approach following the isolation of neuronal stem cells as neurospheres [21] has been employed to isolate nonadherent mammospheres [8]. These mammosphere cells have been shown to differentiate along three mammary epithelial lineages (luminal, myoepithelial, and alveolar) in conditions that favor differentiation, and to clonally generate complex functional structures in three dimensional culture. The stem cell features of mammospheres also include Hoechst dye exclusion and expression of some genes that are involved in stem cell/progenitor cell-specific functions [4]. Unlike type I HBECs, mammosphere cells have not been shown to express ER-α or Oct-4, and to be more susceptible to neoplastic transformation.

Two breast tumor types phenotypically corresponding to two normal HBEC types

Immunohistochemical and mRNA expression profiling studies of large breast cancer cohorts have reproducibly identified a subset (about 15%) of tumors expressing markers of the basal layer of the mammary gland [22]. These tumors are invariably ER negative with high p53 mutations, rarely contain amplified HER2, and are generally high grade/poorly differentiated and associated with poor prognosis. The phenotypes of these cells are similar to those of type II HBECs, which express basal epithelial cell markers and are ER negative [7,13,15]. In contrast, the majority of human breast cancers are ER positive, express luminal cytokeratins (CK8, CK18, and CK19) and harbor a low frequency of p53 mutation [22]. The phenotypes of these tumors are clearly similar to those of the type I HBECs, which express CK18, CK19 and ER-α, and can be neoplastically transformed by HER2/neu oncogene [13,17]. The corresponding phenotypes of these two types of breast tumors with two types of normal breast epithelial cells suggest two different target cells for breast carcinogenesis, namely type I and early type II HBECs, which are highly proliferative and could be precursors for myoepithelial cells. However, common mammary stem or progenitor cells could give rise to two types of breast tumors, as shown by the coexistence of luminal and myoepithelial cells in mouse mammary tumors induced by Wnt signaling [23] and the expression of different lineage-specific markers by cultured breast tumor mammospheres under differentiating conditions [24]. It is possible that tumor stem cell differentiation may involve a wholesale switch in gene expression, similar to the differentiation of type I HBECs [7].

The origin of breast tumor stem cells

In recent years cancer-initiating stem cells have been reported for human leukemia, myeloma, and brain and breast tumors. In most cases, the markers for cancer stem cells are also markers for normal stem cells in the same tissue (i.e. $CD34^+/CD38^-$ for leukemia stem cells and hematopoietic stem cells [25], and $CD133^+$ and $nestin^+$ for brain tumor stem cells and normal neural stem cells [10]), suggesting that the initiating target cells are stem cells and not the committed progenitor cells. The breast cancer-initiating cells have been identified as $CD44^+/CD24^{-/low}Lineage^-$ [11] and have not been cultured as adherent cells. These markers were not found in adherent type I HBEC with stem cell characteristics. In fact, the immortal and tumorigenic type I HBECs were $CD44^-/CD24^+$ [16]. The discrepancy could be due to the selection of $CD44^+/CD24^{-/low}$ cells, which are nonadherent, and the culture of type I HBECs as adherent cells, which would exclude $CD44^+/CD24^-$ cells. Because a subpopulation of type I HBECs was capable of AIG [13], these cells could exhibit a $CD44^+/CD24^-$ phenotype and give rise to the reported breast tumor stem cells.

In vitro propagation of tumor-initiating breast tumor cells has recently been reported [24]. These cells are $CD44^+/CD24^{-/low}$ and Oct-4^+/connexin 43^-; the latter are similar to type I HBECs [3,13]. On the other hand, although mammospheres can be formed by a subpopulation of breast epithelial cells and tumor cells. Some important breast tumor phenotypes such as ER-α and Oct-4 expression and deficiency in GJIC have not been demonstrated in normal mammospheres. The origin of breast cancer from mammosphere cells remains to be demonstrated. Identification and elucidation of the nature of target cells for breast carcinogenesis will help us to develop preventive, early detection, and therapeutic strategies for breast cancer.

The role of $CD44^+/CD24^{-/low}$ cells in tumor progression and clinical outcome

The *in vitro* culture of $CD44^+/CD24^-$ tumor mammospheres was successful for a minority of breast tumor samples (three out of 16) [24]. This indicates either the absence of $CD44^+/CD24^-$ in most breast tumors (13 out of 16) or a limitation of the culture method. In a clinical study, the $CD44^+/CD24^{-/low}$ tumor cells were not found to increase in tumor progression from carcinoma *in situ* to carcinoma but may favor distant metastasis [12]. There was no significant correlation between the prevalence of $CD44^+/CD24^{-/low}$ tumor cells and event-free or overall survival in breast cancer patients. These findings must be verified by further independent studies.

Conclusion

There is substantial evidence in favor of the stem cell theory of breast carcinogenesis, based on the observation of phenotypes common to breast stem cells and tumor cells and on the demonstration that breast epithelial cells with stem cell features were more susceptible to neoplastic transformation. However, the origin of breast tumor stem cells remains unsettled. This may be resolved by identification of a subpopulation of type I HBECs that are nonadherent and with $CD44^+/CD24^-$ expression and/or demonstration that the nonadherent mammosphere cells are target cells for neoplastic transformation. The reported lack of correlation between the prevalence of $CD44^+/CD24^-$ cells and clinical outcomes requires examination in further studies.

Competing interests

CC holds US Patents 5650317, 5814511 and 6140119 which are related to this commentary.

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