

Mammary stem cells: the root of breast cancer?

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Abstract Tissue-specific stem cells play a key role in organ homeostasis. They are relatively well characterized in systems which undergo constant proliferation and production of differentiated cells, including the haemopoietic system, skin and intestine. However, little is known about the role and regulation of stem cells in the mammary gland. This review briefly summarizes the current understanding of the role of breast-specific stem cells in normal and cancerous tissues, and how this may identify new targets for breast cancer prevention and therapy.

Keywords: Breast; Stem cell; Epithelium; Cancer

Introduction

What are stem cells?

Stem cells are defined by their ability to both self-renew and produce progeny that differentiate into all the functional cell types of a particular tissue. They are relatively undifferentiated cells which are long lived and able to undergo either symmetric or asymmetric division to give rise to either identical progeny; a proliferative precursor cell, or both [1]. These properties are essential for the maintenance of normal human tissues in which there is a high cell turnover.

Evidence for mammary stem cells

Tissue-specific stem cells are integral to the development and function of the mammary gland. Cycles of cell proliferation, differentiation, apoptosis and remodelling associated with the menstrual cycle, pregnancy and lactation occur many times throughout the reproductive life of women. These cycles of growth are thought to rely on the presence of a mammary stem cell population.

The functional unit of the breast, the lobule, consists of a terminal duct opening into several acini forming the terminal duct lobular unit (TDLU) which is lined with two layers of cells. The outer layer consists of myoepithelial cells which surround an inner or luminal layer. The majority of breast cancers exhibit a luminal phenotype [2], suggesting that they are derived from a luminal cell precursor [3]. Large contiguous patches of cells within the breast epithelium exhibiting identical X chromosome inactivation [4] suggest that they are derived from the same cell. In mice, transplantation of single cell suspensions or fragments of mammary gland into cleared mouse mammary fat pads can regenerate an entire functional gland [5–7]. Culture of primary human mammary cells suggests the presence of three distinct progenitor cell types; luminal precursors, myoepithelial precursors and multipotent precursors which may be stem cells [8,9]. These studies suggest that mammary glands contain a population of stem cells that are able to give rise to all cell types.

Identifying mammary stem cells

By definition, breast stem cells should rarely divide and persist throughout reproductive life. This means that they can be identified on the basis that they will retain label after the administration of labelled DNA precursors, such as tritiated thymidine or

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bromodeoxyuridine. This approach has been used by a number of groups and shows in the mouse mammary gland, for example, that up to 5% of cells retain label and that these cells also express other putative stem cell markers such as stem cell antigen-1 (Sca-1) or breast cancer resistance protein 1 (Bcrp1) (see below) [10–14]. Electron microscopy has been used to identify small light cells or undifferentiated cells in mouse and rat mammary epithelium [15]. As these light cells divide rarely [15,16], are found in clusters, and can give rise to more differentiated cell types, they are thought to be mammary stem cells [17]. These studies also show that putative stem cells exist in specific physical locations or stem cell niches in both rat [18] and human mammary glands [19]. Stem cell niches are anatomically specialized locations which provide the stem cell with a unique micro-environment necessary for its correct function as demonstrated in other tissues such as skin, bone marrow, gut and hair follicles. As stem cell behaviour appears to be dependent on the stem cell niche [20,21] any attempt to isolate stem cells from a tissue will lead to disruption of that niche and to possible alteration of cell behaviour, which needs to be taken into account when studying their behaviour both *in vitro* and *in vivo*.

Using immunochemical techniques, a population of cells has been isolated from an intermediate position in the human breast that corresponds to the location of the rodent mammary stem cell niche. These cells were negative for markers of luminal or myoepithelial differentiation [8,19] and could give rise to multiple lineages in culture [9]. Finally, the technique of side population (SP) analysis used previously to identify haemopoietic stem cells [22] has been applied to the identification of mammary stem cells. [3,13,23,24]. SP cells are able to efflux the fluorescent dye, Hoechst 33342 by the ABCG2 transporter molecule, also known as Bcrp1. The SP fraction is enriched for stem cells, as it has been demonstrated that mouse mammary SP cells, which also expressed the mouse Sca-1, were able to repopulate cleared mammary fat pads of syngeneic hosts [3,13].

Relevance of stem cells to cancer

It has been suggested that tumours may arise from 'cancer stem cells' (CSCs) [25]. The existence of CSCs may account for the phenotypic heterogeneity seen within solid tumours, which are composed of a mixture of differentiated tumour cell types with limited proliferative capacity and a small population of proliferative, undifferentiated stem cells. Evidence for CSCs has been demonstrated in breast tumours [26] and in leukaemia [27] where transplantation of a subpopulation of tumour cells generated tumours

which were phenotypically identical to the parent tumours. The presence of CSCs has also been seen in glioblastomas [28], which are able to generate differentiated tumour cells. It has been suggested that dissemination of CSCs throughout the body may be the cause of metastasis as the secondary tumours tend to be composed of the same range of cells as the primary tumour [25,29].

Mutation of a stem cell is not the only mechanism for carcinogenesis. It is also possible that a more differentiated, or lineage restricted, progenitor cell could acquire mutations which endow the cell with a stem cell-like capacity for self-renewal [29,30]. Indeed, transformation of different progenitor populations could account for tumour heterogeneity.

What implications does the CSC hypothesis have for anti-cancer therapies? Currently the end point for chemotherapy is a reduction in tumour size, which is attained by using drugs which target actively proliferating cells. However, a CSC may divide infrequently and be refractory to the chemotherapeutic hit. Additionally, stem cells tend to synthesize proteins, such as Bcrp1, which efflux toxic drugs [31,32]. Therefore, for the successful development of new anti-cancer therapies it seems necessary to target stem cells. Both stem cells and cancer cells share similar mechanisms for regulation of self-renewal which include the Notch [33], Hedgehog [34,35] and Wnt [36] pathways. These regulatory mechanisms may offer new therapeutic targets. Wnt is particularly interesting as it has been demonstrated that dysregulation of Wnt signalling leads to increased incidence of epithelial cancers [37,38] and is able to enlarge the haemopoietic stem cell pool [39]. However, targeting regulatory mechanisms common to both normal and CSCs may not provide any significant enhancement of the therapeutic ratio [40]. However, the fact that some leukaemias [41] and most testicular tumours can be cured [42] suggests that targeting CSCs is possible without increasing toxicity to unacceptable levels.

Conclusions

Stem cells are long lived and multipotent, and are essential for development and maintenance of normal tissues by the processes of proliferation and terminal differentiation. Studies have provided evidence for the existence of mammary stem cells in mice, rats and humans. Subsequent identification and isolation of these stem cells has shown their ability to repopulate cleared mammary fat pads and give rise to all the structures seen in normal mature glands. These infrequent and elusive cells have the ability to teach us more about tissue development, tumour susceptibility, how tumour growth is initiated and promoted, and how to prevent or control these processes.

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