

Commentary

Identification of murine mammary stem cells: implications for studies of mammary development and carcinogenesis

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Abstract

The epithelial components of the mammary gland are thought to arise from a stem cell capable of both self-renewal and multi-lineage differentiation. Furthermore, there is increasing evidence that mammary carcinomas originate in these cells or their immediate progeny. The recent identification of murine mammary stem cells should facilitate their molecular characterization and help to elucidate their role in mammary carcinogenesis. In addition, an understanding of the biology of these cells including the pathways that regulate their self-renewal and differentiation may suggest new approaches for the prevention and treatment of breast cancer.

The recent report by Shackleton and colleagues [1] demonstrating the generation of a functional mammary gland in the mouse from a single stem cell has important implications for understanding mammary development and carcinogenesis. The existence of stem cells capable of generating the entire epithelial components of the mammary gland has long been postulated. Stem cells are defined by their ability to undergo self-renewal, as well as lineage specific differentiation. Previous studies providing indirect evidence for the existence of these cells utilized transplantation of retrovirus tagged epithelial fragments into the cleared fat pads of recipient mice [2]. Evidence for the existence of mammary stem and progenitor cells has also been provided by *in vitro* studies. These studies have identified cell populations capable of giving rise to all three epithelial cell types found in the adult gland, ductal and alveolar epithelial cells and myoepithelial cells. Shackleton and colleagues provide more definitive evidence for the existence of such a stem cell by prospectively isolating these cells using cell surface markers and performing transplantation into the cleared mammary fat pads of syngenic mice. They define a mammary repopulating unit based on the ability to regenerate a mammary gland *in vivo*. Cells that are CD29^{hi}CD24⁺ Lin⁻ are enriched for this property. To demonstrate that a single cell contained within this population is able to reconstitute an entire mammary gland they used the Rosa-26 mouse carrying a *LacZ* transgene, which allows for tagging of donor cells. By

performing serial dilutions as well as transplanting single marked cells that were confirmed by visual inspection they calculated that at least one out of 64 cells bearing the phenotype Lin⁻CD29^{hi}CD24⁺ is a stem cell, as defined by its ability to reconstitute an entire mammary gland.

Studies in haematopoietic stem cell biology have been greatly facilitated by the development of suitable animal models in which a single haematopoietic stem cell can reconstitute the entire haematopoietic system of a lethally irradiated mouse [3]. Until the present time, comparable *in vivo* models did not exist for epithelial stem cells. The study of Shackleton and colleagues [1] provides the first model system in which the epithelial components of an entire organ can be generated *in vivo* from a single cell. Both the studies of Shackleton and colleagues [1] and similar studies reported by Stingl and colleagues [4] demonstrate that these cells also display the second defining property of stem cells, the ability to self-renew. To demonstrate this, they performed serial transplantation of mammary repopulating units, calculating that a single mammary repopulating cell is capable of executing at least ten symmetrical self-renewal divisions.

Recent studies have suggested the existence of stem cells in the human mammary gland similar to those in the rodent. These studies have largely consisted of *in vitro* characterization of cells that could give rise to multiple lineages. We demonstrated that non-adherent mammospheres are highly enriched for cells with stem and progenitor cell properties [5]. The ability to transplant human mammary cells into the rodent mammary gland was advanced by the work of Kuperwasser and colleagues [6]. They demonstrated that human mammary epithelial cells could be grown in fat pads of NOD/SCID mice that were humanized by human mammary stromal cells. Building on this work, we have recently reported that mammospheres can regenerate human mammary glands in such a model [7]. These studies highlight the importance of stromal-epithelial interactions in regulating

mammary stem cell self-renewal and differentiation. These interactions define a stem cell 'niche'. This niche is thought to be composed of both cellular as well as extracellular elements. It is postulated that stem cells receive paracrine signals from 'niche' cells that regulate stem cell behavior, including self-renewal and differentiation. The nature of the stem cell 'niche' that regulates behavior of normal and malignant mammary stem cells has recently been reviewed by Bissell and colleagues [8] and Li and colleagues [9]. The ability to transplant both murine and human cells into such a 'niche' should allow the further elucidation of key regulatory pathways for stem cell self-renewal and lineage specific differentiation.

The isolation and characterization of mammary stem cells also has important implications for understanding mammary carcinogenesis. Recent studies in the mammary gland and other organs have given impetus to the 'cancer stem cell hypothesis', which has two interrelated components. The first is that cancers arise from stem cells or their immediate progeny, and the second is that tumors contain a hierarchy of cells, including 'cancer stem cells' that drive tumorigenesis [10]. The study of Shackleton and colleagues [1] supports such a model. They examined the percentage of cells expressing the stem cell phenotype $\text{Lin}^- \text{CD29}^{\text{hi}} \text{CD24}^+$ in MMTV-*wnt* transgenic mice. It has previously been shown that these mice develop carcinomas containing cells that display markers of both epithelial and myoepithelial lineages [11]. Shackleton and colleagues report that there was a 6.4-fold increase in the absolute number of cells bearing the stem cell phenotype in these mice. *Wnt* signaling has been shown to play a role in the self-renewal of several normal stem cells [12]. Interestingly, when Shackleton and colleagues transplanted $\text{CD29}^{\text{hi}} \text{CD24}^+$ cells from these transgenic mice into cleared fat pads of wild-type recipients, the recipients produced hyperplastic outgrowths. This is consistent with the stem cell model in which perturbation of the self-renewal of stem cells gives rise to stem cell expansion and hyperplasia, which in turn provides targets for further transforming events [10]. Indeed, we have found that disruption of Hedgehog signaling in normal human mammary stem/progenitor cells results in the generation of ductal hyperplasia when these cells are transplanted into the humanized cleared fat pads of NOD/SCID mice [7]. Interestingly, expansion of the stem cell compartment was not seen in MMTV-*neu* mice, which develop luminal tumors. These studies suggest that, while MMTV-*wnt* affects a primitive mammary cell, MMTV-*neu* affects a more committed epithelial specific progenitor. These studies are also consistent with our recent characterization of stem cells in human breast cancer, which are characterized as $\text{CD44}^+ \text{CD24}^{\text{lo}} \text{Lin}^-$ [13]. Both Shackleton and colleagues and Stingl and colleagues localized the majority of mammary repopulating cells to the basal compartment in the normal gland, while human breast cancer cells express CD44, a basal marker [13]. This suggests that there may be a link

between normal and tumorigenic breast stem cells. In addition, transformation of different mammary stem or progenitor cells may account for the different molecular subtypes of breast cancer detected in molecular profiling studies [14]. It remains unclear whether markers utilized to identify normal and malignant mammary stem cells play a functional role. CD29 and CD49 recognize alpha-6 beta-1 integrin, a molecule that has also been described as expressed in other stem cells. This integrin may play a role in anchoring stem cells in the stem cell 'niche'. CD44 is a receptor for hyaluronic acid, which has been shown to play a role in tumor migration and metastasis [15]. CD24 has recently been described as a negative regulator of CXCR4, a cytokine receptor important in facilitating breast cancer metastasis [16]. Interestingly, although the studies of Shackleton and colleagues suggest that murine repopulating cells are CD24^+ , more recent studies by this group and others have suggested that high levels of CD24 are expressed on luminal precursors, whereas an intermediate level of expression is found on more primitive mammary repopulating cells [17]. In the studies by Shackleton and colleagues, as well as our own studies, cells displaying lineage specific markers were eliminated to further enrich the stem cell populations. Together, these studies suggest that there are important similarities between markers expressed by both normal and tumorigenic mammary stem cells. Furthermore, these markers may play a functional role in stem cell behavior.

The further identification of markers that identify cells at different stages of mammary development should greatly facilitate our understanding of normal development and carcinogenesis. An understanding of the biology of the cells that drive tumorigenesis has the potential to lead to new therapeutic approaches for breast cancer.

Competing interests

MW is a consultant for and has financial holdings in OncoMed Pharmaceuticals.

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