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Focus On

Breast cancer, stem cells, and the stem cell niche

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Abstract At least four cell types in mouse mammary epithelium, three in human, and three in cow are now known to be proliferation competent. Some evidence indicates that pregnancy may confer proliferative competency on a new cell type. These cells are widely seen as stem and progenitor cells that maintain the epithelium and produce lactational units during pregnancy. Evolutionarily conserved developmental signaling pathways active in germinal and neuronal stem cell proliferation and differentiation in drosophila and mammalian development are implicated in mammary tumorigenesis. In adult tissues this signaling is retained, is regulated by stem cell niches and operates to create new tissue and maintain tissue form and integrity. Disruption of this signaling may abrogate maintenance of the stem cell niche and lead to preneoplastic conditions.

Keywords: Breast cancer; Morphogenesis; Stem cell niche; Stem cells

Introduction

All tissues examined have now been demonstrated to contain a population of cells investigators are calling somatic stem cells [1]. These putative cells are believed to maintain tissue integrity, support wound healing, possess unlimited mitotic potential, and continually renew aged and dying cells in adult animals and plants. Such cells are hypothesized to be long-lived, and to function throughout the life of the individual, and to be specialized for cell division. Somatic stem cells are assumed to be derived from the multipotent stem cells that give rise to embryonic tissues as they are laid down during organogenesis [1]. In adult organs the potential of these undifferentiated cells is reduced to pluripotential status so that they are able to serve as the source for all the cell types for their particular tissue, but their potential is restricted to that tissue. Pluripotency is regulated in a tissue-specific manner, and is limited *in situ* by the number of cell types characteristic of the tissue. It also permits the production and maintenance of a hierarchy of progenitors with more limited potential for producing multiple cell types than the proposed stem cell.

Presently, the only true test for a stem cell is its ability to recreate its tissue of origin in an animal from which that tissue has been ablated (or, in the case of blood, and other critical organs, sublethally ablated). Theoretically, one stem cell should be capable, under the correct conditions, of producing a whole new tissue exactly like the one from which it was derived. However, practically, it is not only mammalian somatic stem cell identification that has eluded us, but also the ability to reproducibly (a) capture a single stem cell, (b) maintain it long enough to transplant it, and (c) successfully introduce it into an environment that will support it. This may be due to an inability of a single stem cell to survive without the presence of particular cells specialized to support, nourish, protect, and instruct it. Such support cells are termed niche cells, because they create a protected residence or 'microenvironment' within which the stem cells can be 'cultivated' as a source of new cells. It is within this

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niche that the committed daughters of the resident stem cell are instructed to specify, proceed with differentiation, participate in tissue-specific morphogenesis and take up a spatial and functional position in the epithelium according to the tissue's specialty.

Cancer and tissue organization

The notion that cancers may start in stem cells was first proposed by Sell and Pierce [2] to be result of maturation arrest in stem cell differentiation. This idea recently gained support when the mixed lineage leukemia (MLL) oncogene transduced into hematopoietic stem or progenitor cells was demonstrated to result in similar leukemia's [3,4], and also with the discovery that tyrosine kinase inhibitors specific for progenitor cell receptors can control leukemias and some lung cancers [5]. In the past decade progress in the spatial and molecular description of the stem cell niche in drosophila and in mammalian tissues [6] has been made. It is clear from this work that the signaling systems involved are evolutionarily conserved, and similar to those which occur during embryonic morphogenesis. The fact that these systems also involve the same pathways that malfunction in tumorigenesis lends strong support to the hypothesis that cancer is a disease that results from the hijacking of developmental pathways that continue to operate in adulthood and that serve to maintain and renew the organ systems of the individual. Cancer biologists view cancer as the disruption of regulation of cell proliferation, apoptosis, angiogenesis, cell differentiation, and cell migration and invasion. These cell behaviors are crucial to the developmental pathways that direct and regulate morphogenesis, compartmentalization, tissue patterning (including cell-position specification), tissue modeling, restriction of cell-fate determination to a few progenitors, and growth control in embryos [7]. These events depend on the coordination of the complex molecular relay systems called signal transduction pathways, and these are similar in both embryonic and adult systems. Mutation and overexpression of various components of signaling pathways have demonstrated they can be involved in initiation, promotion, or progression of mammary tumorigenesis [8–14]. The developmental processes which build the organs remain in effect for maintenance throughout life. Failure of these regulatory pathways in adult tissues can result in the development of cancer instead growth and maintenance of a healthy tissue.

Mammary epithelial stem cells

The presence of stem cells in murine mammary epithelium has been demonstrated by serial transplantation of small tissue pieces [15,16], serially diluted primary epithelial cells [17] or putative stem cells obtained by fluorescence-activated cell sorting (FACS) isolation from primary mammary epithelial cells [18,19]. Light [20,21] and electron microscope studies [22,23] have characterized some of the features of the putative stem and progenitor cell populations in the bovine and murine mammary glands (MG). Putative stem cells (PSC) in the rat mammary epithelium are smaller than luminal or myoepithelial cells, basally located, spare in organelles, take up very little light or electron stain, and are evenly distributed through the epithelium [21,23]. Putative progenitor cell (PPC) types are more differentiated containing more organelles and some are larger [24]. Their identity by morphology depends on their diminished cytoplasmic differentiation, the occasional appearance of mitotic chromosomes within them, as well as the preferential uptake of the thymidine analog bromedeoxyuridine (BrDU) [21]. However, Smith has recently demonstrated the existence of another cell type that is long-lived, frequently mitotic, and that retains the parental DNA strand during mitosis [25]. Since this cell type does not share the morphological characteristics described above, and at the light level appears to be significantly more differentiated and luminally located, this discovery adds a unexpected depth of complexity to a proliferation hierarchy previously believed to be straightforward. The fact that subsets of all cell types also stain positively for proliferating cell nuclear antigen (PCNA) [20] also supports a complex proliferation dynamic for this epithelium. The fact that a subset of parental strand-retaining cells can also stain positively or negatively for either progesterone or estrogen receptor [25] (as can any other mammary epithelial cell type described [26]), reveals why comprehending hormone signaling in this gland and its relationship to cancer has been so difficult. It also raises the question of how a stem cell niche may function in the MG, and whether this putative structure may itself consist of many layers.

The mammary stem cell niche

The concept of a stem cell niche was first proposed by Schofield [27] for the hematopoietic system when it was recognized that spleen colony-forming cells were age-limited, and that this was at variance with the apparent immortality of stem cells. Signaling pathways often require not only the presence of the cells which finalize the outcome of the pathway (proliferation, apoptosis, etc.), but for stem cells, at least one set of cells that create a microenvironment in which stem cells can operate. These cells are specialized to protect stem cells from differentiation signals and to receive, process and deliver the

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proliferation signals. The microenvironment created by the niche cells is termed the stem cell niche [28–31]. Light and electron microscope studies have revealed some of the characteristics of a putative stem cell niche in the murine MG [32].

Access of the PSC to signaling molecules may be regulated in part by luminal and myoepithelial cells as well as the extracellular matrix. In simple niche systems such as drosophila gonads the main components of the stem cell niche are the stem cell and their immediate neighbors [29,33], however, some studies indicate that in MG the extracellular matrix and the surrounding stroma (fibroblasts and fat cells) may be important in delivering or processing proliferation signals to the mammary epithelium [34,35]. Ultrastructural studies in the rat suggest that the cells that neighbor the PSC extend long processes between them and the basement membrane [32]. These extensions vary in thickness, degree to which they limit access to the basement membrane and whether they originate from luminal or myoepithelial cells. Luminal cells immediately next to PSC also participate in other unusual relationships with them: there may be no cell–cell contact for long distances, complete cell contact, but without specialized structures, and wide gaps of space between PSCs and putative niche cells that perhaps, in life, were occupied by a type of substance with high water-retaining capacity [32]. All of these characteristics are present in nulliparous gland and throughout pregnancy and involution and are present in parous MG, suggesting that the niche is highly plastic and constantly responding to the environment.

Recently, we described a structure in nulliparous MG ducts at the light microscope level that resembles that of the stem cell niches described in gut epithelium and developing brain [20]. This structure consisted of PSC and/or PPC enclosed by extraordinarily thin, deeply staining and elongated luminal cells (Fig. 1). Although immunohistochemical stains for signaling molecules have not yet been optimized for MG so that specific cell morphology is preserved in the process, specific cell population and proliferation-based evidence in this study suggested that PPC, which are enclosed within the putative niche with the PSC [20], are the morphogenetic unit of the adult mammary epithelium.

Mammary microenvironments may determine niche function

In the adult, the function of the MG stem cell niche in the ducts may differ from that in lobules, since the ductal system is complete after puberty, and whereas ducts may require a stem cell niche for general maintenance, lobulogenesis operates on a periodic basis

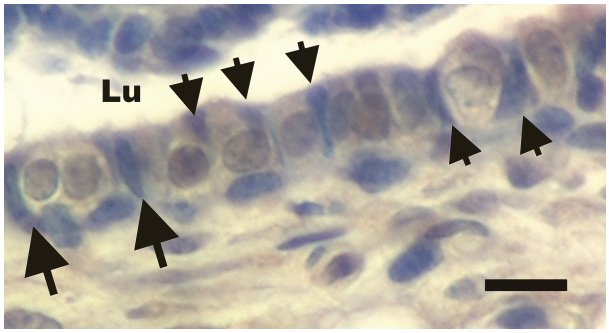

Figure 1.
Stem cell niches in a wildtype FVB mouse mammary duct. Staining is anti-PCNA (brown color) and hematoxylin. Light staining cells are putative differentiating luminal cells which have a pronounced rectangular shape in this section, and progenitor cells (larger, rounded cells). Putative niches are seen in repeating units flanked by thin elongated deeply stained luminal cells (arrows). Bar ~10 μm.

dependent on the estrus and reproductive cycles. Just as the intestinal stem cell niche generates the intestinal villus [6], the main product of the adult mammary stem cell niche is the lobule, the actual milk gland itself. In rodents lobulogenesis occurs at branch points along the sides and at the termini of ducts, and involves a clearly different system of spatial cues and cellular addressing than that evidently involved in generating and maintaining the ducts. Results of a comparison of cell type populations in ducts and lobules of wildtype FVB mice showed that population sizes differed significantly among all cellular morphotypes within both structures. Further, all cell populations were smaller in the ducts than in the lobules whilst in the preneoplastic state in two transgenic mouse models (c-Myc and transforming growth factorα (TGFα)) over-expression of either oncogene had a differential effect on both the population sizes and the proliferation dynamics of all the five cellular morphotypes in both epithelial microenvironments [20]. This effect was more pronounced during the high progesterone phase of the estrus cycle, and differed significantly between ducts and lobules both in normal and transgenic animals. Specifically, c-Myc significantly increases all cell populations in ducts and decreases them in lobules relative to the wildtype, whereas TGFα has a more complex differential effect on all cell populations in both microenvironments. These results plus reports that c-Myc affects stem cell niche signaling in epidermis [36–38] lead us to propose that loss of the proliferation–differentiation regulation in the mammary stem cell niche unbalances cell population sizes, and changes rates of differentiation progression and PPC migration out of the stem cell niche. The incompletely differentiated cells seen in c-Myc transgenic epithelium may be incapable of

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organizing new stem cell niches, and instead grow into the ductal lumen [20]. Composition of extracellular matrix is essential to proper organization and function in every tissue, and is synthesized and maintained continuously in healthy tissues. It is possible that c-Myc or TGFα over-expressing cells cannot produce or organize appropriate extracellular matrix components that will aid in proper differentiation. This may abrogate formation of new stem cell niches leading to the dysplastic morphogenesis seen in MMTV (mouse mammary tumor virus)-c-Myc ducts and aberrant lobulogenesis in MT-TGFα mice. The loss of the regulation conferred by the stem cell niche may lead in turn to inappropriate signaling that results in neoplastic processes. Both c-Myc and TGFα are commonly overexpressed in human breast cancers, and may be important in the dysregulated morphogenesis seen in those cancers as well.

Clearly, breast cancer research is witnessing important breakthroughs in understanding mechanisms of the disease, and in this decade an important proportion of that understanding rests on the new information being contributed by basic scientific research that recognizes and utilizes knowledge gained from the study of developmental processes. Research in developing systems is informing cancer research in other organs, and new and continuing investigation into the role of stem cells and niche regulation in adult systems will rely more and more on knowledge gained from the study of growth regulation in the corresponding developing systems.

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