



MINI-REVIEW

Mammary Stem Cells

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The mammary gland is a dynamic organ that undergoes extensive morphological changes through development, puberty, pregnancy, lactation and involution. During pregnancy, the steroid hormones estrogen, progesterone and prolactin regulate the development of alveolar sacs (lobules) lined with luminal cells that produce and secrete milk. Elongated myoepithelial cells form a layer between the luminal cells and the basement membrane, thus constituting the basal cell population. After lactation, the gland involutes, losing much of the complex lobular structure to resemble its virgin state.¹ This process is regulated by mammary stem cells (MaSCs) and lineage-restricted progenitors, which both function to maintain glandular homeostasis while also being poised to undergo extensive expansion and differentiation when required. The hierarchical arrangement and molecular regulation of these MaSCs and progenitors is not fully understood.

Defining the Mammary Stem Cell

Over 50 years ago, DeOme et al. described a model system in which transplantation of normal mammary epithelial tissue segments from donor mice into the epithelium-free (cleared) mammary fat pad of a recipient mouse led to the regeneration of the entire organ.² Subsequent studies showed that transplantation of any segment of the mammary epithelium,³⁻⁶ or even a single mouse mammary epithelial cell⁷ into the cleared fat pad could generate the ductal and lobular components of the mammary epithelium. Together, these findings suggest that functional MaSCs are widely distributed throughout the adult organ. The term mammary repopulating unit (MRU) has been coined to describe cells with these stem cell properties. MRUs occur with the highest frequency in the terminal end bud (the dilated end of the developing ducts during puberty) and with the lowest frequency in lactating alveoli.⁵ The phenotype of mouse MRUs was first correlated with *in vivo* repopulating ability by Welm and colleagues, who demonstrated that expression of *Sca-1* enriches for MRUs.⁸ Subsequent studies demonstrated that the *SCA-1*^{low} fraction of the *SCA-1*⁺ population is enriched for MRUs, and that most MRUs have a basal phenotype (EPCAM⁺CD24⁺CD29^{hi}CD49^{thi}).^{9,10}

Unexpectedly, MRUs in mice and humans express neither the

estrogen receptor (ER α) nor the progesterone receptor (PR). It is widely accepted that these steroid hormones exert many of their effects in a paracrine fashion by binding to their respective receptors in differentiated luminal cells, triggering secretion of RANKL and WNT4 that act on the more primitive ER α -PR⁻ cells to effect a 14-fold fluctuation in MRU frequency over the mouse estrus cycle.¹¹⁻¹⁹ Steroid hormones may directly influence mammary epithelial cell growth by promoting proliferation of PR⁺ progenitor cells²⁰ or through amplification of estrogen signalling by ER α ⁺ luminal cells.¹³

The detection of human MRUs has been much more difficult than that of mouse MRUs. Early experiments used *in vitro* colony-forming unit (CFU) assays to detect primitive cells in the human mammary epithelium and demonstrated the existence of three distinct progenitors: the luminal-restricted progenitor (EPCAM^{hi}CD49^fMUC1⁺), the myoepithelial-restricted progenitor (EPCAM^{low}CD49^fCD10⁺) and the bipotent progenitor (EPCAM^{low}CD49^{thi}MUC1⁺).²¹⁻²⁴ Serial passaging of the colonies generated by bipotent progenitors has shown that the myoepithelial-restricted progenitor is descended from the bipotent progenitor.²² Human mammary cells do not grow well when transplanted into cleared fat pads, presumably due to inappropriate epithelial-stromal interactions.²⁵ To circumvent this, investigators have transplanted single-cell suspensions of human mammary epithelial cells (HMECs) into humanized fat pads (i.e. fat pads pre-inoculated with human mammary fibroblasts) and have embedded HMECs within collagen gels and/or Matrigel® prior to transplant.²⁶⁻²⁸ HMECs transplanted under the renal capsule recapitulate histologically normal-looking human mammary epithelium, complete with polarized β -casein⁺ luminal cells (when the host is made pregnant), ER⁺ luminal cells, and smooth muscle actin-expressing myoepithelial cells.²⁸ Unfortunately, the generation of an epithelial outgrowth *in vivo* does not imply self-renewal capacity in the progenitor cell. To add additional rigor to the assay, gels can be removed from the recipient mouse 4 weeks after implantation, dissociated, and the cells seeded into a CFU assay. In addition to quantifying MRUs, the CFU assay indirectly detects cells that can generate epithelial outgrowths *in vivo* as well as primitive daughter progenitor cells, including



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DOCUMENT #29017 VERSION 5.0.1 SEPTEMBER 2015

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bipotent progenitors. Flow sorting of freshly dissociated human breast tissue shows that human MRUs have an EPCAM^{low}CD49^{hi} phenotype, implying that MRUs are a type of basal cell.²⁸ This phenotype is identical to that of the bipotent progenitors, but it is not known whether MRUs and bipotent progenitors otherwise overlap. Aldehyde dehydrogenase (ALDH), an enzyme whose activity can be detected by flow cytometry using the ALDEFLUOR™ kit, was originally reported to be a robust marker for identifying mammary stem cells in normal human breast tissue²⁹ but a recent report indicates that ALDH expression may be restricted to a subset of luminal progenitor cells.³⁰ It is not yet known if these ALDH⁺ luminal cells are analogous to the luminal-restricted stem cells identified in the mouse mammary gland. Another recent surrogate assay for detecting primitive mammary cells is the sphere assay, which has also been used for other tissues.³¹⁻³³ Most cells seeded into non-adherent cell culture systems in serum-free media will undergo anoikis; however, it is thought that primitive mammary cells can survive and proliferate to generate clusters termed “mammospheres”. Mammospheres display some self-renewal ability upon disaggregation and are enriched for multipotent epithelial progenitors³¹, but it is not yet known which type(s) of mammary stem cell contributes to this phenotype.

Influence of Model System on Stem Cell Identification

Although MRUs are present throughout the mammary epithelium, the specific identity of these cells remains elusive, due in part to unresolved differences between data gathered through transplantation of purified mammary epithelial cell populations and data gathered from lineage-tracing experiments. Following dissociation of mammary tissue, the epithelial cell fraction can be enriched by immunomagnetic depletion of non-epithelial cells using antibodies including BP-1, CD31, CD45 and TER119. Subsequent sorting of these epithelial cells using EPCAM or CD24 with CD49f allows for further segregation of the different epithelial cell fractions: luminal cells are EPCAM^{high} and CD49f^{low}, basal cells are CD49f^{high}, and MRUs are enriched in the 20% of basal cells expressing the highest levels of EPCAM³⁴. Interestingly, culturing basal cells in the presence of the actin cytoskeleton-disrupting Rho-kinase inhibitor Y-27632 for 7 days resulted in a dramatic 460-fold expansion of MRUs, yielding an MRU frequency of approximately 1 in 80 cells. MRUs could be derived with high efficiency from both EPCAM^{high} and EPCAM^{low} basal cell populations following culture on feeders with Y-27632, suggesting that there was de novo acquisition of MRU potential during the short-term culture period³⁴ and that the nature of dissociation and culture of mammary epithelial cells may alter the

progenitor status or “stemness” of these cells. Similar “conditional reprogramming” of mature epithelial cells has been demonstrated using feeders and Rho kinase inhibition in other tissue models.³⁵

An alternative means of investigating the identity of MaSCs is through the use of in vivo lineage tracing. A recent study from Dr. Visvader's lab in Melbourne used novel high-resolution 3D imaging with expression of fluorescent proteins under the control of promoters for the luminal cell marker *Elf5* or the basal cell markers Keratin 5 and Keratin 14 (K14) to visualize mouse mammary glands at the cellular level. With these experiments, Rios and colleagues demonstrated that LGR5⁺ cells contribute to maintenance of the luminal and myoepithelial compartments, indicating that a small fraction of LGR5⁺ basal cells may constitute a bipotent MaSC pool. These researchers were also able to demonstrate the existence of restricted luminal progenitors and bipotent stem cells from the basal cell component, both of which contribute widely to the developing and adult mammary gland.³⁶ This finding is in contrast to previous lineage-tracing results that suggested that while basal cells can generate all cells of the mammary gland during prenatal development, the post-natal gland is maintained by luminal- and basal- (myoepithelial)-restricted progenitors.³⁷ The discrepancy may be related in part to Van Keymeulen's use of the K14 promoter that may preferentially label basal cell progenitors, and Rios' use of a transient low-dose tamoxifen exposure, which was designed to reduce off-target effects of tamoxifen on the mammary gland.³⁶

Breast Cancer Stem Cells

Understanding the stem and progenitor cell hierarchy of the mammary gland has significant implications for the identification of tumor-initiating cells in breast cancer. Seeding mammary epithelial cells into in vitro CFU assays reveals that a large population of CFUs reside within the luminal cell compartment and that most of these progenitor cells have a CD24^{hi}SCA-1^{ER} phenotype.³⁸⁻⁴⁰ Additional marker sets have been used more recently to identify subsets of luminal cells with colony-forming ability. Luminal cells with a CD49b⁺CD14b⁺SCA-1^{ALDH1} phenotype exhibit high colony-forming ability, with alveolar progenitors being distinguished by high C-KIT levels within this population.⁴¹ Human mammary tumors have stem cell and non-stem cell components, but only the stem cell fraction can generate new tumors upon transplantation into immune-deficient hosts.⁴² At least five distinct molecular subtypes of breast cancer have been identified through gene expression profiling studies,⁴³ and there is increasing evidence that different pools of stem or progenitor cells may constitute the CSC in each of these different tumor types.^{12,44} In many of these tumor types, luminal progenitors are associated with malignant transformation. Recent studies from the Eaves lab

in Vancouver have demonstrated that luminal progenitor cells have short telomeres and the unique ability among mammary epithelial cells to accumulate and withstand high reactive oxygen species levels^{45,46}, thus imparting on these cells multiple mechanisms of accruing DNA damage. Interestingly, women carrying mutations in the BRCA1 gene, a mutation normally associated with basal-like breast tumors, show an expanded luminal progenitor population and gene expression profiling indicates that basal breast tumors and breast tissue heterozygous for a BRCA1 mutation are more similar to normal luminal progenitor cells than any other mammary epithelial cell subset.¹² Finally, a recent study has shown that the $\Delta Np63$ isoform of P63 has a functional role in regulating tumor-initiating cell activity associated with the basal subtype of breast cancer through increasing WNT signalling in luminal progenitors.⁴⁷ Together, these findings suggest that the luminal progenitor population is a target for transformation in a number of breast tumor subtypes.

Ultimately, the ability to prospectively identify, isolate and culture human mammary stem and progenitor cells will improve our understanding of the development of this unique organ and our success in defining therapeutic strategies for targeting cells-of-origin in breast cancer.



WALLCHART

Assays for Human Mammary Stem and Progenitor Cells
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