

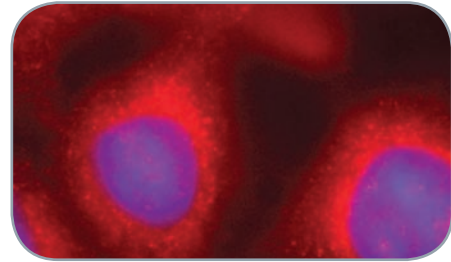
# MINI-REVIEW

## MAMMARY STEM CELLS

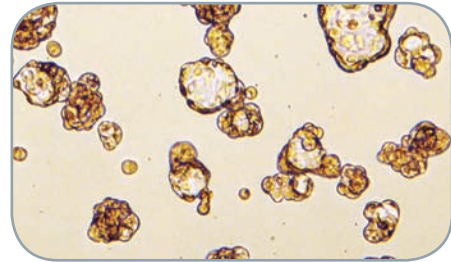
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### The Mammary Gland

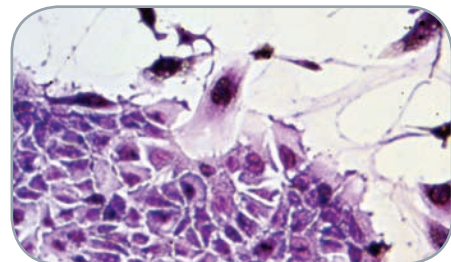
The mammary gland is a compound tubulo-alveolar gland composed of a series of branched ducts that drain sac-like alveoli (lobules) during lactation. In the mouse, the mammary epithelium is embedded within a mammary fat pad, whereas in humans, it is embedded within a fibrous and fatty connective tissue. It is the fibrous component that is in immediate association with the epithelium. In both species, the mammary epithelium is composed of two lineages of epithelial cells: the luminal cells and the myoepithelial cells. The luminal cells are cuboidal/columnar cells that line the ducts and alveoli, and are the cells responsible for milk production during lactation. The myoepithelial cells are specialized contractile epithelial cells that express smooth muscle actin, and are situated basal to the luminal cells and adjacent to the basement membrane. Approximately 3 - 8% of mammary epithelial cells do not have a terminally differentiated phenotype, and are considered to reside higher within the mammary epithelial cell hierarchy.<sup>1,2</sup> Generation and maintenance of the mammary epithelium is hypothesized to be via the mammary stem cell (MaSC), which is defined here as the cell that can generate both the ductal and lobular structures of the mammary gland, can generate all the cell lineages of the mammary epithelium, and can self-renew. The MaSC is of interest to the breast cancer biologist as the cancer stem cell theory suggests that it is the stem cell, or some of its more immediate descendants, that are the targets for malignant transformation.<sup>3</sup>



Human mammary epithelial cells cultured in EpiCult™-B and stained for CD10 (red) and DAPI (blue).



Human mammary epithelial cells cultured as mammospheres in MammoCult™.



Mouse mammary epithelial cells cultured in EpiCult™-B (mouse).

## Mouse Mammary Stem Cells

In 1998, Kordon and Smith demonstrated that a single mouse mammary epithelial cell could recapitulate both the ductal and lobular portions of the mammary epithelium as well as renew itself, thereby fulfilling the criteria of a MaSC.<sup>4</sup> MaSCs are known to be distributed throughout the mammary tree since transplantation of any segment of the epithelium into surgically cleared (epithelium-free) fat pads recapitulates the mammary epithelial tree, with a maximum of seven transplant generations before loss of repopulating ability.<sup>5-8</sup>

Mammary repopulating units (MRUs), the mammary cells that generate ductal-lobular outgrowths upon transplantation into cleared fat pads, occur with the highest frequency in the terminal end buds (the dilated end of the developing ducts during puberty), and with the lowest frequency in lactating alveoli.<sup>7</sup> Previous electron microscopic studies have identified small electron-lucent cells (small light cells or SLCs) as potential candidates for MaSCs and their immediate offspring (primary progenitors).<sup>12</sup> These cells are basally positioned within the mammary epithelium and occur at a frequency of 3%, except in senescent epithelium where they are absent.<sup>9</sup> These cells have also been observed to undergo mitotic division, giving rise to cells of both luminal and myoepithelial phenotypes, suggesting they are not lymphocytes traversing through the epithelium. To date, no functional evidence of stem cell activity has yet been reported for cells morphologically defined as SLCs and strategies to isolate these cells have not been established.

Another potential candidate for mammary stem cells are the cap cells that line the terminal end buds of the elongating mammary ducts during adolescence.<sup>10-12</sup> The cap cells, which are only present in the terminal end buds, have a phenotype intermediate between luminal and myoepithelial cells as they express both luminal and myoepithelial-related proteins at low levels. These cells have been postulated by some investigators to represent the stem cells of the rodent mammary gland,<sup>10-12</sup> although others have suggested that these cells are merely myoepithelial progenitors that pave the way for ductal elongation during development.<sup>13</sup> Like the SLC, the transitory cap cells have not been demonstrated in functional assays to have any ability to regenerate mammary epithelial structures.

The phenotype of MRUs was first correlated to *in vivo* repopulating ability by Welm and colleagues, who demonstrated that expression of SCA1 enriches for MRUs.<sup>14</sup> Subsequent studies demonstrated that it is the SCA1<sup>lo</sup> fraction of the SCA1<sup>+</sup> population that is enriched for MRUs, and that most MRUs have a CD45<sup>TER119</sup>CD31<sup>(lin)</sup>CD24<sup>+</sup>CD29<sup>hi</sup>CD49<sup>fh</sup> phenotype and can be enriched to a purity of

approximately 1 MRU in every 20 - 90 sorted cells.<sup>15,16</sup> These MRUs meet the functional definition of a MaSC since transplantation of a single cell can generate mammary outgrowths in both primary and secondary transplants.<sup>16</sup> An immunocytochemical analysis of these MRU-enriched cells demonstrates that they do not express the estrogen receptor (ER) or the progesterone receptor (PR).<sup>17</sup> Yet interestingly, despite not expressing ER or PR, MRU numbers fluctuate 14-fold during the mouse estrus cycle, with maximal MRU numbers during the luteal dioestrus phase when progesterone levels are maximal.<sup>18</sup> A small number of MRUs can also be detected within the luminal (lin<sup>-</sup>CD24<sup>hi</sup>) cell compartment, but the full potential of these MRUs as compared to the MRUs in the basal cell compartment has not been established as of yet.<sup>19</sup> Seeding of mammary epithelial cells into *in vitro* colony-forming cell (CFC) assays reveals that a large population of CFCs reside within the luminal cell compartment, and that most of these CFCs are ER<sup>-</sup>.<sup>15,16,20</sup> A subpopulation of CFCs that have the ability to generate myoepithelial cells have also been reported to be present in the basal (lin<sup>-</sup>CD24<sup>lo</sup>) compartment.<sup>21</sup> The transplantation of single cell suspensions of mammary epithelial cells at limiting dilutions into cleared fat pads has demonstrated the existence of ductal-restricted and lobular-restricted progenitors, both of which are derived from the mammary stem cell.<sup>22-23</sup> The frequency and phenotypic profile of these two structurally-restricted progenitors is not known.

Another unique progenitor recently described is the parity-induced epithelial progenitor that arises following the first pregnancy.<sup>24,25</sup> These cells, which have previously undergone lactogenic differentiation, do not undergo apoptosis during mammary gland remodeling following pregnancy, but instead survive and are thought to act as alveolar precursors in subsequent pregnancies.

## Human Mammary Stem Cells

Early experiments examining the presence of primitive cells within the mammary epithelium involved the detection of CFCs by the use of *in vitro* assays. Such an analysis has demonstrated the existence of three distinct progenitors within the human mammary epithelium: the luminal-restricted progenitor (EpCAM<sup>hi</sup>CD49f<sup>hi</sup>MUC1<sup>+</sup>), the myoepithelial-restricted progenitor, and the bipotent progenitor (EpCAM<sup>lo</sup>CD49f<sup>hi</sup>MUC1<sup>+</sup>), a progenitor that can generate both luminal and myoepithelial cells.<sup>25-28</sup> Serial passaging of the colonies generated by bipotent progenitors has demonstrated that the myoepithelial-restricted progenitor is a descendant of the bipotent progenitor.<sup>26</sup>

The detection of human MRUs has been much more difficult than their mouse counterparts since human mammary cells do not grow well when transplanted into cleared fat pads, presumably due to inappropriate epithelial-stromal interactions.<sup>29</sup> To circumvent this, investigators have transplanted single cell suspensions of HMECs into humanized fat pads (i.e. fat pads pre-inoculated with human mammary fibroblasts) or have embedded the HMECs within collagen gels and/or Matrigel<sup>TM</sup> and placed these cells either subcutaneously or under the renal capsule of immune-deficient mice.<sup>30-32</sup> HMECs transplanted under the renal capsule recapitulate histologically normal-looking human mammary epithelium, complete with polarized  $\beta$ -casein<sup>+</sup> luminal cells (when the host is made pregnant), ER<sup>+</sup> luminal cells, and smooth muscle actin-expressing myoepithelial cells.<sup>32</sup> Unfortunately the presence of an epithelial outgrowth *in vivo* may represent the progeny of a multi-lineage progenitor that does not have any self-renewal capacity. To add an additional level of rigor to the assay, the gels are removed from the recipient mouse 4 weeks after implantation and dissociated, and the liberated cells seeded into a CFC assay. In addition to making the MRU assay quantitative, the CFC assay indirectly detects cells that can generate epithelial outgrowths *in vivo* that contain primitive daughter progenitor cells, including bipotent progenitors. Flow sorting of freshly dissociated human breast tissue demonstrates that human MRUs have an EpCAM<sup>lo</sup>CD49f<sup>hi</sup> phenotype, which suggests that MRUs are a type of basal cell.<sup>32</sup> This phenotype is identical to that of the bipotent progenitors; however, it is not known if MRUs and bipotent progenitors are overlapping populations. A recent report has also demonstrated that human MRUs express high levels of aldehyde dehydrogenase (ALDH).<sup>33</sup>

While it has been shown that human mammary tumors have stem cell and non-stem cell components, only the stem cell fraction can generate new tumors upon transplantation into immune-deficient hosts.<sup>34</sup> These tumor stem cells were described as having an EpCAM<sup>+</sup>CD44<sup>+</sup>CD24<sup>lo</sup> phenotype, with low expression of the luminal-

specific protein CD24 suggesting a basal cell origin of tumor stem cells. Interestingly, women carrying mutations in the BRCA1 gene show an expanded luminal progenitor population with growth factor-independent cell proliferation *in vitro*. Gene expression profiling revealed that breast tissue heterozygous for a BRCA1 mutation and basal breast tumors share similarities with normal luminal progenitor cells more so than with any other subset, including the stem cell-enriched population. These new findings suggest that the luminal progenitor population is a target for transformation in BRCA1-associated basal tumors.<sup>35</sup>

Breast cancer stem cells in some, but not all, breast tumors and cell lines can be isolated based on their high expression of ALDH.<sup>33, 35-37</sup> The presence of ALDH<sup>hi</sup> breast cancer stem cells has also been correlated with poor clinical outcome.<sup>35</sup>

Human mammary epithelial cells can be maintained in serum-free suspension cultures. The clusters of cells obtained have been called "mammospheres" (not to be confused with the differentiated alveolar structures generated in 3-dimensional matrices) due to their similarity to neurospheres, as described for the neural system.<sup>38-41</sup> These mammospheres display some self-renewal ability upon disaggregation and are enriched for multi-potent epithelial progenitors.

The mammosphere assay was successfully used to establish long-term cultures enriched in tumorigenic cells from invasive tumor samples. The mammospheres formed under these conditions were called tumorspheres. They showed an increase in CD44/CD24<sup>lo</sup> cells, over-expression of neo-angiogenic and cytoprotective factors, expression of primitive embryonic stem cell marker OCT4 and displayed high tumorigenic potential in NOD-SCID mice.<sup>42</sup> It is not clear whether the mammosphere or tumorsphere-initiating cells are human mammary stem cells, but if so, this assay would represent an important tool for mammary gland research.

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