

**Mouse Mammary Gland Stem cells: Location, Location, Location!**

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Mammary epithelium has demonstrated long term repopulating capacity consistent with the presence of resident adult stem cells. We used novel computational microscopy methods to characterize long-term, label-retaining cells (LRC), a putative stem cell population, in the mouse mammary gland. Multiscale analysis showed that the proximal region of the larger mammary ducts, close to the site of embryonic origin of the epithelium, was enriched in LRC. These cells divided during mammary development, were relatively quiescent at homeostasis, and rarely colocalized with differentiation markers. Furthermore, they exhibited distinct nuclear features with respect to differentiated cells. Spatial analysis of LRC distribution suggested that the proximal epithelium contained 3.5-times more stem cells than the distal epithelium. We tested this prediction using limiting-dilution transplantation assays. Approximately 2.5-fold fewer proximal epithelial cells were required to regenerate the gland, confirming the presence of a proximal stem cell zone. This model provides new quantitative tools and spatial criteria in the search for a mammary stem cell niche.

**Take-home points**

- 1) Mammary LRC rarely colocalize with differentiation markers (e.g. progesterone receptor) and have a distinct set of nuclear features (size, shape, chromatin texture) with respect to differentiated mammary epithelial cells.
- 2) Mammary LRC are differentially distributed across the mammary ductal tree, with increased frequency (3.5-fold) in areas proximal to the site of embryonic origin of the gland.
- 3) Functional analysis (limiting-dilution transplantation assays) reveals that proximal mammary epithelial cells are more efficient (2.5-fold) at regenerating ductal outgrowths than distal cells, indicating the presence of a stem cell zone in the proximal mammary gland.