Multi-Scale *In Situ* Sorting Reveals a Proximal Stem Cell Zone in the Mouse Mammary Gland

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Exposures to environmental agents can have an effect on mammary gland development and may affect breast cancer risk. A major question arises from epidemiological analysis of one of the few known human carcinogens, ionizing radiation: Why is there a window of susceptibility during adolescence? Mammary gland stem cells have been hypothesized to be the primary targets for carcinogenesis in several model systems. A variety of experimental models support the notion that the demonstrated regenerative capacity of mammary epithelium is consistent with the presence of adult stem cells (ASC). The goal of this study was to identify the characteristics of mammary ASC; future studies will determine their response to radiation and other environmental carcinogens.

We used novel computational microscopy and multiscale analysis to characterize putative ASC distribution and features in the mouse mammary gland. These cells divided during mammary development, were relatively quiescent at homeostasis, and rarely colocalized with differentiation markers. Feature analysis identified a distinct ASC nuclear morphology compared to differentiated cells. Spatial analysis suggested that ASC were 3.5-times more frequent in the region of large mammary ducts proximal to the nipple, which is the site of embryonic origin of the epithelium, than in the distal region. We tested this prediction using limiting-dilution transplantation assays which showed that the regenerative potential of proximal epithelial cells was enriched by approximately 2.5-fold. Thus there is a defined architecture of ASC distribution in mammary gland consisting of a proximal ASC zone. This study provides new quantitative tools and spatial criteria that will facilitate the study of ASC and their role in breast carcinogenesis.