On Murine Mammary Epithelial Stem Cells: Discovery, Function, and Current Status

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Remarkable progress has been made in the past decade in the isolation and characterization of mouse mammary stem cells and progenitors, as nicely reviewed in the article by Visvader and Smith (2011). Following in the footsteps of the hematopoietic system and analogous to bone marrow transplantation, the mammary gland can be reconstituted following transplantation of cells into the cleared mammary fat pad (see review by Medina 2011). Taking advantage of these similarities as well as the availability of genetically engineered mice (GEM), our laboratory initially used magnetic bead and fluorescence-activated cell sorting (FACS) and SCA enhanced green fluorescent protein (EGFP) knock-in mice to identify mammary gland progenitors (Welm et al. 2002). We also attempted to identify and isolate quiescent cells using a BrdU label retention strategy that had been successfully applied in the epidermal and intestinal epithelium. Subsequently, the identification of several cell-surface markers expressed on mammary stem cells and progenitors has resulted in an explosion in the field, and helped to define a preliminary mammary cell lineage hierarchy. These studies on the normal mammary gland have also provided the basis for hypotheses into potential mechanisms accounting for the heterogeneity of breast cancer subtypes (Behbod and Rosen 2004).

One intrinsic difference between the hematopoietic system and the mammary gland, how-

ever, is the requirement for tissue dissociation in the latter case to facilitate the isolation of single cells required for FACS sorting. Even when using freshly isolated cells, there is a concern that these rather lengthy dissociation protocols may alter the expression of cell-surface molecules and properties of cells following disruption of the mammary gland architecture. Even short-term cell culture of primary mammary epithelial cells may alter the expression of cell-surface molecules. At present, single gene markers of mammary stem cells have not been identified, so the application of knock-in mice, e.g., the use of LGR5-EGFP to identify intestinal stem cells and perform lineage-tracing experiments (Barker et al. 2007), has not been feasible. One alternative approach may be to use pathway reporters, as recently described by Zeng and Nusse (2010), who used an axin-lacZ knock-in mouse to identify cells with canonical Wnt signaling with increased mammary repopulating activity. We have used a similar approach in a p53-null mouse mammary cancer model following lentiviral transduction with a Wnt reporter construct to identify cells with enhanced canonical Wnt signaling. These cells displayed a significant overlap with cell-surface markers in the basal-like tumors shown to enrich for tumor-initiating cells (Zhang et al. 2010).

The use of multiple pathway reporters with different fluorescent reporters may provide a

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new approach to complement the current dependence on cell-surface markers. Fluorescent reporters also have the potential to help precisely visualize and model the location of mammary stem cells and progenitors in situ using multiphoton and other sophisticated microscopic techniques. The ability to visualize single stem cells in their niche environment and to follow both symmetric versus asymmetric division ultimately will be required for the next advances in the field. Recent studies on the paracrine effects of the steroid hormones, estrogen and progesterone, on mammary gland stem cells and progenitors illustrate the need to understand the spatial relationships among the various epithelial and stromal cell types present in the mammary gland. These studies will need to include cells from the immune system such as macrophages, neutrophils, etc., and derivatives of mesenchymal stem cells. Hopefully, in the near future it may be feasible to reconstitute and study these interactions in vitro, but for the present time this can be studied in GEM models. In addition, there is increasing evidence for the coexistence of quiescent and active adult stem cells in mammals (Li and Clevers 2010), but these distinct populations and their spatial and temporal relationships in the mammary gland remain to be discovered. Application of single-cell analysis using newly developed microfluidic platforms has the potential to help elucidate the potential heterogeneity of signaling pathways and gene expression in mammary stem cells and progenitors. Finally, there is a critical need for lineage-tracing experiments in the normal mammary gland to validate the proposed hierarchy for stem cells and progenitors,

as well as to identify the cells of origin for different subtypes of breast cancer. Comparative studies of the murine and human stem cell populations in both the normal mammary gland and different breast cancer subtypes hold enormous potential for the future. Thus, despite the remarkable progress in this field, much remains to be done.

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