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Epithelial Plasticity In The Mammary Gland

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Abstract

Many epithelial tissues rely on multipotent stem cells for the proper development and maintenance of their diverse cell lineages. Nevertheless, the identification of multipotent stem cell populations within the mammary gland has been a point of contention over the past decade. In this review, we provide a critical overview of the various lineage-tracing studies performed to address this issue and conclude that although multipotent stem cells exist in the embryonic mammary placode, the postnatal mammary gland instead contains distinct unipotent progenitor populations that contribute to stage-specific development and homeostasis. This begs the question of why differentiated mammary epithelial cells can exhibit stem cell behavior in culture, and we speculate that such reprogramming potential is repressed in situ under normal conditions but revealed in vitro and might drive breast cancer development.

Mammary epithelial cells have limited plasticity in vivo

The mammary gland is a highly branched ductal structure composed of a double layer of cells, in which a layer of basal myoepithelial cells encloses the tubular luminal epithelial cell sheet. Unlike most epithelial organs, mammary glands develop postnatally, and undergo complex epithelial remodeling throughout puberty, pregnancy, lactation and weaning. Several epithelial organs arise from and are maintained by multipotent stem cells, and early studies suggested that stage-specific development of the mammary gland is likewise driven by multipotent mammary stem cells (MaSCs). However, despite intensive investigation, the identity and potency of MaSCs remains contentious. Prospective enrichment using generic markers demonstrated the existence of MaSCs in the context of a transplantation/regeneration assay^{1,2}. These presumptive MaSCs are within the basal population; however, no markers were described that uniquely identifies them. This reliance on generic markers has led to confusion in the literature, with basal markers such as CD49f and CD44 frequently being described erroneously as stem cell markers.

A concern with using transplantation assays is that the cells to be tested are removed from their normal microenvironment, which might drastically alter behavior. Lineage-tracing approaches avoid this issue by enabling fate-mapping of cells in situ, without perturbation, and their application has led to a different view of mammary gland development. Strikingly, the keratin-14 (K14) promoter, which is expressed in all basal cells of the mouse mammary

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gland, did not detect a multipotent stem cell population in vivo³ and lineage-tracing using an Axin2-lacZ marker, smooth muscle actin or Lgr6 reporter strategies also failed to detect multipotent stem cells in the postnatal mouse⁴⁻⁶. Interestingly, lineage-tracing using either the Lgr5 or Axin2 promoter marked only luminal cells when activated in newborn pups and only myoepithelial cells when activated at a later time, but did not identify any bipotent stem cells^{4, 7}.

These results were later questioned by a lineage-tracing study using a Keratin-5 (K5) promoter, which also marks basal cells⁸, as well as an additional study that claimed to identify rare protein C receptor-positive (ProCR⁺) cells in the ducts that are multipotent⁹. These ProCR⁺ cells are scattered throughout the ducts in the basal layer, and lineage-tracing indicated that they can generate all lineages of the mammary epithelium⁹. However, these cells do not proliferate rapidly and could provide only a limited contribution to mammary gland homeostasis¹⁰. Moreover, the reconstitution experiments in this study were performed using Procr⁺ cells mixed with 50% Matrigel, which can generate artefactual outgrowth¹¹. A more recent quantitative analysis using saturation labeling with lineage markers strongly argues that these results were an artifact caused by promoter leakiness, and that all cells within the postnatal mammary gland arise solely from unipotent progenitors rather than multipotent stem cells¹². Two additional recent studies used a different approach to generate stochastic genetic labelling and unbiased lineage-tracing methods that permanently label specific single clones, ultimately concluding that postnatal luminal and basal cells are lineage-restricted^{13, 14} (Figure 1B). While several publications claim that these lineages arise from “unipotent stem cells”, we feel that this is a confusing term for what is really a progenitor, and that the term “stem cell” should be reserved for cells that exhibit multipotency.

Luminal cells can be subdivided into estrogen (ER)⁺/progesterone receptor (PR)⁺ and ER⁻/PR⁻ cells. Lineage-tracing of the luminal cell population has revealed that ER⁺ progeny are restricted to the ER⁺ lineage and, likewise, ER⁻ progeny are restricted to the ER⁻ lineage during pregnancy, lactation and involution¹⁵⁻¹⁷. Furthermore, Wap-Cre, which is active only during pregnancy in the ER⁻ alveolar cells, marked only ER⁻ labeled cells after two pregnancies¹⁸. Taken together, these data suggest that even within the luminal lineage, the ER⁺ and ER⁻ subgroups are sustained by separate pools of progenitors rather than by a common stem cell (Figure 1B).

Interestingly, in the embryo, cells from the rudimentary duct can display multipotency and give rise to all lineages in the postnatal mammary gland^{3, 16}, although the Axin2 reporter marked only luminal progenitors in the embryo⁴. The mammary placode contains MaSCs that express basal and luminal markers, which are believed to give rise to both cell types (Figure 1A). After birth, however, only lineage-restricted progenitors remain that contribute to mammary gland homeostasis (Figure 1B). Nevertheless, for unknown reasons, these progenitors retain the remarkable ability to revert to a multipotent stem cell state when isolated from their niches in the mammary gland (Figure 1C).

One problem with many of the lineage-tracing studies described above is the use of Tamoxifen to activate the Cre recombinase. Tamoxifen is an ER antagonist, which strongly

influences mammary gland homeostasis and suppresses ductal outgrowth during puberty. This effect needs to be considered in the interpretation of short-term lineage tracing^{8, 19}. Doxycycline-inducible models are a better choice for lineage analysis in the mammary gland¹², but are subject to leakiness and require more components, which complicates breeding. Another limitation is that short-term expression of pathway-specific promoters is often inefficient, which can lead to labeling that does not represent the whole population. This problem was resolved by Wuidart and colleagues who saturated the entire basal cell population¹². However, a recent report showed that the enzymatic digestion method used to prepare mammary ducts for 3D imaging can deplete myoepithelial cells and cause structural damage to ducts, and potentially might also deplete rare bi-lineage clones²⁰. New methods of micro-dissection or clearing, together with whole-organ 3D imaging analysis and mathematical modeling^{8, 12, 13, 21–23}, will undoubtedly provide a clearer picture of mammary gland cell fate during normal development.

Despite the recent progress that has contributed valuable insights to our understanding of mammary epithelial cell (MEC) behavior, several important questions remain unaddressed: 1) Why does MEC lineage restriction occur prior to mammary branching morphogenesis? 2) Why is postnatal mammary cell plasticity restricted while the progenitor cell populations of other epithelial tissues (e.g., prostate¹² and epidermis²⁴) maintain multipotency throughout development and homeostasis? 3) How is the prenatal to postnatal shift in mammary plasticity regulated – what are the stimuli that actively repress multipotency? 4) Is MEC lineage restriction a tumor suppressive mechanism, and, if so, is de-repression of multipotency a significant step in breast cancer initiation? Identifying the key regulators of epithelial plasticity would greatly enrich our understanding of tissue stem cell behavior, which could ultimately inform the development of more sophisticated and targeted approaches to both breast cancer prevention/therapy and regenerative medicine.

MECs can be reprogrammed into stem cells ex vivo

Early transplantation studies all demonstrated that cells isolated from the murine mammary gland could display multipotency^{2, 5, 25}. Later work showed that cells known to be lineage-restricted in vivo, such as mature myoepithelial cells, could be reprogrammed in vitro into multipotent stem cells which, even in limiting numbers, were capable of generating functional mammary ductal trees de novo^{1, 3, 4}. Whereas the process of isolation from the mammary gland seems to be sufficient for basal cell reprogramming, passaging the cells in vitro substantially increased the efficiency of conversion to stem cells⁵ and these traits were strongly enhanced by perturbation of the actomyosin network, TGF β inhibition, as well as by induction of Wnt, PR, ER, and ErbB signaling^{5, 26–30} (Figure 1C). In addition, some data have supported the notion that the extracellular matrix, stroma and/or luminal cells may supply signals that actively repress myoepithelial stemness; however, the key underlying mechanism behind myoepithelial reprogramming ex vivo remains elusive^{3, 31, 32}.

Surprisingly, although wild type luminal cells alone are unable to regenerate mammary glands upon transplantation and were assumed to be an irreversibly committed cell population, recent studies have revealed their remarkable reprogramming capacity. Transient co-expression of Slug (an epithelial-mesenchymal transition factor) plus Sox9 (a

transcription factor involved in development), or transient YAP/TAZ activation (transcription factors implicated in mechanotransduction and organ size control), can efficiently convert differentiated luminal cells into MaSCs in vitro^{33, 34} (Figure 1C). However, the mechanisms through which this transition occurs remain unclear.

These findings may provide important insight with respect to breast cancer development, as the majority of breast cancers are believed to arise from luminal precursors, including those that have a basal phenotype. For example, BRCA1 mutant cancers, which have a basal-like signature, may originate from the dedifferentiation of luminal progenitors³⁵. Furthermore, other recent work demonstrated that polyoma middle T (PyMT) antigen or ErbB2 signaling activation in differentiated luminal cells can reprogram them to become bipotent, which can give rise to myoepithelial cells³⁶. These findings hint at the possibility that oncogenic activation or tumor suppressor loss-of-function within MECs could drive reactivation of cell plasticity to promote tumorigenesis. Several recent studies support this notion by demonstrating that PIK3CA activation or p53 loss in either lineage-restricted myoepithelial or luminal cells promotes their dedifferentiation into a stem-like state and subsequent mammary tumor formation^{37–40} (Figure 1C). Furthermore, other studies have associated epigenetic reprogramming, hypoxia and DNA damage with MEC dedifferentiation, which are all stimuli that have been linked to breast cancer^{41–43}. Taken together, this collection of recent studies underscores how MECs harbor extraordinary reprogramming potential that is likely derepressed and exploited during breast tumorigenesis.

Conclusions

Work over the past decade has dramatically enhanced our understanding of the remarkable cellular complexity within the mammary epithelium. While it is now clear that distinct epithelial lineages are generated by unipotent progenitors throughout postnatal mammary morphogenesis and homeostasis, it remains to be understood whether the epithelial plasticity that is revealed by culture in vitro plays any role in the intact gland under non-homeostatic conditions, such as for regeneration following wound-healing, response to stress, or in tumorigenesis. In addition, although quiescent stem cell populations have been identified within the mammary epithelium, their contribution to tissue maintenance has not been adequately addressed²⁹. Future investigation focusing on these intriguing unanswered questions may yield invaluable insights into drivers of stem cell reprogramming. This could ultimately help optimize both therapeutic approaches to epithelial cancers as well as stem cell-based therapies.

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Annotations

(*) special interest

(**) outstanding interest

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Figure 1. Postnatal mammary epithelial cells are unipotent in vivo but can be reprogrammed into multipotent stem cells ex vivo

1. Mammary epithelial cell (MEC) hierarchy before and after birth. 1A. During embryogenesis, multipotent mammary stem cells (MaSCs) give rise to committed basal and luminal cell progenitors. **1B.** In the postnatal mammary gland, committed unipotent progenitors generate each cell type. **1C.** Upon isolation and culture ex vivo, differentiated MECs can be reprogrammed into multipotent stem cells. While isolation alone is sufficient to reprogram myoepithelial cells, certain factors can either promote or inhibit this conversion. Conversely, luminal cells in culture require induction by specific factors for reprogramming. These ‘multipotency factors’ may behave in situ as oncogenic signals that drive breast cancer.