Cell Stem Cell
Previews

Stem Cell Maintenance of the Mammary Gland: It Takes Two

Patricia J. Keller,[1,2] Lisa M. Arendt,[1,2] and Charlotte Kuperwasser[1,2,*]
[1]Department of Anatomy and Cellular Biology, Sackler School, Tufts University School of Medicine, Boston, MA 02111, USA
[2]Molecular Oncology Research Institute, Tufts Medical Center, Boston, MA 02111, USA
*Correspondence: charlotte.kuperwasser@tufts.edu
DOI 10.1016/j.stem.2011.11.008

Transplantation assays suggest that multipotent stem cells maintain the two lineages of the mammary gland. Recently in *Nature*, Van Keymeulen et al. (2011) used lineage tracing to discover unipotent stem cells that maintain the bulk of the mouse mammary gland after birth and during pregnancy.

The mammary gland undergoes extensive development following birth, from glandular expansion during puberty to full lobuloalveolar differentiation for lactation. Two main cell lineages are present within mammary epithelium: luminal cells that line the interior of ducts and alveoli and express hormone receptors and cytokeratins (CKs) 8, 18, and 19, and contractile basal/myoepithelial (ME) cells, which are localized between the luminal cells and the basement membrane and express smooth muscle actin (SMA), as well as CKs 5 and 14. The luminal lineage also encompasses the terminally differentiated cells of the lobuloalveolar units, which secrete milk during lactation.

The enormous cellular output needed to meet the regenerative demands of successive pregnancies and lactations suggests the presence of multipotent stem cells that sustain the two lineages (Figure 1A). However, a recent study by Van Keymeulen, Blanpain, and colleagues has radically revised our understanding of the established hierarchy of the adult mammary gland by suggesting that, unlike other ''renewable'' epithelial tissues known to be maintained by multipotent stem cells (e.g., skin or intestine), the mammary gland is maintained by two separate populations of unipotent progenitor cells (Van Keymeulen et al., 2011).

Current models of the mammary epithelial hierarchy derive mainly from studies that have used transplantation of dissociated epithelial cells into fat pads cleared of endogenous epithelium. Multipotent progenitor cells have been described that contribute to ductal and alveolar morphogenesis and exhibit self-renewal properties in serial transplantations based on either $CD24^{Med}/CD29^{Hi}$ or $CD24^{+}/CD49f^{Hi}$ immunophenotypes (Shackleton et al., 2006; Stingl et al., 2006), or based on genetically marked cells that emerge during pregnancy to contribute to alveolar development (parity-induced cells; Wagner et al., 2002). In their study, Van Keymeulen and colleagues used an inducible genetic linage tracing strategy to study the progenitor behavior of cells at defined time points: embryogenesis, after birth, during puberty, and through multiple lactations. Stem/progenitor activity was monitored by YFP expressed by lineage-specific cytokeratin promoters in either basal/ME or luminal cells after induction by doxycycline or tamoxifen administration. Embryonic induction of YFP controlled by the CK14 promoter demonstrated that all cells in the mammary gland were derived from an initial CK14+ population, confirming previous reports localizing multipotent progenitors to the basal/ME lineage (Visvader, 2009). However, when induced during puberty, CK14 promoter-driven YFP marked only CK14+/SMA+ myoepithelial cells and not CK8+/CK19+ luminal cells. Similar results were seen when YFP expression was induced at puberty by CK5 promoter activity. In contrast, when CK8 and CK18 promoters were used to drive YFP expression at puberty, only CK8+/CK19+ luminal lineage cells were marked. Taken together, these results indicate that lineage restriction is established shortly following birth. Low-dose treatment with doxycycline or tamoxifen to label isolated CK14+ or CK8+ cells, followed by several rounds of pregnancies, indicated that lineage-restricted progenitors were maintained and were able to clonally expand through lactation and involution; labeled CK18+ cells did not clonally expand, indicating that CK18 may mark more differentiated cells.

This provocative study provides the first in situ evidence that facultative luminal and basal/ME cell progenitors exist and that together, they meet the regenerative potential of the adult mammary gland. Additionally, while not completely ruling out the presence of rare multipotent stem cells, it suggests that in adult mice, these cells do not contribute significantly to the maintenance of the gland. There are some limitations to the study. A complete lineage trace of the luminal epithelium during embryonic development was not performed, so it is unclear whether CK8+ cells, or likely, a dual positive cell is contributing to the formation of both lineages in early development. Additionally, clonal analyses would have been strengthened if they had been performed in combination with a marker of proliferation to support evidence for expansion of the normally quiescent myoepithelial layer. Nevertheless, these results revise our thinking about the mouse mammary hierarchy (Figure 1B).

But what are we to make of the wealth of evidence for multipotent progenitors from cleared fat pad transplantation studies and in vitro colony forming assays? In this study, the authors demonstrated that, while endogenous CK14+ and CK8+ cells were lineage restricted, when transplanted into cleared mammary fat pads alone or in an excess of $CD24^{+}CD29^{Lo}$ luminal cells, the $CD24^{+}CD29^{Hi}$ fraction (enriched in CK14+ cells) was able to regenerate the mammary tree and contributed to both the luminal and basal/ME lineages. However, when YFP+ basal/ME cells were transplanted along with unmarked luminal cells from glands

where YFP was induced in CK14-expressing cells at puberty, the YFP+ CK14+ cells were once again restricted to the regeneration of only basal/ME cells. These results suggest that CK14+ basal/ME cells retain the potential to revert to a bipotent "embryonic mode" under certain conditions. Similar phenomenon have been seen in other tissues where separate pools of stem cells can have different roles depending on whether they are contributing to homeostatic maintenance or are enhancing regeneration in response to injury or in a transplantation assay (Barker et al., 2010). Likewise, Van Keymeulen et al. suggest that either CK14+ cells can be called upon to adopt multipotent behavior or that dormant multipotent cells are "reactivated" under conditions of complete gland regeneration. Hence, they suggest that caution is warranted when assigning stem cell activity to transplanted cells.

Lastly, what are the implications of this study for our understanding of the human breast hierarchy and how it may influence cancer? Human mammary glands have a more heterogeneous structure than their murine counterparts, with fibrous divisions into lobules, which expand during pregnancy and do not fully regress following involution. Second, although the murine mammary gland has restricted basal/ME expression of CK14 and luminal expression of CK8/18, in the human gland, expression is more varied. CK14 can be identified within luminal cells and can differ significantly within the basal/ME cells of lobules as compared with ducts; some areas can even lack expression of both types of keratins, suggesting that the human mammary hierarchy is likely more complex (Petersen and Polyak, 2010). For breast cancer, it is becoming evident that lineage is both instructive and somewhat plastic for determining tumor phenotype. Evidence from mouse and human tumor models suggests that luminal progenitor cells may be the cells of origin for basal-like breast tumors characterized by expression of CK14 and CK5, while basal/ME cells can adopt dedifferentiated states to form metaplastic tumors that have lost identity with breast tissue (Lim et al., 2009; Molyneux et al., 2010; Keller et al., 2011). It remains to be determined if this plasticity reflects transformation of rare multipotent cells within luminal and basal/ME lineages, transforming genetic events that influence differentiation, or a combination of factors.

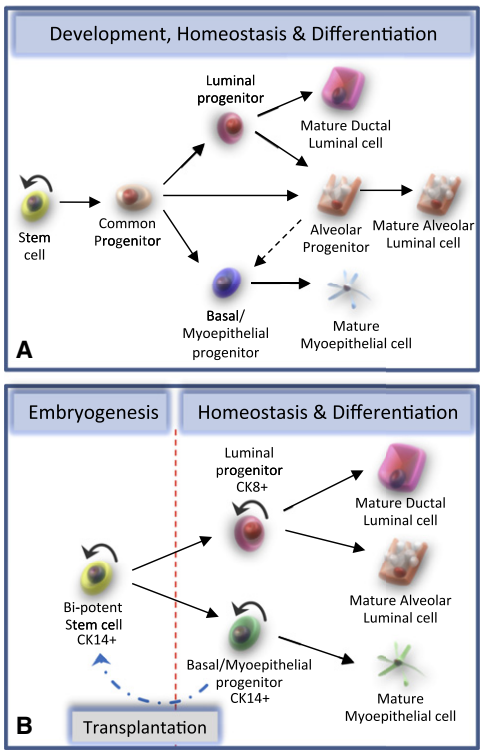

Figure 1. Rethinking the Mouse Mammary Hierarchy

(A) Classical model of mammary differentiation (Visvader, 2009) wherein a common progenitor cell maintains two lineage-specific progenitors as well as alveolar progenitors. Alveolar progenitors have also been proposed to have bipotent potential during pregnancy (dashed arrow). (B) Revised model of mammary differentiation (Van Keymeulen et al., 2011) in which bipotent progenitors only participate in embryonic development of the gland or are reactivated during transplantation of basal/ME progenitors (dashed arrow); luminal and basal/ME lineages are maintained by self-renewing unipotent progenitors in adults.

REFERENCES

Barker, N., Bartfeld, S., and Clevers, H. (2010). Cell Stem Cell *7*, 656–670.

Keller, P.J., Arendt, L.M., Skibinski, A., Logvinenko, T., Klebba, I., Dong, S., Smith, A.E., Prat, A., Perou, C.M., Gilmore, H., et al. (2011). Proc. Natl. Acad. Sci. USA, in press. Published online September 21, 2011. 10.1073/pnas.1017626108.

Lim, E., Vaillant, F., Wu, D., Forrest, N.C., Pal, B., Hart, A.H., Asselin-Labat, M.L., Gyorki, D.E., Ward, T., Partanen, A., et al; kConFab. (2009). Nat. Med. *15*, 907–913.

Molyneux, G., Geyer, F.C., Magnay, F.A., McCarthy, A., Kendrick, H., Natrajan, R., Mackay, A., Grigoriadis, A., Tutt, A., Ashworth, A., et al. (2010). Cell Stem Cell *7*, 403–417.

Petersen, O.W., and Polyak, K. (2010). Cold Spring Harb. Perspect. Biol. *2*, a003160.

Shackleton, M., Vaillant, F., Simpson, K.J., Stingl, J., Smyth, G.K., Asselin-Labat, M.L., Wu, L., Lindeman, G.J., and Visvader, J.E. (2006). Nature *439*, 84–88.

Stingl, J., Eirew, P., Ricketson, I., Shackleton, M., Vaillant, F., Choi, D., Li, H.I., and Eaves, C.J. (2006). Nature *439*, 993–997.

Van Keymeulen, A., Rocha, A.S., Ousset, M., Beck, B., Bouvencourt, G., Rock, J., Sharma, N., Dekoninck, S., and Blanpain, C. (2011). Nature *479*, 189–193.

Visvader, J.E. (2009). Genes Dev. *23*, 2563–2577.

Wagner, K.U., Boulanger, C.A., Henry, M.D., Sgagias, M., Hennighausen, L., and Smith, G.H. (2002). Development *129*, 1377–1386.