The mammary stem cell conundrum: is it unipotent or multipotent?

Purna A Joshi¹,² and Rama Khokha*¹,³

Abstract
Exploring the normal biology and regulation of stem cells has the promise to yield insights into the etiological roots and survival of breast cancer cells. Many studies have supported the existence of a multipotent mammary stem cell that regenerates all aspects of glandular development. However, Van Keymeulen and colleagues (2011) illustrated the presence of lineage-restricted unipotent stem cells that self-renew and collaborate in postnatal mammary development, whereas multipotent stem cells were found only during embryonic mammogenesis. This prompts a re-evaluation of currently accepted mammary stem cell dynamics and conceivably its impact on the evolution of different breast cancer subtypes.

The capacity of the mammary gland for rapid growth and regeneration is attributed to mammary stem cells (MaSCs). Mamopoiesis initiates in the embryo but the majority of glandular development occurs during puberty. Recurrent reproductive cycles elicit transient but significant alveolar epithelial expansion, whereas pregnancy involves heightened lobuloalveologenesis and lactational differentiation. Deome and colleagues [1] were the first to show that tissue transplants from several portions of the gland form an entire gland in vivo in epithelium-divested fat pads, suggesting the existence of MaSCs. Transplantation of tissue fragments or dispersed cells has since become a routine functional assay in the mammary gland field. Limiting-dilution transplants of cells have demonstrated the capacity of single cells to clonally expand to form a functional mammary gland and self-renew in serial transplants, supporting the existence of a multipotent MaSC [2].

Bilayered mammary ducts are composed of inner luminal epithelial and outer basal/myoepithelial cells. When mammary cells are purified by fluorescence-activated cell sorting (FACS) using surface markers that segregate luminal (CD24med+/CD49fhi/CD29lo) and basal (CD24med+/CD49fhi/CD29hi) cells and are transplanted, basal cells generate robust functional mammary outgrowths whereas luminal cells lack this capacity [3,4]. Indeed, a single sorted basal cell has been shown to generate an entire mammary gland constituting ducts and alveoli and serially transplant in vivo. This illustrates the multipotent and self-renewal capacity of the basal MaSC. Injections of sorted/unsorted cells in limiting dilutions allow deductions of MaSC numbers in varying experimental conditions [5,6]. Interestingly, human breast stem cells capable of in vivo reconstitution were also found in basal cells (CD49f+EpCAMneg-low) but not in luminal cells [7].

Van Keymeulen and colleagues [8] reported the existence of lineage-restricted unipotent stem cells in both luminal and basal epithelial populations and challenged prevailing work on the contribution of multipotent MaSCs to postnatal gland development. The authors performed lineage-tracing experiments of embryonic, pubertal, adult, pregnant, and involuting mammary glands, employing K14-rtTA/TetO-Cre/Rosa-YFP and K5-CreER/Rosa-YFP mice for tracking basal cells and K8-CreER/Rosa-YFP and K18-CreER/Rosa-YFP mice for tracking luminal cells. Induction of K14-driven YFP in embryos led to labeling of both luminal and basal cells at puberty, implying that embryonic K14+ cells are multipotent. Postnatal YFP induction showed exclusive labeling of basal cells that clonally expanded during puberty and pregnancy. Similar results were obtained with K5-CreER, indicating that K14+/K5+ cells are unipotent and do not contribute to luminal cell progeny. In contrast, YFP driven by K8/K18 luminal promoters labeled only luminal cells that clonally expanded in K8-CreER mice but not in K18-CreER mice.

The differentiation potential of both epithelial lineages was determined by using transplantation assays. Unsorted cells from basal-specific Cre lines generated outgrowths in which YFP+ cells were predominantly

*Correspondence: rkhokha@uhnresearch.ca
¹Ontario Cancer Institute, 610 University Avenue, Toronto, ON, M5G 2M9, Canada
Full list of author information is available at the end of the article

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basal, although YFP+ clones were also rarely seen in luminal cells. YFP+ cells from the luminal-specific Cre line remained restricted to the luminal layer similar to that observed in the intact gland. This YFP chimerism was preserved in secondary grafts that the authors cite as evidence of self-renewal in these unipotent cells. When FACS-purified YFP+ basal or luminal cells were transplanted, basal, but not luminal, cells were capable of reconstituting a mammary gland, recapitulating previous findings that originally defined multipotent basal MaSCs. In mixing experiments, reducing the luminal/basal cell ratio increased the potential of basal cells to generate luminal cells. The study argues that the experimental setting of the transplantation assay forces differentiation of basal MaSCs into both epithelial lineages while the intact gland relies on lineage-restricted unipotent stem cells.

Lineage-tracing experiments have proven valuable in uncovering new insights into stem cells in other tissue contexts such as the skin and intestine, and Van Keymeulen and colleagues [8] have harnessed this technique to address a fundamental question about stem cell differentiation potential. Their findings bring new perspective to the MaSC field. Previous work has adhered to transplantation as a reliable gold-standard stem cell assay. The lack of detection of a multipotent basal MaSC in lineage-tracing experiments of the intact postnatal gland now thrusts scepticism on the contribution of basal multipotent stem cells to postnatal gland regeneration. Although the study broadens our conceptualization of the MaSC and raises questions about the relevance of transplantation as a functional stem cell readout, facets of this study warrant further discussion. Stem cells in other tissues such as the hematopoietic system have been shown to possess varying degrees of self-renewal, including short-term, long-term, and even intermediate repopulating potentials [9]. The basal and luminal unipotent cells were shown to maintain their chimerism in secondary grafts that the authors demonstrate to be self-renewal but whether these cells have short- or long-term potential is unclear. Second, luminal cells, though unipotent, were unable to regenerate a gland independent of basal cells in transplantsations, whereas basal cells possess this intrinsic property, implying a hierarchy of stem cell capacity. Luminal cells clearly rely on basal cells for cues that are likely paracrine to facilitate their unipotency in the intact gland. Furthermore, several groups have identified dual-positive cells (K14+ with K8/K18/K19) in the human breast, suggesting the existence of bipotent progenitors and candidate stem cell zones [10-12]. Dual-positive cells, as well as markers that tag basal cells, have also been noted in situ in the murine gland within the luminal compartment of terminal end buds and alveoli [13,14]. Van Keymeulen and colleagues [8] found Lgr5+ cells, an intestinal stem cell marker, predominantly in basal mammary cells but additionally in luminal cells. It is conceivable that these are indicative of a bipotent cell population but this possibility has been overlooked in their lineage-tracing experiments, perhaps because of low K14/K5 expression levels in dual-positive cells.

Identifying epithelial populations that have regenerative capacity and defining their extrinsic and intrinsic regulatory mechanisms are relevant to understanding not only the normal development but also the etiology of breast cancers in which transformed cells share properties akin to those of stem cells, notably self-renewal [15]. Given the heterogeneity of breast cancers, it is surmised that specific mammary cells are cells of origin for different cancer subtypes. It will be important to understand whether the likely targets of transformation are initially unipotent or multipotent and whether the mutation repertoire influences their differentiation potential and self-renewal capacity.

Abbreviations
FACS, fluorescence-activated cell sorting; MaSC, mammary stem cell.

Competing interests
The authors declare that they have no competing interests.

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Author details
1. Ontario Cancer Institute, 610 University Avenue, Toronto, ON, MSG 2M9, Canada. 2. Department of Laboratory Medicine and Pathobiology, Faculty of Medicine, University of Toronto, Medical Sciences Building, 1 King’s College Circle, 6th floor, Toronto, ON, M5S 1A8, Canada. 3. Department of Medical Biophysics, University of Toronto, Ontario Cancer Institute, Princess Margaret Hospital, 610 University Avenue, Room 7-411, Toronto, ON, MSG 2M9, Canada.

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