Viewpoint
Highway to heaven: mammary gland development and differentiation

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Abstract

In recent years, the mammary gland epithelium has been shown to be a mixture of differentiated cell populations in a hierarchical relationship with their stem and progenitor cells. However, the mechanisms that regulate their cellular differentiation processes are still unclear. The identification of genes that govern stem and progenitor cell expansion, or that determine daughter cell fate, will be of crucial interest for understanding breast cancer diversity and, ultimately, improving treatment. Two recent analyses have identified some of the key genes that regulate these processes, lighting up the highway to normal mammary gland development.

In the mammary epithelium, recent successes in identifying stem and progenitor cells and lineage-specific transcription factors have raised the possibility of a much deeper under-standing of what controls lineage commitment and cell fate. We have some way to go before we can approach the current level of understanding of haematopoietic lineage commitment. Nevertheless, every new report adds another piece to the puzzle and two recent publications have done just that.

Pietersen and co-workers have described the expression of Bmi1 in mammary cells and its crucial role in mouse mammary gland biology [1]. Bmi1 is a member of the polycomb group of proteins and a regulator of stem/progenitor cell function in other systems [2,3]. However, Pietersen and co-workers found that Bmi1 was expressed in all mammary epithelial cells and reported the profound inhibition of mammary gland development in a *Bmi1* knockout mouse model, as well as a reduction of the mammary stem cell activity and premature lobuloalveolar differentiation. Interestingly, the co-deletion of *Ink4a/Arf* (repressors of the cell cycle down-regulated by Bmi1) partially rescued normal mammary gland development, whereas pregnancy did so completely. Thus, Bmi1 regulates stem and progenitor cell proliferation not only through the inhibition of *Ink4a/Arf*, but also through the inhibition of other genes related to differentiation processes that are signifi-cantly up-regulated by pregnancy.

While *Bmi1* is a gene ubiquitously expressed in mammary cells, other genes are differentially expressed between cell populations and are important for lineage commitment and cell-fate. Raouf and co-workers [4] have recently compared the gene expression profiles of four human mammary cell populations isolated using cell-surface marker expression. Genes from the NOTCH signalling pathway, known to regulate cell fate decisions in other systems [5-7], were described as differentially expressed between two cell populations with different colony forming capacities: undifferentiated or bipotent colony forming cells (CFCs) and luminal-restricted CFCs. *NOTCH4* was highly expressed in bipotent CFCs and markedly down-regulated in luminal-restricted CFCs, whereas an opposite pattern was found for *NOTCH3* and *HES6*, as well as for *NOTCH1* and *NOTCH2* (although the changes were less dramatic for the latter two genes). Functional studies in which NOTCH3 signalling was blocked in bipotent CFCs showed a substantial decrease in formation of luminal-type colonies and an increase in myoepithelial-type colonies, leading the authors to propose *NOTCH3* as one of the key genes for the luminal cell commitment. Although it was not directly demonstrated, it seems likely that the bipotent CFCs correspond to stem and/or multilineage progenitor cells whereas the luminal CFCs correspond to a lineage-restricted progenitor population.

These studies have highlighted two classes of genes driving mammary epithelial cell differentiation: genes accelerating or putting brakes on the development and differentiation processes ('traffic lights'); and genes defining directions in these processes ('road signs') (Figure 1). *Bmi1* is a promoter of stem and progenitor cell expansion but blocks differen-tiation, preventing its premature initiation [1]. Interestingly, both studies agreed in describing *Bmi1* as commonly expressed in all mammary cells [1,4]. In addition, pregnancy favours both cell expansion and differentiation, co-operating with *Bmi1* in regulating stem cell kinetics but opposing its

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CFC, colony forming cell.

Figure 1
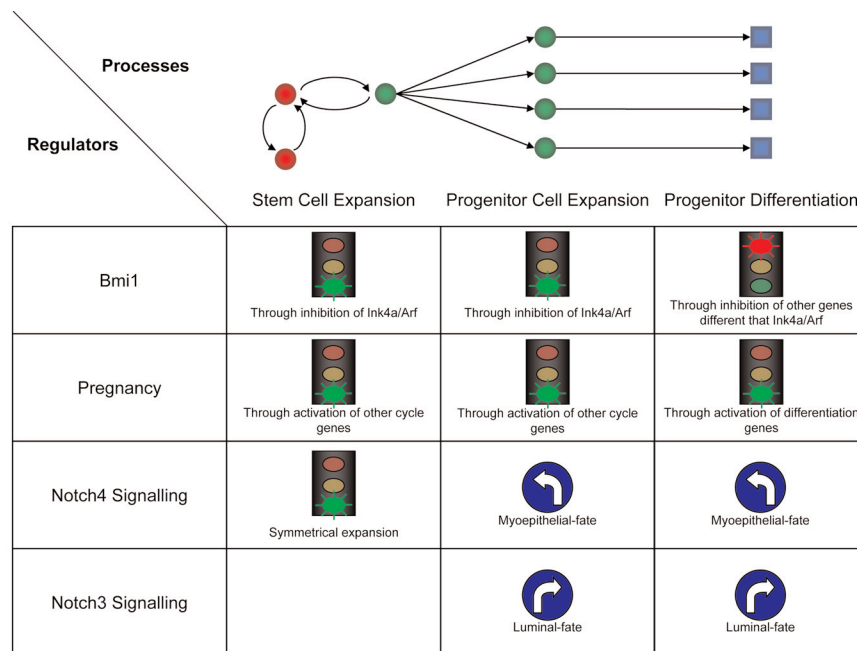
The highway code of mammary gland biology. A schematic representation of the processes involved are depicted in the top: stem cells (red) divide symmetrically (giving rise to two stem cells) or asymmetrically (generating one stem cell and one progenitor cell) [11]. Progenitor cells proliferate (green; progenitor cell expansion) before initiating their maturation to differentiated cells (blue; progenitor differentiation). These processes may be accelerated (represented by green traffic lights), stopped (red traffic lights), or orientated through specific directions (direction arrows) under the regulation of driver genes, either *Bmi1*, genes up-regulated by pregnancy, or the Notch signalling pathway.

effects in differentiation [1]. On the other hand, the NOTCH signalling pathway determines mammary cell fate, as in other tissues [6,7]. In previous studies on human mammospheres, NOTCH4 was shown to be essential for maintaining stem/progenitor cell activity and to direct progenitor cells to myoepithelial cell commitment [8], indicating a dual role for *NOTCH4* as a 'traffic light' gene in stem cells but a 'road sign' gene in progenitor cells. Raouf and colleagues have confirmed the importance of NOTCH signalling pathways in mammary gland biology, showing that the luminal cell lineage is defined by down-regulation of NOTCH4 and the increase of NOTCH3. This NOTCH signalling role in cell-fate determination is also supported by conditional knockout mouse models of Notch effectors [9]. Although an interaction between Notch and Bmi1 has not been shown in these studies, a complex interacting model may exist with the involvement of Hedgehog and Wnt signalling pathways for the self-renewal and differentiation in mammary stem cells, as previously proposed [10].

Given that both traffic lights and road signs must be phased correctly for smooth fate determination, it is important to be able to integrate studies that address stem/progenitor cell kinetics and lineage commitment in both human and rodent systems. However, the different cell-sorting methods currently in use to isolate mammary cell populations, and the differing

terminology used to describe them, confound our ability to create coherent models. Once the cell markers we use have been fully validated, the mammary stem cell community should be encouraged to develop common isolation protocols and nomenclature to facilitate the integration of data.

The mammary stem cell field has changed almost beyond recognition in the last five years. We are now in a position to go further than simply identifying stem cells and to truly begin to understand the molecular control of cell fate in the stem-progenitor-differentiated cell hierarchy. This is not simply an academic exercise. We feel (as no doubt do many others working in the field) that the different gene expression profiles of different breast cancer subtypes reflect real underlying differences in the cells of origin of these tumours and in the differentiation potential of these cells [11-13]. Therefore, we feel that molecular pathology data can only be fully interpreted and understood by reference to normal mammary epithelial cell subtypes and the regulation of their differentiation. The more we understand lineage selection and fate determination in the normal mammary epithelium, the closer we get to a comprehensive understanding of the biology of breast cancer subtypes.

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

References

1. Pietersen AM, Evers B, Prasad AA, Tanger E, Cornelissen-Steijger P, Jonkers J, van Lohuizen M: **Bmi1 regulates stem cells and proliferation and differentiation of committed cells in mammary epithelium.** *Curr Biol* 2008, **18:**1094-1099.
2. Valk-Lingbeek ME, Bruggeman SW, van Lohuizen M: **Stem cells and cancer; the polycomb connection.** *Cell* 2004, **118:**409-418.
3. Lobo NA, Shimono Y, Qian D, Clarke MF: **The biology of cancer stem cells.** *Annu Rev Cell Dev Biol* 2007, **23:**675-699.
4. Raouf A, Zhao Y, To K, Stingl J, Delaney A, Barbara M, Iscove N, Jones S, McKinney S, Emerman J, Aparicio S, Marra M, Eaves C: **Transcriptome analysis of the normal human mammary cell commitment and differentiation process.** *Cell Stem Cell* 2008, **3:**109-118.
5. Bellavia D, Checquolo S, Campese AF, Felli MP, Gulino A, Screpanti I: **Notch3: from subtle structural differences to functional diversity.** *Oncogene* 2008, **27:**5092-5098.
6. Osborne BA, Minter LM: **Notch signalling during peripheral T-cell activation and differentiation.** *Nat Rev Immunol* 2007, **7:**64-75.
7. Yu X, Zou J, Ye Z, Hammond H, Chen G, Tokunaga A, Mali P, Li YM, Civin C, Gaiano N, Cheng L: **Notch signaling activation in human embryonic stem cells is required for embryonic, but not trophoblastic, lineage commitment.** *Cell Stem Cell* 2008, **2:**461-471.
8. Dontu G, Jackson KW, McNicholas E, Kawamura MJ, Abdallah WM, Wicha MS: **Role of Notch signaling in cell-fate determination of human mammary stem/progenitor cells.** *Breast Cancer Res* 2004, **6:**R605-615.
9. Buono KD, Robinson GW, Martin C, Shi S, Stanley P, Tanigaki K, Honjo T, Hennighausen L: **The canonical Notch/RBP-J signaling pathway controls the balance of cell lineages in mammary epithelium during pregnancy.** *Dev Biol* 2006, **293:**565-580.
10. Liu S, Dontu G, Wicha MS: **Mammary stem cells, self-renewal pathways, and carcinogenesis.** *Breast Cancer Res* 2005, **7:**86-95.
11. Molyneux G, Regan J, Smalley MJ: **Mammary stem cells and breast cancer.** *Cell Mol Life Sci* 2007, **64:**3248-3260.
12. Melchor L, Benitez J: **An integrative hypothesis about the origin and development of sporadic and familial breast cancer subtypes.** *Carcinogenesis* 2008, **29:**1475-1482.
13. Dontu G, Al-Hajj M, Abdallah WM, Clarke MF, Wicha MS: **Stem cells in normal breast development and breast cancer.** *Cell Prolif* 2003, **36(Suppl 1):**59-72.