DEVELOPMENT

# Mammary lineage restriction in development

The establishment of the two distinct lineages that form the branched epithelial ductal tree of the mammary gland is a complex and essential developmental process. Two independent studies now describe the switch from multipotency to unipotency as an embryonic process and outline mechanisms of early lineage restriction.

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Embryonic mammary epithelial cells (MECs) are a unique cell population comprised of undifferentiated and highly plastic progenitor cells that ultimately give rise to all postnatal MECs[1,2]. Lineage tracing studies have previously indicated that embryonic mouse mammary cells are multipotent in vivo[1]. Like other organs, mammary glands form during prenatal growth and continue to develop after birth from the descendants of these multipotent prenatal mammary cells. The rudimentary branched epithelium of the mammary gland present at birth holds the capacity for rapid ductal development (puberty), milk production (lactogenesis) and regeneration (involution), processes that are enabled by epithelial cellular plasticity and lineage restriction. The two principal lineages of the mammary gland consist of basal cells with contractile muscle and epithelial properties that determine the basal membrane and encapsulate the ductal-facing luminal cells[2,3]. Luminal cells are polarised and consist of oestrogen receptor positive (ER+) or negative (ER−) ductal cells and secretory ER− alveolar cells[3]. During normal tissue homeostasis these lineages are known to be maintained predominantly by lineage-restricted progenitor/stem cells but, until now, it had not been fully elucidated when lineage restriction of multipotent mammary cells occurs.

In this issue of *Nature Cell Biology*, Lilja et al.[4] and Wuidart et al.[5] examine the switch of multipotent stem/progenitor cells to unipotency (Fig. 1a). Utilising the multi-colour Confetti reporter mouse and clonal analysis during embryonic mammary gland development, these authors investigate the kinetics of stem and progenitor cells and both show that transition from multipotency to unipotency occurs surprisingly early during prenatal stages of development. The two manuscripts from the Blanpain and Fre laboratories arrive at similar conclusions using complementary approaches. Each group also identifies a key complementary lineage-restricting regulator that is capable not only of driving embryonic mammary

cells towards a single lineage, but also of reprogramming mature postnatal MECs to switch from one lineage to the other.

Building on previous work demonstrating that keratin 14 (K14) expressing cells of the embryonic mammary gland can develop into both basal and luminal lineages[1], Wuidart et al. have further characterised the temporal switch from embryonic multipotency to lineage restriction. They associated multipotency with the simultaneous expression of basal and luminal hybrid gene signatures, and identified the transcription factor p63 as promoter of the basal lineage in multipotent progenitors[5]. p63 is a known regulator of epithelial transcriptional profiles and its accumulation in a *Rbpj* conditional knock-out mouse model induced a basal profile in luminal cells, which included keratin 5 (K5) expression[6].

Lilja et al. determined E15.5 as the embryonic timepoint when multipotent MECs become lineage restricted. The authors identified Notch1 as a potential marker of bipotency in the nascent mammary bud and an orchestrator of cell fate during late embryonic development, dictating progression towards ER− luminal progenitors[4]. Notch signalling is ubiquitous in higher eukaryotes and activated during stem cell maintenance and differentiation in a multitude of tissues[7]. Notch1 activation in mammary stem cells dictates luminal lineage selection and promotes luminal progenitor cell expansion, which has been associated with tumourgenesis[7].

Lilja et al. analysed Notch1-expressing cells in the embryonic mammary gland using a double fluorescent reporter line (N1CreERT2/R26mTmG), which revealed Notch1 receptor expression in the majority of cells of the mammary bud at E13.5 and E15.5. All Notch1 positive cells at the E13.5 stage were positive for K5, K14 and p63 basal markers, alongside the luminal marker keratin 8 (K8). Fluorescence-activated cell sorting analysis of the Notch1 positive progeny suggested that, at the population level, descendants of Notch1 positive cells labelled at this stage

show no lineage bias. Wuidart et al. reached the same conclusion after analysing gene signatures of E14.0-stage Lgr5+ embryonic mammary progenitor cells that showed co-expression of basal and luminal markers. Progeny from K14+ cells labelled at E13.0 also showed no lineage bias.

The single-cell RNA sequencing analysis performed by Wuidart et al. provided some tantalising insights into the signalling used by embryonic mammary progenitor cells at E14, which showed a hybrid, basal-luminal embryonic mammary progenitor gene signature during normal development. Bulk RNA sequencing of postnatal MECs indicated that luminal cells also went through a transient hybrid multipotent state after ΔNp63-induced cell fate reprograming into basal cells, as shown by Wuidart et al. Lilja et al. observed the same for basal cells that switched to a luminal fate after ectopic expression of active Notch1 (Fig. 1b). However, single-cell RNA sequencing analysis of MECs has inherent caveats. It is not yet clear whether removal of MECs from their microenvironment during the experimental process alters their gene signatures. Basal MECs harbour the ability to acquire a multipotent stem cell state without additional manipulation, after isolation from their niche within the mammary gland[1]. This potential limitation needs to be considered in all single-cell analyses of mammary progenitor cells. Stem and progenitor cells are thought to exist in a spectrum of cell states and it is likely that other types of embryonic progenitor cells are present in addition to those described in the two manuscripts, for instance cells that do not express Lgr5 or Notch1.

Both studies implicate cell-autonomous processes in the initiation of a mammary cell progenitor state. A number of classic tissue recombination studies, in which embryonic epithelial and mesenchymal (embryonic stroma) tissues from heterotypic organs are separated, recombined and allowed to develop in organotypic culture (reviewed in[8]), have shown that non-cell autonomous signals from the embryonic mammary mesenchyme
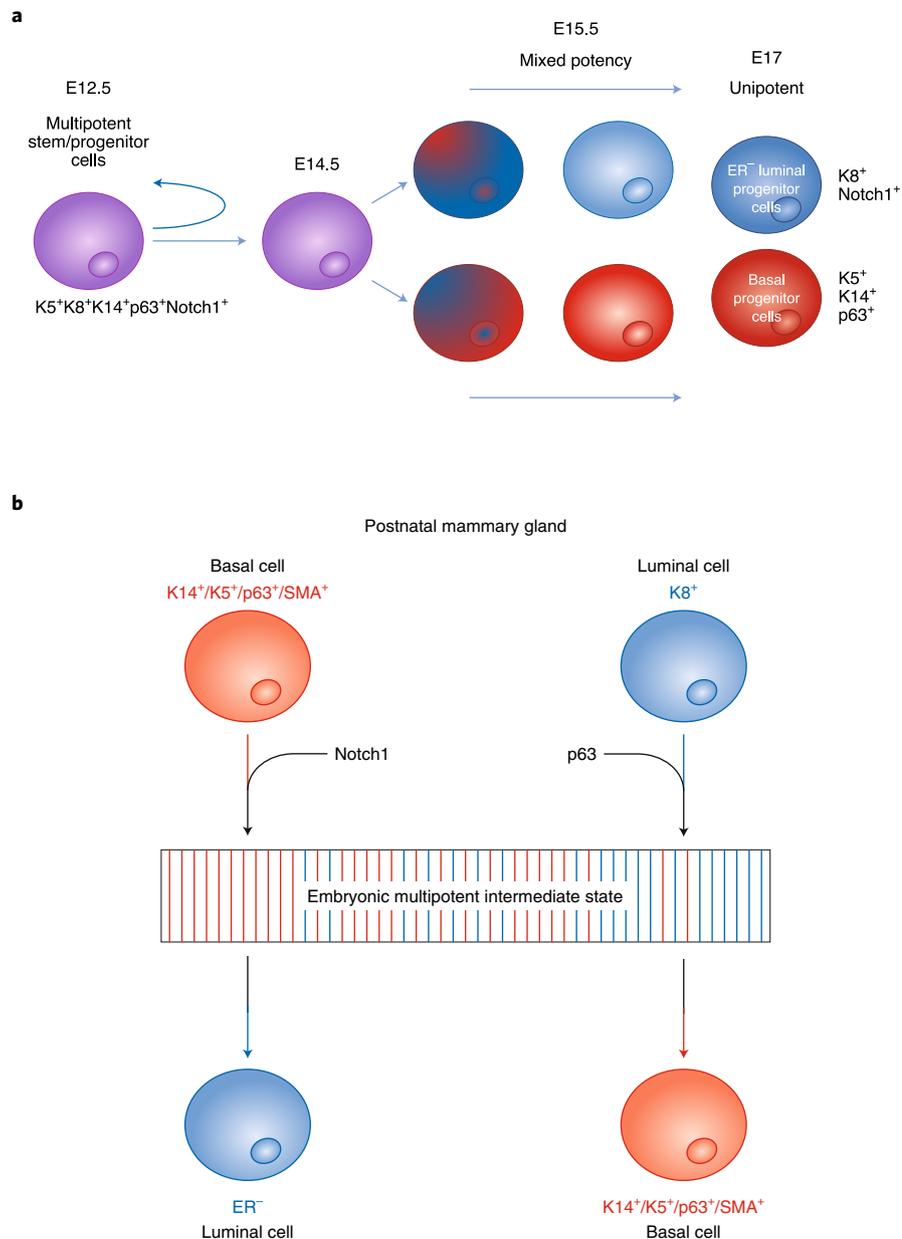
**Fig. 1 | Schematic representation of embryonic bifurcation of the mammary lineage and reprogramming of mammary epithelial cells after lineage restriction. a,** Prenatal lineage restriction of multipotent embryonic mammary epithelial cells. Multipotent mammary progenitor/stem cells exist in the embryonic mammary gland at mid-gestation and give rise to unipotent basal and luminal progenitor cells prior to birth. Multipotent mammary progenitor/stem cells co-express markers from the basal and luminal lineages. Embryonic mammary progenitor cells become biased towards one lineage as development proceeds and adopt marker profiles and location within the epithelium accordingly. **b,** In vivo reprogramming of postnatal MECs. MECs in the postnatal mammary glad are unipotent. Both luminal and basal (myoepithelial) cells can be reprogrammed to switch lineage by p63 and Notch1, respectively, after passing through an intermediate state similar to that found in multipotent mammary stem/progenitor cells that are abundant in the early stages of embryonic mammary gland development. SMA, smooth muscle actin.

the signatures of multipotent embryonic mammary progenitors and postnatal MECs undergoing cell fate specification described in these two studies. As of yet, no clear-cut markers exist to discern embryonic MEC identity from other embryonic epithelial organs and unambiguously show that a cell has committed completely to an embryonic mammary fate.

Plasticity, defined as the ability of cells to dynamically change their state, is crucial for many processes during mammary gland development and tissue homeostasis. However, in breast cancer pathogenesis, dysregulated plasticity produces cellular heterogeneity and might complicate therapies, in particular as the disease progresses. Breast cancer treatments are routinely prescribed to patients based on the expression of a small number of key markers present in the primary tumour (such as ER, PR (progesterone receptor) and HER2 (human epidermal growth factor 2)) and these marker profiles can be distinct from those expressed by disseminated lesions or recurrent disease[9]. A better understanding of the reprogramming potential of postnatal cells and plasticity regulators may eventually lead to improvements in cell state manipulation and therapeutic approaches for breast cancers, including stem-cell-based therapies. In addition, the contribution of rare multipotent cells to normal mammary gland development and homeostasis has been debated, and their physiological function remains unclear[2].

The reactivation of embryonic programmes in adult cells has also been implicated in cancer[10]. Studies using mouse models of breast cancer report that mutations in the phosphatidylinositol 3-kinase catalytic subunit (PIK3CA) disrupt lineage restrictions of luminal and basal progenitor cells[11,12]. These suggest that a gain of multipotency, a unique feature of embryonic mammary progenitor cells, may be a common occurrence in breast cancer. In support of this notion, *SOX11*, a transcription factor highly expressed by embryonic mammary progenitor cells including those profiled by Wuidart et al., is not expressed in the normal postnatal breast, but is upregulated in aggressive breast cancers, including triple negative and HER2+ sub-types[10]. SOX11 is also expressed in ER− and HER2+ pre-invasive ductal carcinoma in situ lesions and promotes invasive growth in both three-dimensional in vitro assays and mouse models of these ER− lesions[13]. Taken together, these findings raise the question of whether MEC-lineage restriction represents a tumour suppressive mechanism and

are instructive and can confer mammary cell identity to non-mammary epithelium, even across species. It is unclear which stromal factors are involved in mammary induction and these types of experiments have not yet

been subjected to rigorous modern molecular analyses. It will be interesting to see how the molecular signatures of embryonic cells that have initially been specified through mammary tissue interactions compare to

whether reactivation of multipotency is a crucial step in breast cancer initiation. Stemness features are associated with oncogenic dedifferentiation across multiple cancer types, and have been put forward as a hallmark of cancer[14].

Finally, regulation of the prenatal to postnatal shift in mammary plasticity might also be an important subject for further study. In epithelial tissues, p63 is regulated by the epigenetic regulator KMT2D (also known as MLL4) in a genome-wide manner[15], which suggests the Notch1–p63 axis postulated by these papers could be part of an epigenetic switch during development. MECs harbour reprogramming potential that is realised during breast tumourigenesis. What are the signals that reactivate multipotency? Identifying the key regulators of mammary epithelial plasticity will reveal basic mechanisms underlying tissue-specific stem cell behaviour, which could lead to the development of targeted approaches to expand breast cancer prevention and therapeutic options. ❐

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