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Modeling the complexity of mammary gland in vitro

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A new method models mammary gland in vitro during physiological and pathological conditions.

Organoid culture technology has become one of the most useful techniques to generate mini-organs in vitro, allowing researchers to study the mechanisms of organ development, tissue maintenance, regulation of cell fate, disease states and cancer[1]. However, the complex architecture of some organs, their three-dimensional organization and their extrinsic regulation by hormonal cues remain difficult to recapitulate in vitro. One such area of research is the mammary gland (MG) which is a branched epithelial structure composed of basal and luminal cells (Fig. 1a). The MG is regulated by hormonal cues such as estrogen and prolactin that ensure the postnatal development of the MG during puberty and expansion and differentiation of luminal cells into alveolar

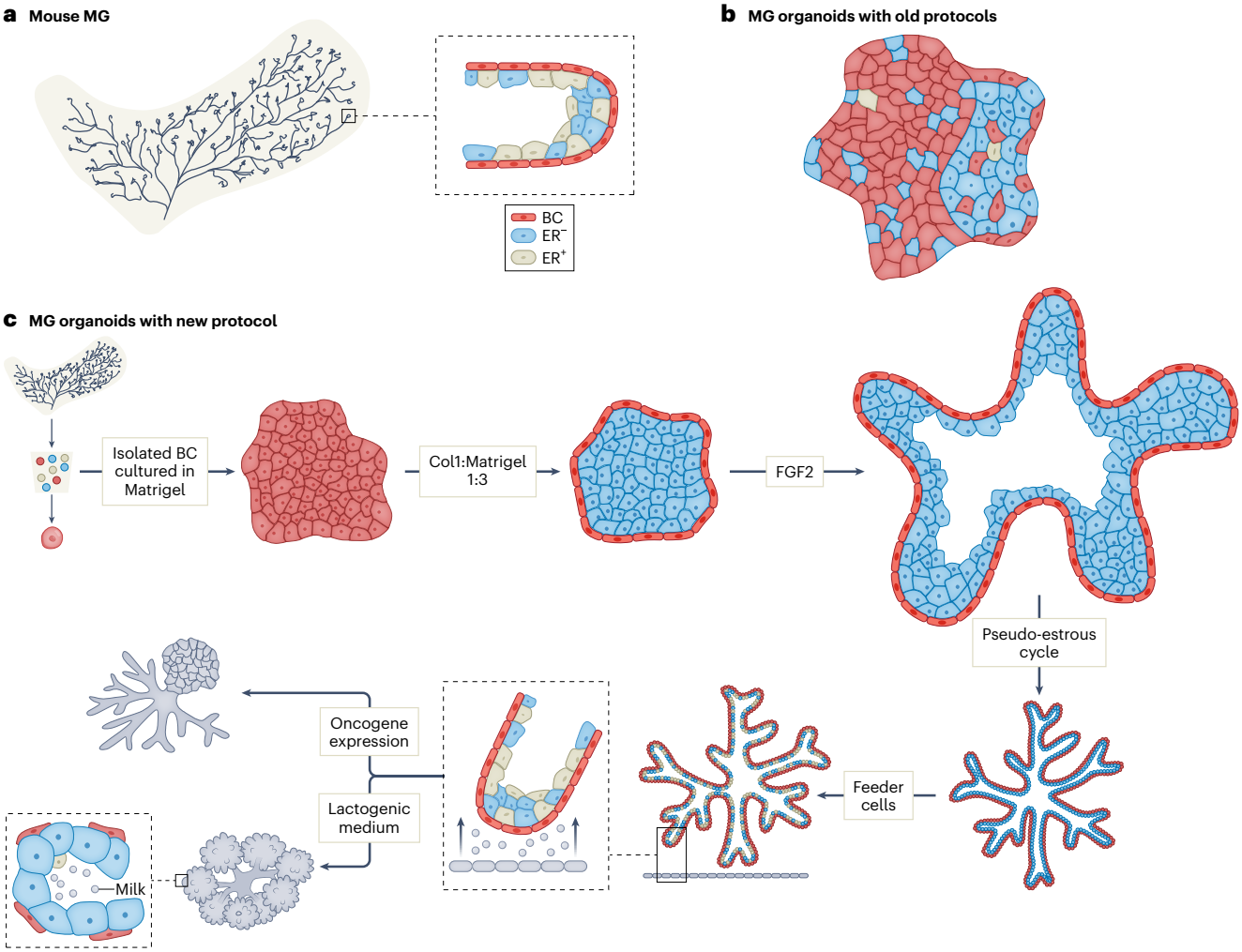

Fig. 1 | Cellular composition and structural complexity of mammary gland in vivo and in vitro. a, Mammary gland (MG) in vivo is a branched epithelium that presents an outer layer of basal cells (BCs) and an inner layer of luminal ER$^+$ and luminal ER$^-$ cells. **b**, MG organoids generated with previous methods are roundish, do not always present a very precise segregation of BCs and luminal cells (LCs) and contain a low percentage of ER$^+$ LCs. **c**, The new stepwise method described here ensures the generation of multibranched mini-MGs that recapitulate the molecular, cellular and structural complexity of the MG in vivo. Col1, type I collagen.

milk-producing cells during pregnancy and lactation[2]. Several methods have been developed to make MG organoids in vitro[3,4], but they have failed to mimic the defined highly branched structure. These organoids could not respond to hormonal cues mimicking pubertal development, menstrual cycles and pregnancy (Fig. 1b). Now, in this issue of *Nature Methods*, Yuan and colleagues report a new method to generate mouse mammary glands in vitro that overcomes these technical hurdles. These MG organoids are almost indistinguishable from in vivo MG and recapitulate the whole complexity of the in vivo organ, from architectural, cellular and molecular points of view, thus offering us new ways to tackle key open questions in the field[5].

The work of Yuan and colleagues describes a new approach to convert the roundish and unorganized cellular structures obtained by the previous methods into multibranched, bilayered organoids able to undergo alveolar differentiation (Fig. 1c). The first step is to generate a primordial sphere that resembles the mammary placode, which is the first morphological features during MG embryonic development[2], by isolating adult basal cells (BCs) and culturing them on Matrigel. These primordial colonies lack proper apicobasal polarity and usually cease to grow after reaching a certain size (Fig. 1c).

First, the authors modified the composition of the extracellular microenvironment by optimizing the type I collagen:Matrigel ratio to 1:3, which increased organoid size, and the addition of fibroblast growth factor 2 (FGF2), a well-known morphogen of MG development[6], which induced symmetry breaking and the branching of MG organoids (Fig. 1c). The authors also induced a pseudo-estrous cycle by administrating oscillating concentrations of estrogen and progesterone to mimic the physiological hormonal cycle. This allowed better elongation, differentiation and side branching of the MG, although at this stage these organoids still lacked the luminal cells (LCs) expressing the estrogen receptor (ER^+) observed in vivo (Fig. 1c). Next, the team cultured the MG organoids on Transwell plate coated with fibroblast feeder cells to mimic the role of stromal fibroblasts that support the growth of mammary epithelium through paracrine signals[7]. They observed that MG organoids started branching into a tree-like structure and could recapitulate the normal cellular compositions of in vivo MGs containing BCs, ER^+ LCs and ER^- LCs as shown by immunostaining and single cell RNA-seq (Fig. 1c).

By isolating and transplanting the MG epithelial cells from the organoids into mammary mesenchyme, the team then showed that the organoids maintain the self-renewing capacities of these cells and their ability to re-form a normal MG upon transplantation in vivo.

Yuan et al. then demonstrated the functional properties of these MG organoids. Upon the administration of lactogenic hormones such as prolactin, these organoids undergo alveolar differentiation and produce milk of the same lipid composition of normal milk (Fig. 1c). The addition of oxytocin promotes the secretion of oil droplets into the lumen, and upon withdrawal of the lactogenic medium the organoids involute and go back to their pre-lactogenic appearance (Fig. 1c).

The authors then explored the cell fate regulation of MG epithelial cells in these organoids. One of the main problems of the existing protocols is that most of the epithelial cells do not properly maintain their in vivo fate and present lineage infidelity[3]. The MG develops from multipotent progenitors that give rise BCs and LCs, which become lineage restricted postnatally[8–10]. Different conditions, such as transplantation, LC ablation and oncogene expression, reactivate BC multipotency[10–12]. Using this new organoid method, the authors used lineage tracing to show that BCs are multipotent at early time points and undergo lineage restriction as they mature due to tumor necrosis factor signaling from LCs, as occurs in vivo[12]. Finally, the authors transduced the MG organoids with the conditional polyoma middle T oncoprotein to induce oncogene expression and study the early steps of tumor initiation in vitro. These tumor-like structures when transplanted in vivo could form tumor tissue in immunodeficient mice, demonstrating the utility of these models for the study of tumorigenesis (Fig. 1c).

While this study is a breakthrough for the field, this methodology can still be further improved by increasing the complexity of the stromal cells by adding fat cells, immune cells or endothelial cells (or the molecules they secrete) to the organoids to better explore how the cross-talk between epithelial cells and the different cell types of the microenvironment regulates MG biology, as well as adapting this approach to study human MG epithelial cells.

In conclusion, the work of Yuan and colleagues described an elegant method to generate mouse mini-MG from organoid culture in vitro that can recapitulate every feature of the MG in vivo. These mini-MG are genetically modifiable and retain their ability to give rise to normal MG upon transplantation in vivo, opening avenues to address key open questions in the field of MG and breast cancer formation. Using CRISPR–Cas9 genetic editing of these new organoids, it will now be possible to define at a much larger scale how genes differentially expressed by the distinct populations of epithelial cells regulate MG morphogenesis, pubertal expansion, reproductive cycles and lactation, as well as tumor initiation. Lineage tracing and clonal analysis using real time imaging will reveal the cellular behavior and proliferative dynamic of the different cell lineages during MG expansion and branching in vitro, as well as how these processes are deregulated upon oncogenic hits, providing insights into the mechanisms regulating branching morphogenesis and the early steps of tumor initiation.

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Competing interests

The authors declare no competing interests.