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Organoid models for mammary gland dynamics and breast cancer

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Abstract

The mammary gland is a highly dynamic tissue that undergoes repeated cycles of growth and involution during pregnancy and menstruation. It is also the site from which breast cancers emerge. Organoids provide an in vitro model that preserves several of the cellular, structural and microenvironmental features that dictate mammary gland function in vivo, and have greatly advanced our understanding of glandular biology. Their tractability for genetic manipulation, live imaging and high throughput screening have facilitated investigation into the mechanisms of glandular morphogenesis, structural maintenance, tumor progression, and invasion. Opportunities remain to enhance cellular and structural complexity of mammary organoid models, including incorporating additional cell types and hormone signaling.

Introduction

Unlike other tissues in the body, the mammary gland undergoes the majority of its development after birth (Fig 1a) when a highly branched ductal epithelium emerges from a rudimentary embryonic ductal tree. The branched ductal epithelium comprises three distinct epithelial lineages. Lining the luminal space are two populations of luminal cells (LEP), which are responsible for milk production and hormone sensing. LEPs are surrounded by an outer population of contractile myoepithelial cells (MEP) that serve in milk ejection during breast-feeding (Fig. 1b). The epithelium is surrounded by a basement membrane and is embedded in a complex stroma containing fibroblasts, nerves, vasculature, lymphatics, immune cells and adipocytes (Fig. 1b). Signals from the surrounding non-epithelial cells, extracellular matrix (ECM) and hormones further guide the structure and function of the mammary gland. The epithelium is also the site where most breast cancers originate (Fig. 1c), a disease that affects 1 in 8 women in the United States.

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The mammary gland has been studied extensively as a model tissue, encompassing multiple cell types that undergo large-scale morphological changes in response to stimuli. Early histological studies established changes in tissue composition and structure during development, reproductive cycle and breast cancer [1], but these were only snapshots of highly dynamic processes. In contrast, lineage tracing studies in genetically engineered mouse models (GEMM) provided insight into lineage specification and developmental dynamics [2], but may not accurately reflect human biology [1]. Three-dimensional cell culture of immortalized human mammary epithelial cells can provide insight into human-specific cell biology and have established the importance of structural cues and paracrine signaling in the breast [3]. Frequently referred to as “acini” or “cysts”, these tissue models, however, lack the multiple cell types found in vivo, and suffer from molecular and physical changes associated with long-term cell culture and immortalization. Therefore, the several primary tissue-derived mammary organoid models, which retain more in vivo-like cellular heterogeneity and a more physiologically relevant structure, represent an important advance for understanding the dynamic remodeling in the breast [4–6]. Like immortalized cell lines cultured in vitro, organoids are amenable to advanced imaging and perturbation techniques, providing an exciting venue for studying cell and tissue dynamics. In this review, we will summarize key aspects of mammary gland biology and corresponding organoid models (Fig. 2). Additionally, we will discuss emerging applications where organoids will be transformative for studying both the biology and diseases of the breast. We restrict the scope to organoids sourced from primary human or mouse tissue and stem/progenitor cells that are capable of self-organization and at least limited self-renewal [7].

Establishment of mammary organoid models

Mammary organoids are most commonly established from murine or human mammary epithelial fragments that were micro-dissected or mechanically and enzymatically digested, and then embedded in a reconstituted ECM [6,8]. While murine organoids have been popular due to the availability of powerful genetically engineered mouse models, human-derived organoids sourced from reduction mammoplasty, prophylactic mastectomy, breast biopsy, or resected cancerous tissues are increasingly providing important insight into human biology. Additionally, organoids have been successfully established from dissociated single or reaggregated primary mammary epithelial cells [5,6,9–14]. Importantly, different regions and cell types differ in their organoid-forming potential [9,15,16]. Human pluripotent stem cell (hPSC)-derived mammary-like organoids present the potential for a human model for mammary development and cancer whilst circumventing the requirement for primary tissue [17]. Each of these methods for organoid generation has distinct advantages and disadvantages pertaining to cell compositional control, structural integrity and functionality that must be considered before choosing the right model system (Fig. 3). These models further require additional validation to establish appropriate tissue architecture, cell composition and signaling states [16]. In particular, many of these organoid systems should be benchmarked against primary tissue using techniques like single-cell RNA- or ATAC-seq to better establish in vivo-like cell-type and cell-state diversity [18–21].

Organoid models for mammary gland development and morphogenesis

The earliest stages of mammary development occur prenatally, starting as an ectodermal placode that subsequently gives rise to a rudimentary ductal tree [1]. During puberty, hormonal signaling induces ductal elongation and branching morphogenesis. In the adult, cycling hormone levels accompanying the menstrual cycle cause repeated but small-scale growth and regressions of this ductal network. During pregnancy, however, as hormone levels spike, there is pronounced elaboration of the network prior to lactation. Upon weaning, the network involutes to return to a resting, but somewhat altered ductal structure [22]. Some organoids maintain hormone receptor expression (which is typically lost in 2D culture) over long periods [6,23], but their ability to drive growth and involution in response to hormone levels has not been firmly demonstrated. The primary function of the mammary gland is milk production and delivery to the nipple, and organoid models have begun to recapitulate elements of this process in vitro. While milk protein production by mouse LEP has previously been induced *in vitro* [12], the generation of human organoid models with the correct morphology and cell composition that model lactation and involution has only recently been described [24]. As the expulsion of milk from the gland requires the contractile function of MEP, organoids can additionally be used as a screening system for identifying the Ca2+-dependent mechanisms which drive contractility and milk secretion [25].

Organoids undergo dynamic shape changes in response to growth factors and ECM cues, similar to ductal elongation, branching and invasion seen in vivo [8,26,27]. GEMM-derived organoids expressing fluorescent reporters or Cre-drivers from lineage-specific promoters allow cell type-specific genetic manipulation and facilitate spatiotemporal characterization of ductal elongation and branching dynamics in exquisite detail. Fluorescence time lapse microscopy of organoids established that ductal elongation is driven by collective migration of the epithelial cells, reinforced by radial intercalation, and balanced by interfacial tensions along the migrating cell's edges [28,29]. Further, it was shown that cell stratification at the terminal end bud occurs through the loss of apico-basal polarity and oriented cell divisions [30–35].

The three main cell lineages of the mammary epithelium emerge during development and considerable debate surrounds how these lineages are maintained in the adult. Whether there exists a dedicated (as opposed to facultative) bipotent stem cell, the existence of cell trans-differentiation, and the physical and molecular basis for lineage specification remain unclear, in part because in vivo and ex vivo models sometimes provide contradictory results [2,36]. Organoids will be a powerful tool for resolving this debate as they provide precise control on cell composition and microenvironment, and are amenable to live imaging to track cell and tissue dynamics. As pointed out previously, however, more rigorous characterization of cell lineages within different organoid models will be necessary before their potential can be realized in this area.

Organoid models for breast cancer

Breast cancer is a constellation of molecularly distinct diseases. The major molecular subtypes – including luminal A, luminal B, basal, and her2(+) – are also heterogenous with respect to their gene expression and cellular composition [37]. While recurring mutations in breast cancers are described [38], their impact on cellular signaling, tumor heterogeneity, cancer progression and treatment outcome is not well understood. Organoids present a unique platform to address some of these existing gaps in knowledge. Breast cancer subtype-specific GEMMs exist [39], and organoids derived from these have revealed the importance of collective motility, cell adhesion and matrix interactions on morphogenesis and invasion [10,40–43]. Gain and loss of function studies in organoids, using small molecules and genetics, further established the role of specific genes (e.g. Twist1, Trps1, and E-cadherin) or cell behaviors (cell adhesion and epithelial to mesenchymal transition) in tumor invasion and metastasis [10,42–47].

Recently, organoid biobanks were established from normal and tumor-derived human breast tissue. This resource is amenable to live imaging, as well as genetic and chemical perturbations. These organoids retain their distinct molecular subtypes and elements of the cellular heterogeneity found in vivo [6,11,48]. While they can be cultured for extended periods, it is important to be conscious of molecular and phenotypic drift after passaging, which might alter tumor characteristics or drug response [49]. Human tissue-derived tumor organoids will enable the characterization of dynamic changes to cellular composition, tissue morphology, mechanics, marker expression, and drug susceptibility that coincide with the progression of different tumor types. Additionally, these organoids could allow quantitative analysis of how environmental factors such as aging, injury, hormone therapy, nutrition, and radiation exposure can impact breast cancer risk [47,50]. With sufficient validation, these systems will ultimately be useful guides to develop personalized treatment strategies for patients.

Organoid models for structural dynamics in the breast

The bilayered structure of the mammary epithelium is not only critical to milk secretion and movement, but is a key structural and dynamic barrier to breast cancer progression [51]. Hence, understanding the physical and molecular processes that maintain this structure is an ongoing topic of research. Primary tissue fragments maintain the bilayered structure in vitro, enabling quantitative characterization of changes in tissue organization and shape in response to genetic and microenvironmental perturbations [52,53]. Reconstituted organoids from human reduction mammoplasty tissue established that this bilayered structure arises from a robust program of self-organization [5,54]. Like in many other tissues [55], lineage-specific cell-cell and cell-ECM interfacial tensions in the mammary gland determine the relative mechanical energy of these interfaces and are primarily responsible for driving cells toward the correct luminal or basal position within the tissue. Specifically, the highly unfavorable LEP-ECM interface excludes LEP from the basal tissue layer, while a highly favorable MEP-ECM interface maintains MEP in the basal layer [5].

Breast cancers typically originate in the luminal compartment, and progress through a series of defined structural changes (Fig. 1c), identified as inflection points in risk for breast cancer patients [56]. Less than 30% of ductal carcinoma in situ (DCIS) lesions progress to a more dangerous invasive ductal carcinoma (IDC), characterized by the translocation of transformed LEP past MEP [57,58]. The observation that LEP must translocate past MEP to invade led to the hypothesis that MEP represent a physical barrier to invasion. Using murine organoid models, MEP were recently shown to drastically reduce the dissemination of invasive cancer cells in a dose-dependent manner by undergoing dynamic rearrangement to exclude LEP from the basal layer when they contact ECM [10]. In addition to validating the long-standing hypothesis regarding MEP as structural tumor suppressors, these studies further established the importance of cell dynamics in the process. Understanding the physical and molecular underpinnings of structural maintenance and breakdown may provide key insight into disease progression.

Organoid models for microenvironmental and stromal interactions

Microenvironmental cues from the ECM, stromal and immune cells tightly regulate the mammary epithelium [59]. Alterations to the tissue microenvironment during cancer progression are well established, including changes in ECM stiffness and organization, as well as the composition of the immune cell infiltrate [60]. Previously, patient-derived xenografts and mouse models have been popular for studying these cancer-associated microenvironmental changes, but they provide only minimal control over tumor composition and structure, take time to develop, and are challenging for studying dynamic responses to drugs or stimuli. Many of these challenges can be overcome using organoids that incorporate microenvironmental stimuli from stromal cells and hormone signaling, and are an important frontier for both normal and breast cancer organoids.

Organoids have also proven useful for interrogating the role of epithelial-stromal interactions during glandular morphogenesis [53]. For example, remodeling of the collagenous ECM by the epithelium [61] can reinforce the orientation of collective migration independent of stromal cells, though this is dependent on ECM composition, orientation and adhesion [53,62,63]. Additionally, organoids can be used to study how external signals from hormones or stromal cells can induce ECM changes observed in breast cancer. In one recent example, hormone stimulation of patient-derived organoids identified changes in ECM-related gene expression in patients with BRCA mutations [64]. Considerable effort is being devoted to adding key stromal cell types to co-culture models with epithelial organoids, including fibroblasts, immune cells, and endothelial cells. These efforts are particularly important because the stromal compartment differs in structure and composition between human and mouse (e.g., mouse fibroblasts express estrogen receptors) [1], and cannot be recapitulated in xenograft models. Some progress has been made in this direction. For example, FGF derived from fibroblasts was shown to directly impact epithelial branching and proliferation [29,65–67]. Moving forward, a major goal of organoid-based research will be to add additional stromal cell types, such as immune and endothelial cells, to better mimic the paracrine signaling networks the characterize glandular biology in vivo. A functional immune compartment is also of great interest to the immuno-oncology research community.

Conclusions

Modeling mammary morphogenesis necessitates the correct epithelial structure, ECM organization, cellular composition, as well as multiple stromal cell types. Existing organoid models have begun to bring together the necessary components. These emerging models are shedding light on how signals and forces in the epithelial microenvironment collaborate to generate the complex tissue dynamics observed in vivo, and will undoubtedly be useful in understanding how genomic and microenvironmental changes alter these processes in breast cancer. Looking forward, research should be focused toward the goal of increasing organoid reproducibility, uniformity, complexity and structural accuracy. Further emphasis should be placed on carefully characterizing newly developed organoid models using single cell analysis tools (e.g. scRNA-seq, ATAC-seq, CyTOF, etc.), and benchmarking any findings against in vivo data. These will be important for establishing an optimal human experimental model for studying the signaling and mechanical interactions that occur between mammary epithelial cells, transformed cells, and the surrounding stroma.

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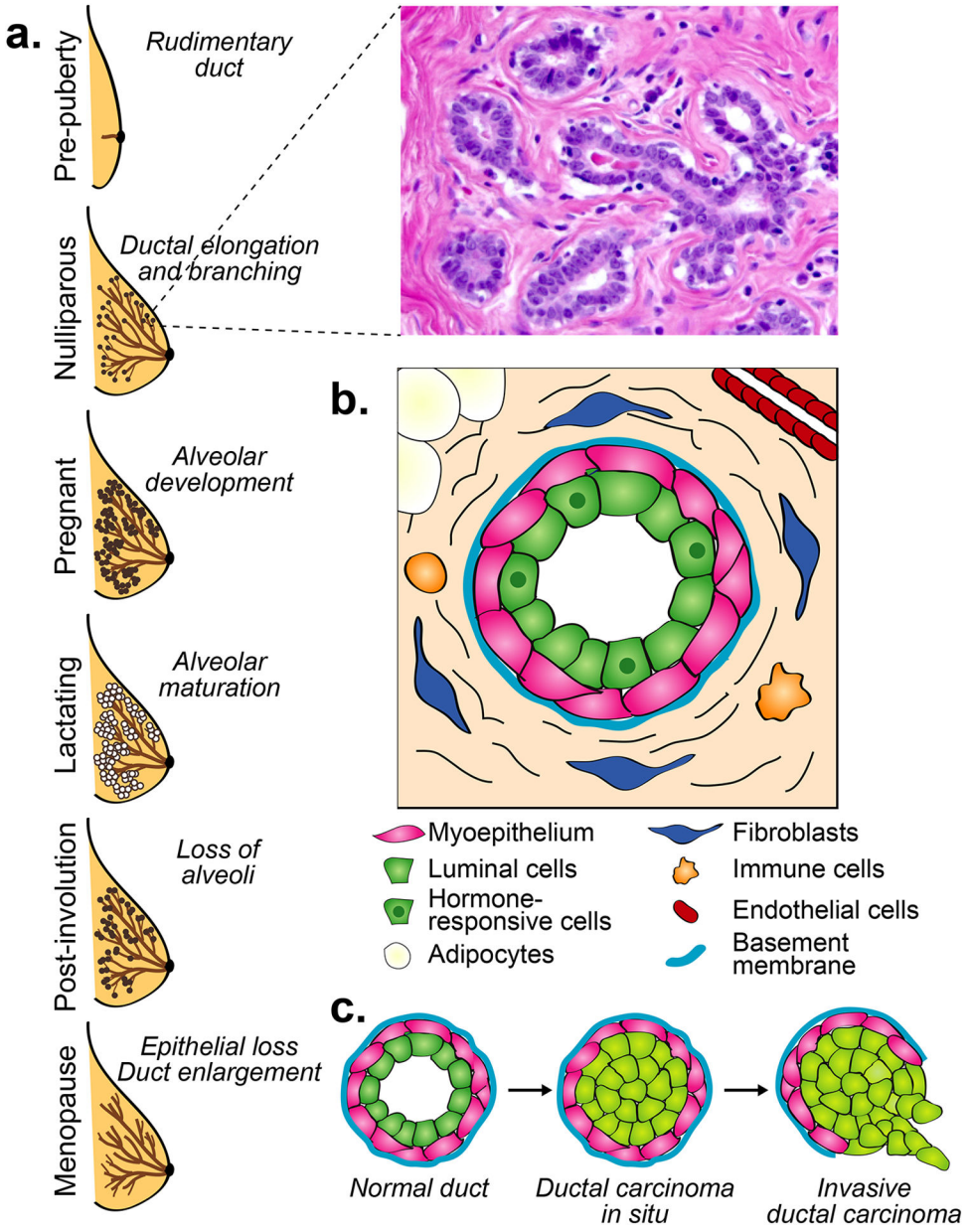

Figure 1:

a. The mammary gland undergoes extensive remodeling during a woman's lifetime, particularly during pubertal development and pregnancy. During puberty, the rudimentary epithelial tree elongates and branches to infiltrate the stroma (mainly adipocytic in mouse and fibroblastic in human), resulting in a lobular tree. Further maturation of the gland occurs during pregnancy, when hormones drive the growth of the terminal lobular ductal units into alveoli, wherein the luminal epithelial cells can differentiate into milk-secreting cells for lactation. After weaning, the process of involution remodels the mammary gland, including the collapse of the alveoli and extensive apoptosis. **b.** The structure of the mammary gland comprises a bilayered epithelium surrounded by a variety of stromal cell types. How tissue homeostasis is maintained remains an active area of research, including questions about

lineage specification, tissue composition and structure, differences in the ducts vs alveoli as well as how they change with time and age, autocrine and paracrine signaling, and response to stimuli (e.g. hormones, drugs). **c.** Histologically, breast cancer progresses through distinct stages, beginning with the filling of the lumen in ductal carcinoma in situ (DCIS), to invasion of the proliferative cancerous cells past the myoepithelium, and finally to metastasis.

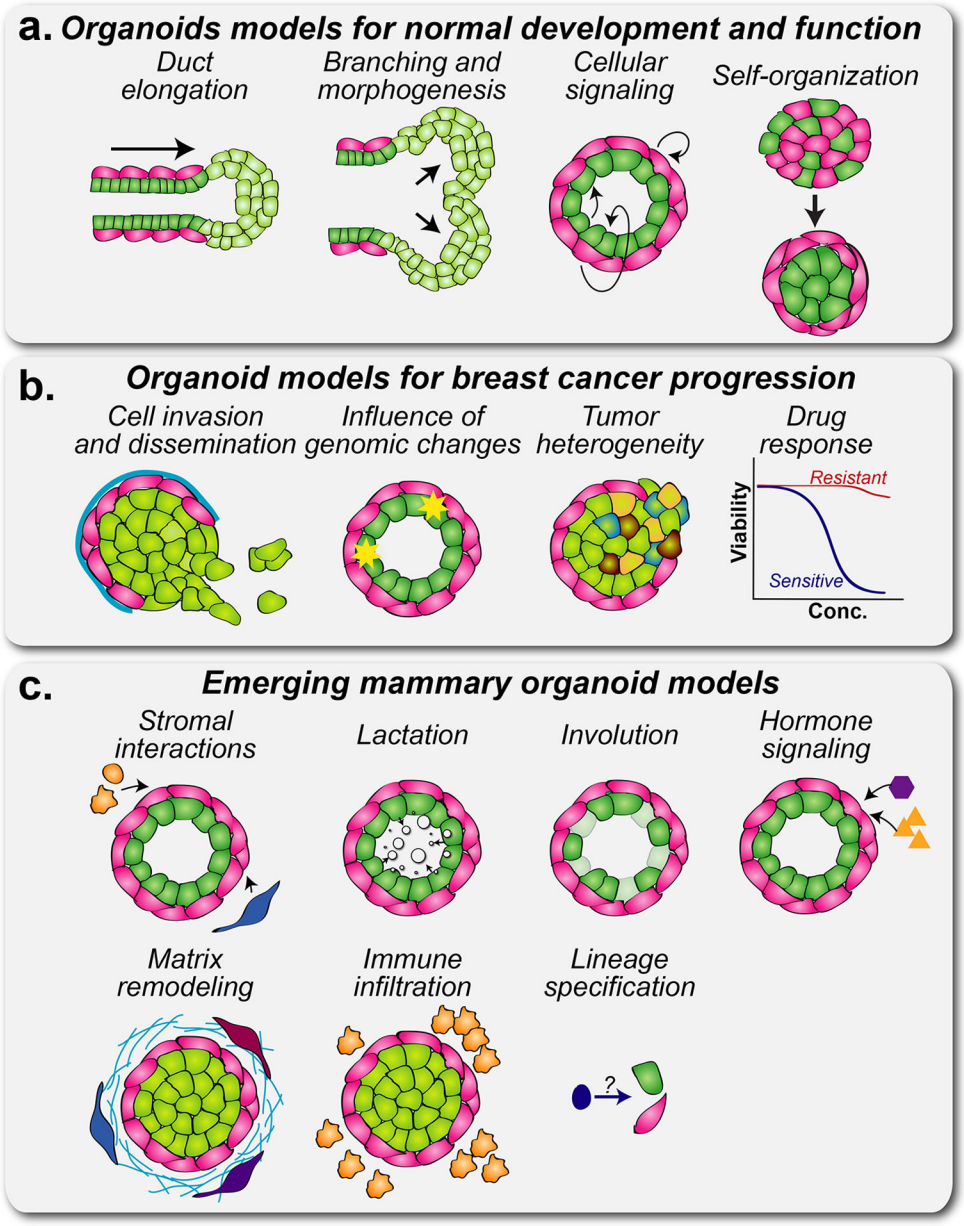

Figure 2:

Many organoid models of the mammary epithelium have been established to study the biology of the mammary gland, both in development and tissue homeostasis (**a**), as well as breast cancer progression (**b**). We also highlighted emerging models that could leverage the capabilities of organoid cultures to address active questions in the mammary field (**c**).

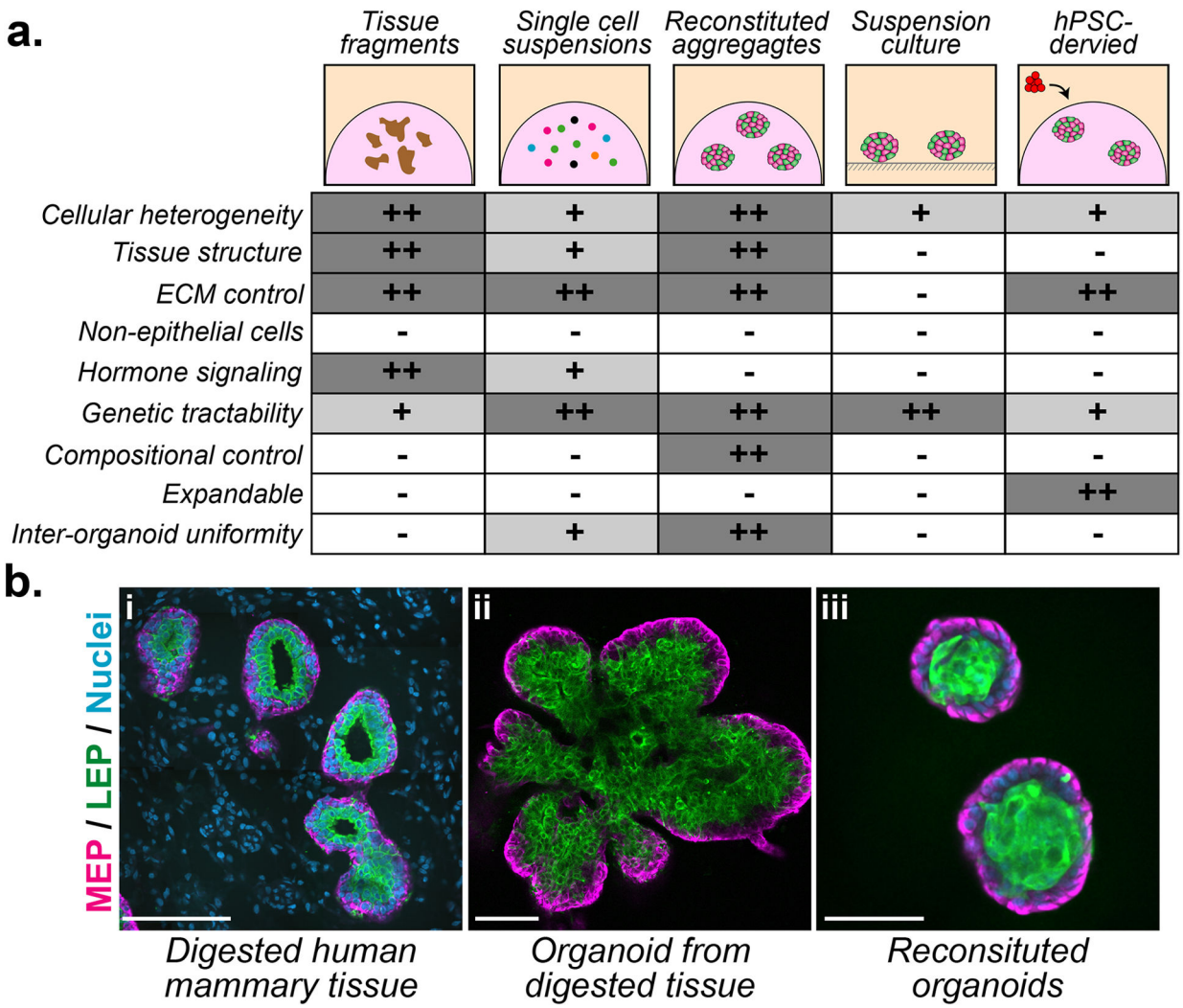

Figure 3:

a. Mammary organoids have been established from various tissue sources including human and murine primary tissue, breast tumors and stem cells, which are processed as tissue fragments, cellular aggregates or single cells. Each method for organoid preparation has its distinct benefits and limitations as summarized here, which must be considered when choosing the appropriate organoid model for the study. Darker colors represent higher similarity to the corresponding feature of the in vivo gland. **b.** Representative images of digested human mammary tissue **(i)**, organoids derived from tissue fragments **(ii)** and reconstituted organoids made by aggregating mammary epithelial cells **(iii)**. These methods have been commonly used for mammary organoid preparation. The tissue and organoids have been stained for luminal (Keratin-19) and myoepithelial (Keratin-14) markers to highlight incorporation of multiple cell types and maintenance of the in vivo-like bilayered tissue structure. Scale bar: 100 μm.