Modeling Human Mammary Epithelial Lineages Using Patient-Derived Organoid Cultures

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INTRODUCTION

Organoids have become a valuable tool for modeling both normal and tumor cell biology. However, culturing human breast epithelial cells—especially estrogen receptor (ER)-positive luminal cells—remains difficult. Current culture methods fail to reliably maintain luminal cells, which are believed to be the cells of origin for most breast cancers. To address this limitation, we have developed MammoCult™ Organoid Kit (Human), a modular culture system that supports the growth of multilineage organoids as well as lineage-restricted basal and luminal cell-derived organoids. The data presented here demonstrate that MammoCult™ Organoid Kit (Human) offers a robust, in vitro model system for studying human mammary gland biology.

METHODS

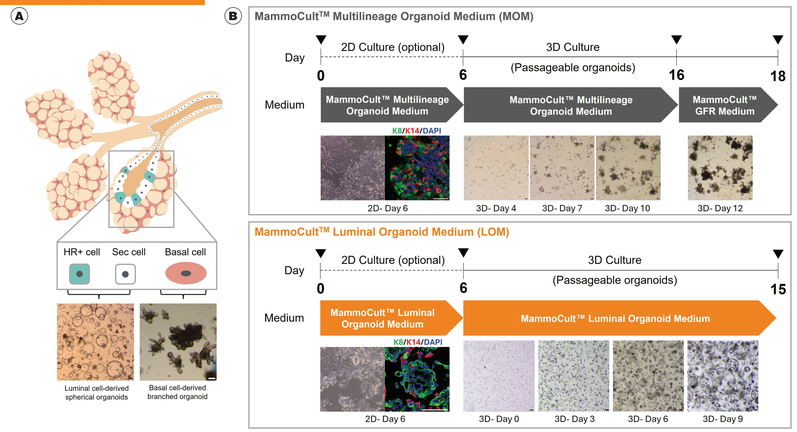


FIGURE 1. A Modular Medium Enables the Culture of Distinct Mammary Epithelial Cell Populations

(A) Illustration of the 3 lineages in the human mammary epithelium: hormone-responsive (HR), secretory (Sec), and basal (myoepithelial). By combining different basal media with different supplements, two formulations of the MammoCult™ Organoid Kit (Human) can be prepared: Multilineage Organoid Medium (MOM) and Luminal Organoid Medium (LOM). **(B)** Primary human breast cells from reduction mammoplasty tissues from both pre- and postmenopausal donors may optionally be seeded into collagen 1-coated 6-well plates and expanded in 2D culture in MammoCult™ MOM (top) or MammoCult™ LOM (bottom) for up to 2 passages. Cells may then be seeded into 3D organoid culture in 100 µL Matrigel® domes (with or without collagen 1) on non-tissue culture treated 24-well plates and grown in the same medium. Alternatively, freshly dissociated or cryopreserved human mammary cells may be seeded directly into 3D organoid culture and grown in either MammoCult™ MOM or MammoCult™ LOM. Organoids were serially passaged as single cells in MammoCult™ MOM for up to 2 passages (n = 3) or in MammoCult™ LOM for up to 5 passages (n = 3). To promote the formation of a lumen and lobules in branched organoids (top), the medium was switched on Day 10 to Growth Factor Reduced (GFR) MammoCult™ MOM. Scale bar = 100 µm; Keratin (K) 14 (red), K8 (green), DAPI (blue).

RESULTS

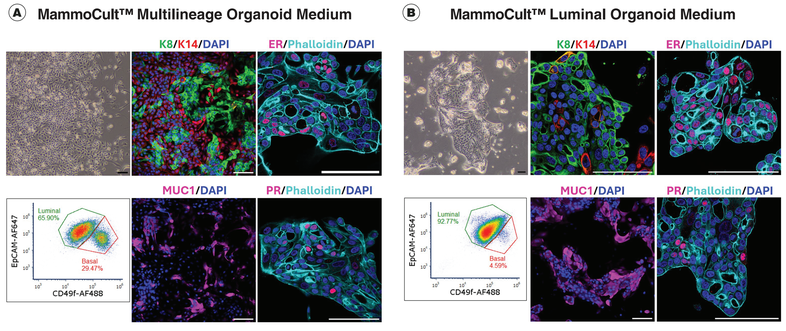


FIGURE 2. Expansion of Human Mammary Epithelial Cells in Adherent 2D Cell Culture

Representative light and fluorescence microscopy images showing 2D cultures of primary human breast epithelial cells on day 5 (passage 2). **(A)** Culturing cells in MammoCult™ MOM gave rise to a mixed population of K8-expressing luminal cells (green) and K14-expressing basal cells (red; n = 4). A representative flow cytometry plot of EpCAM vs CD49f confirmed the presence of the luminal and basal cell populations. Cells maintained the expression of estrogen receptor (ER), progesterone receptor (PR), and mucin 1 (MUC 1). **(B)** Culturing cells in MammoCult™ LOM positively selected for K8-expressing luminal cells growing in tightly packed clusters (n = 6). A flow cytometry plot of EpCAM vs CD49f revealed a predominantly luminal population. These cells also expressed ER, PR, and MUC1, consistent with a luminal cell identity. K14-expressing basal cells were rarely observed. DAPI (blue). Scale bars = 100 µm.

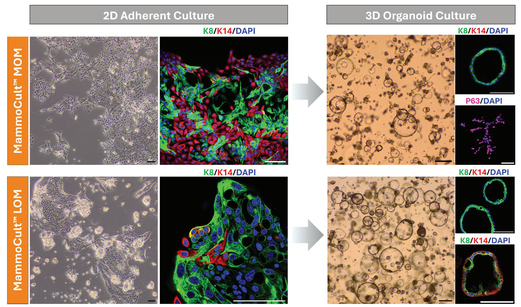


FIGURE 3. MammoCult™ Organoid Kit (Human) Supported a 2D to 3D Cell Culture Workflow

Human mammary epithelial cells were grown in 2D adherent cell culture to passage 2 in either MammoCult™ MOM (top) or LOM (bottom) and subsequently passaged into 3D organoid culture (right). Light and fluorescence microscopy images revealed distinct organoid morphologies reflective of their cell of origin; basal cells generated branched structures, whereas luminal cells generated hollow spherical structures (n = 4). Immunocytochemistry was performed to detect luminal markers MUC1 and K8 and basal markers K14 and P63. Scale bars = 100 µm.

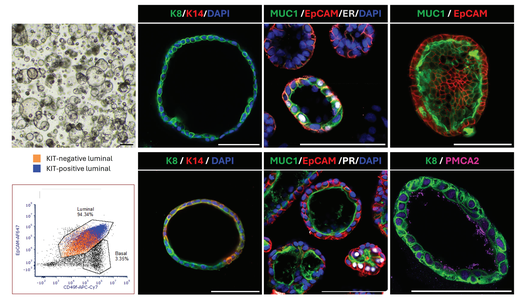


FIGURE 4. Luminal Cell-Derived Mammary Organoids Expressed Representative Luminal Cell-Lineage Specific Proteins

Pure luminal organoid cultures (passage 1) were generated from non-sorted primary human mammary epithelial cells seeded in MammoCult™ LOM. The majority of organoids stained positive for the luminal markers MUC1, K8, ER, PR, and PMCA2 (n = 6 - 12). The presence of secretory lineage luminal cells was confirmed by expression of KIT (n = 10) and, more rarely, by co-expression of both K8 and K14 (n = 3). Scale bars = 100 µm. DAPI (blue).

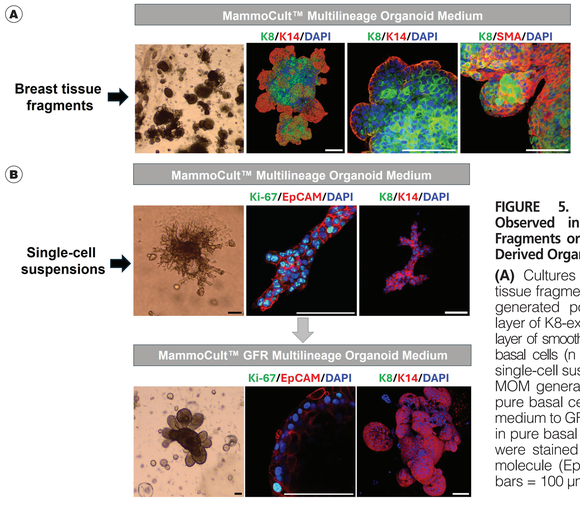


FIGURE 5. Multilineage Organoids Were Observed in Cultures Derived from Tissue Fragments or Cell Aggregates While Single-Cell Derived Organoids Remained Lineage-Restricted

(A) Cultures derived from dissociated breast tissue fragments seeded in MammoCult™ MOM generated polarized organoids with an inner layer of K8-expressing luminal cells and an outer layer of smooth muscle actin (SMA)/K14- expressing basal cells (n = 5). **(B)** Organoid cultures from single-cell suspensions seeded in MammoCult™ MOM generated a mixture of pure luminal and pure basal cell organoids. Changing the culture medium to GFR MOM promoted lumen formation in pure basal cell organoids (n = 10). Organoids were stained for Ki-67, epithelial cell adhesion molecule (EpCAM), K14, K8, and DAPI. Scale bars = 100 µm.

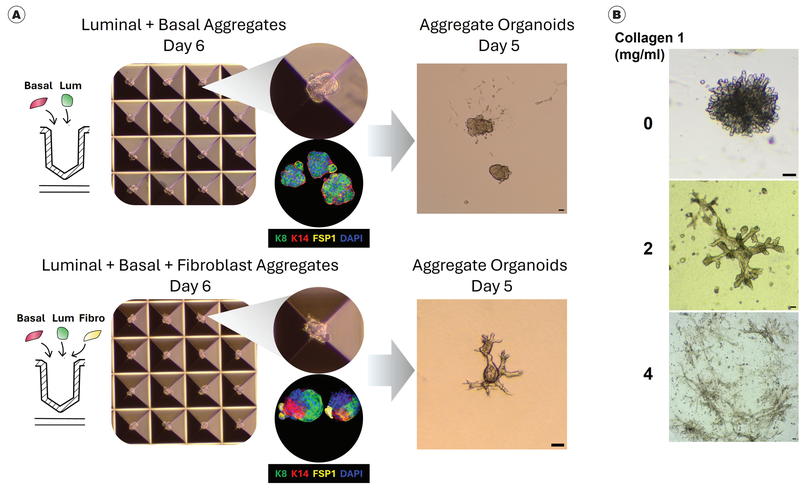


FIGURE 6. Branching Morphogenesis Was Dependent upon the Presence of Fibroblasts and Collagen 1

(A) Human mammary epithelial cells were sorted into three lineages and recombined into aggregates using AggreWell™400 Microwell 24-well Culture Plates. After 6 days in MammoCult™ MOM, aggregates were stained for K8, K14, and fibroblast specific protein 1 (FSP1). Upon transfer to organoid culture, aggregates containing fibroblasts displayed increased branching and ductal elongation, unlike aggregates lacking fibroblasts (n = 3). **(B)** Non-sorted mammary epithelial cells were seeded into 3D organoid culture in Matrigel® domes supplemented with increasing concentrations of collagen 1 (n = 3). The degree of organoid branching was proportional to the concentration of collagen 1. Scale bars = 100 µm.

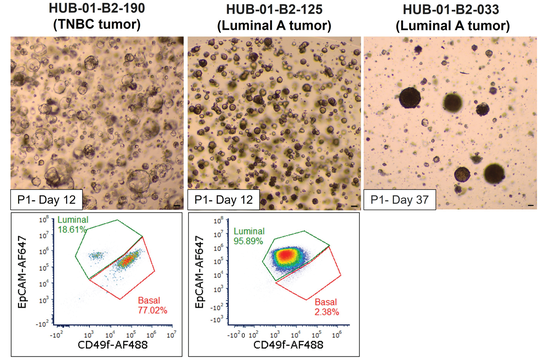


FIGURE 7. MammoCult™ Luminal Organoid Medium Supported the Growth of Breast Cancer Organoid Lines

Representative light microscopy images (top) of a triple-negative breast cancer (TNBC) and two luminal A tumor organoid lines that were propagated to passage 1 in MammoCult™ LOM. Flow cytometry plots (bottom) of EpCAM vs CD49f revealed a predominantly luminal population in the Luminal A tumor organoid culture and a predominantly basal population of cells in the TNBC tumor organoid, as would be expected for each respective tumor. Scale bars = 100 µm.

Summary

- MammoCult™ Organoid Kit (Human) is a modular kit that produces organoid morphologies specific to their cell of origin: basal cells form branched structures and luminal cells form hollow spheres.
- MammoCult™ Organoid Kit (Human) supports a 2D-to-3D cell culture workflow.
- MammoCult™ Luminal Organoid Medium overcomes the challenge of reliably expanding and maintaining estrogen receptor-positive (ER+) and progesterone receptor-positive (PR+) luminal cells in vitro, offering a powerful model for studying human mammary gland biology.
- Luminal Organoid Medium can support the growth of breast cancer organoid lines.

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