Establishing a long-term versatile culture system for functional mouse mammary organoids from single cells

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Abstract

The mammary gland serves as a pivotal model for studying stem cell dynamics and breast cancer, the most prevalent malignancy worldwide. Developing a long-term organoid culture system to study the normal physiology and pathophysiology of mammary glands in vitro is of paramount importance. However, current organoid systems lack the morphological and functional fidelity required to model its complex physiology. Here we present a detailed Protocol to establish a long-term, dynamic three-dimensional culture system for mouse mammary organoids, which we call 'mini-glands', that recapitulates in vivo morphogenesis and functional cycles. This method uses basal stem cells to generate organoids through sequential phases: sphere formation, polarity induction, symmetry breaking, branching morphogenesis and pseudoestrous cycle simulation. The resulting 'mini-glands' replicate the natural gland's branched architecture and undergo developmental stages mimicking puberty, pregnancy, lactation and involution. Furthermore, the system enables lineage tracing of cell fate transitions and oncogenic transformation studies via genetic manipulation. By bridging the gap between in vitro models and in vivo complexity, this platform advances studies in mammary gland biology, breast cancer initiation and therapeutic screening. The Protocol can be readily performed by researchers with basic experience in mammalian cell culture and requires no specialized instrumentation. A full culture cycle typically takes ~2 weeks to produce mature, highly branched 'mini-glands'.