Original Research Article
Mammary Gland Cell Culture of *Macaca fascicularis* as a Reservoir for Stem Cells

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Abstract
The mammary gland contains adult stem cells that are capable of self-renewal and are likely target for neoplastic transformation leading to breast cancer. In this study, we developed a cell culture derived from the mammary glands of cynomolgus monkeys (*Macaca fascicularis)* (MfMC) and furthermore identified the expression of markers for stemness and estrogen receptor-associated activities. We found that the primary culture can be successfully subcultured to at least 3 passages, primarily epithelial-like in morphology, the cultured cells remained heterogenous in phenotype as they expressed epithelial cell markers *CD24*, *CK18*, and marker for fibroblast *S1004A*. Importantly, the cell population also consistently expressed the markers of mammary stem cells (*ITGB1* or CD29 and *ITGA6* or CD49f), mesenchymal stem cells (*CD73* and *CD105*) and pluripotency (NANOG, OCT4, *SOX2*). In addition to this, the cells were also positive for Estrogen Receptor (ER), and ER-activated marker Trefoil Factor 1, suggesting an estrogen responsiveness of the culture model. These results indicate that our cell culture model is a reliable model for acquiring a population of cells with mammary stem cell properties and that these cultures may also serve as a reservoir from which more purified populations of stem cell populations can be isolated in the future.

Keywords breast cancer, mammary gland, nonhuman primate, stem cells

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Mammosphere Culture of Mammary Cells from Cynomolgus Macaques (*Macaca fascicularis*)

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Abstract

The mammary gland contains adult stem cells that are capable of self-renewal. Although these cells hold an important role in the biology and pathology of the breast, the studies of mammary stem cells are few due to the difficulty of acquiring and expanding undifferentiated adult stem cell populations. In this study, we developed mammosphere cultures from frozen mammary cells of nulliparous cynomolgus macaques (Macaca fascicularis) as a culture system to enrich mammary stem cells. Small samples of mammary tissues were collected by surgical biopsy; cells were cultured in epithelial cell growth medium and cryopreserved. Cryopreserved cells were cultured into mammospheres, and the expression of markers for stemness was evaluated by using quantitative PCR analysis. Cells were further differentiated by using 2D and 3D approaches to evaluate morphology and organoid budding, respectively. The study showed that mammosphere culture resulted in an increase in the expression of mammary stem cell markers with each passage. In contrast, markers for epithelial cells and pluripotency decreased across multiple passages. The 2D differentiation of the cells showed heterogeneous morphology, whereas 3D differentiation allowed for organoid formation. The results indicate that mammospheres can be successfully developed from frozen mammary cells derived from breast tissue collected from nulliparous cynomolgus macaques through surgical biopsy. Because mammosphere cultures allow for the enrichment of a mammary stem cell population, this refined method provides a model for the in vitro or ex vivo study of mammary stem cells.

Abbreviations: MaSC, mammary stem cells; qRT-PCR, quantitative real-time RT–PCR.

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