Mammosphere Culture Supports Short But Not Long-Term Propagation of Human Mammary Epithelial Progenitors

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Introduction

Investigation of mammary stem cells and their regulation has been greatly limited by a lack of methods to isolate and propagate these cells in vitro. Recently, evidence has demonstrated that primitive human mammary cells may be cultured in suspension as multicellular clusters, or “mammospheres” (1,2). Mammospheres were reported to include cells whose phenotypic and functional properties indicated their primitive bipotential origin. Presence of primitive cells has been detected over multiple generations of mammospheres, indicating the potential of this system to preserve cells capable of self-renewal. In this study we have looked at a dynamic change in the number of mammosphere-forming units, colony-forming cells (CFC) as well as relative distribution of progenitors (luminal, myoepithelial and bipotential) over multiple generations of mammospheres. The purpose of the study was to evaluate the capacity of this culture system to preserve primitive mammary epithelial progenitors.

Experimental Method

FIGURE 1: Media Evaluation and Long-Term Culture Analysis

Single cells obtained from dissociated reduced mammary gland samples were plated in mammosphere culture containing MammoCult® or control medium* and CFC assay in Epithelial medium to measure progenitor content within the start population. After 7 days mammospheres were dissociated, a fraction of the sample was used for CFC analysis while the rest of the suspension was used to generate new mammospheres. This step was repeated every 7 days.

* Control medium was prepared according to Demu et al. (3)

Results

FIGURE 2: Relative Numbers of Mammosphere-Forming Units and Colony-Forming Cells Detected in the Control Medium Compared to MammoCult®

Over two passages MammoCult® medium produced higher number of mammospheres containing higher progenitor content compared to the control medium, A) Mammosphere numbers obtained from the control medium were multiplied by 100%, B) CFCs detected in mammospheres generated in each passage (2 passages tested) in control medium were divided by the number of CFCs in mammospheres generated in each passage in MammoCult®.

Conclusions

- MammoCult® is a specialized, defined, serum-free medium that supports formation of mammospheres from single-cell suspensions enriched in human mammary epithelial cells.
- In MammoCult®, cells from dissociated human mammary gland samples form clusters of >60µm in diameter in culture at a frequency of 0.51 ± 0.29 (n=15).
- Cells (clonal analysis not shown) from dissociated mammospheres have the ability to differentiate in standard colony-forming cell assay conditions and generate colonies containing both lineages of mammary epithelial cells.
- To date, MammoCult® is the best medium for the culture of mammospheres and maintenance of mammary epithelial progenitors.

Select Publications:
2. Gineis et al. 2007, Cell Stem Cell: 35-48