Immortalization of Primary-Derived Human Salivary Stem/Progenitor Cells

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Objective: Patients receiving head and neck cancer treatment suffer from radiation-induced soft tissue damage, leading to a decrease in salivary flow detrimental to their oral health and overall quality of life. To overcome the limited lifetime of our primary-derived human salivary stem/progenitor cells (hS/PCs), we aim to immortalize cells from three patients. The goal of immortalization is to surpass the Hayflick Limit and avoid mechanisms that will result in replicative senescence and possible cell apoptosis. The Hayflick Limit relates to the number of replication cycles and is based on the gradual shortening of telomeric DNA. Immortalized cell lines are a powerful tool for biological, biochemical, and biological growth, differentiation, and aging studies.

Experimental Methods: Primary hS/PCs were isolated from the parotid gland of a 38 year-old female, expanded, and cultured to passage 4. Cells ($2x10^5$ cells/mL) were plated in 6-well plates in hS/PCs growth media. The day after plating, increased concentrations of G418 sulfate (0, 50, 100, 150, 200, 400, 500, 600, 1000 µg/mL) were added to the growth media to determine selection conditions. Every 48 hours, freshly prepared selection media was replaced, and cultured for up to 7 days. Cell growth and morphology were observed daily.

Results: After 7 days, the minimum antibiotic concentration of 100 µg/mL showed complete cell death. No differences in hS/PC cell morphology were observed, and uniform epithelial patterns remained.

Conclusions: Determining the minimum concentration of G418 sulfate is a crucial step before using a selection antibiotic to kill hS/PCs not transfected with Lenti-Myc T58A virus to generate stable cell lines. Successful generation of immortalized hS/PCs will give rise to a limitless cell source for salivary gland tissue engineering and stem cell therapy studies.

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