**PRIMARY AIRWAY EPITHELIAL CELLS THAT ARE CONDITIONALLY REPROGRAMMED MAINTAIN LINEAGE, PHENOTYPIC AND FUNCTIONAL CHARACTERISTICS**

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**Introduction:** The airway epithelium is critical in the disease pathogenesis of inflammatory airway diseases including asthma and cystic fibrosis (CF). Research progression in these areas is reliant on accurate and reflective modelling and primary airway epithelial cultures remain the gold standard However, advancements are restricted due to; limited access to airway sample, number of cells obtained and limited expansion capacity of primary cells.

**Aim:** To compare the current method of primary airway epithelial cell culture to a conditionally reprogrammed methodology utilising irradiated NIH-3T3 fibroblast feeder cells and medium containing ROCK inhibitor.

**Methods:** Primary epithelial cells from healthy, asthmatic and CF children were co-cultured with an irradiated fibroblast feeder cell in F-medium containing 10 µM ROCK inhibitor. Population doubling times, cell yield and passage longevity were compared to the group’s gold standard method of culture (Basal Epithelial Growth Medium; BEGM). Epithelial lineage was confirmed by qPCR and immunocytochemistry using cytokeratin (CK)-19 and vimentin. Phenotypic characterisation was assessed via terminal differentiation at air-liquid interface (ALI) and confocal analysis. Functional characteristics investigated included; wound repair capacity via scratch wound assays, ion transport via Ussing chamber and response to inflammatory stimulation via ELISA.

**Results:** Healthy and disease airway epithelial cells (AECs) that were conditionally reprogrammed were successfully established, expanded and maintained over at least 7 passages. Furthermore, a subset of cultures from all phenotypes were easily maintained beyond this. Archetypal cobblestone morphology was maintained over the conditional reprogramming period. Doubling times (n=4; BEGM 12.10 days ± 3.896; Co-culture 2.470 days ± 0.8376 p=0.0149), cell yield (n=4 BEGM 25,579 cells/cm2 ±6,858; Co-culture 68,532 cells/cm2 ± 23,643; p=0.0188) and passage longevity (n=4 BEGM 1.75 passages ± 0.9574; Co-culture 13 passages ± 6.976 p=0.0085) all improved under the conditional reprogramming methodology. Proliferation of these cells was also quicker and population doublings maintained for longer periods when compared to those grown in BEGM. Furthermore, epithelial cell-lineage was maintained throughout culture life in both healthy and disease cohorts. Cells that were conditionally reprogrammed successfully achieved terminal differentiation, had beating cilia and tight junction formation. CFTR function as measured by Ussing chamber was representative of both healthy and CF AECs. Scratch wound repair studies revealed an inability of asthmatic epithelial cells to fully repair. Finally, ELISA revealed an inflammatory signature pattern response in AECs derived from children with CF.

**Conclusions** The combination of F-medium and irradiated NIH-3T3 fibroblast feeder cells, conditionally reprogrammed AECs while maintaining lineage, phenotypic and functional characteristics.

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