Abstract must remain in Arial, 11 point font. Maximum 250 words for the abstract body and please retain the provided subheadings (Introduction & Aims; Methods etc.).

PERSONALIZED CELL CULTURE MODEL FOR HIGH-THROUGHPUT SCREENING FOLLOWING CFTR CORRECTION

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Introduction & Aims: Small molecules can correct CFTR production or function and greatly improve CF outcomes. Rarer mutations are hindered by the lack of appropriate primary culture models. Modifying primary airway epithelial cells (AECs) from children with CF modified to express a fluorescence marker that allows functional CFTR assessment would provide a culture-based high-throughput screening platform. We compared methodologies to achieve stable fluorescence in primary AEC.

Methods: Primary AECs were obtained from children (CF and non-CF) by bronchial brushing and cultured using a conditional reprogramming methodology. Cells were transfected with a pcDNA3.1 plasmid via Lipofectamine® or Nucleofector™ device. Alternatively, AEC were transduced by a replication-deficient retroviral vector with fluorescent tag. After 48 hours, cells expressing fluorescence were selected by fluorescence activated cell sorting (FACS) flow cytometry and then expanded further in culture to assess stability of gene expression.

Results: Primary AECs were amenable to all three methodologies. Transfection by Lipofectamine® was 24.1% efficient and the best electroporation protocol achieved a slightly higher efficiency (30.0%). Replication deficient retrovirus was most effective with 78.9% positive cells. There was no difference in transfection efficiency observed between non-CF and CF AECs. Cells could be successfully cultured after FACS selection, but only AEC transduced by retroviral vector maintained their fluorescence through two passages of cultures.

Conclusion: Retroviral vector could stably induce fluorescence into primary AECs. We are constructing a retroviral vector containing the YFP gene and validating the CFTR functional assay across a range of CFTR mutations.

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