Cystic fibrosis rescued by reprogramming the porosome secretory machinery

Based on:

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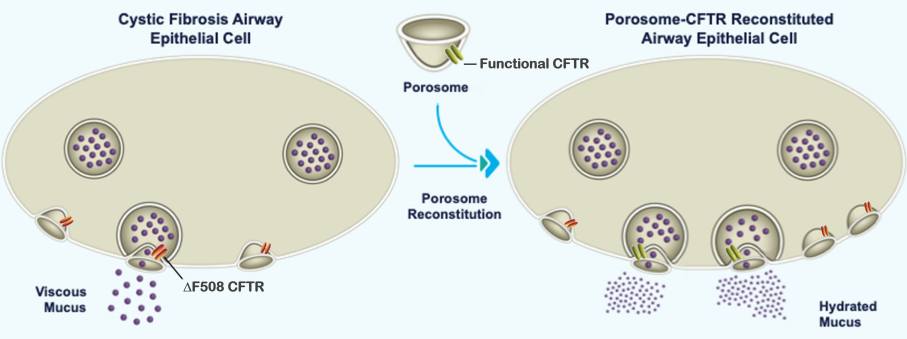
INTRODUCTION: Cystic fibrosis (CF) is a genetic disorder resulting from mutations in the CF Transmembrane Conductance Regulator (CFTR) gene that codes for a chloride transporting channel at the cell plasma membrane. In CF, highly viscous mucus is secreted in the airways, leading to lung infections and respiratory failure. A major challenge in treating all CF patients has been the presence of more than 2,000 different CFTR mutations, although the ΔF508 CFTR is the most common, accounting for approximately 70% of all CFTR mutations. CFTR is among the 34 proteins present in the 100 nm porosome secretory machinery, involved in mucin secretion in the human airway epithelia. Reconstitution of functional porosomes having normal CFTR, therefore holds great promise in treating CF with all different types of CFTR mutations.

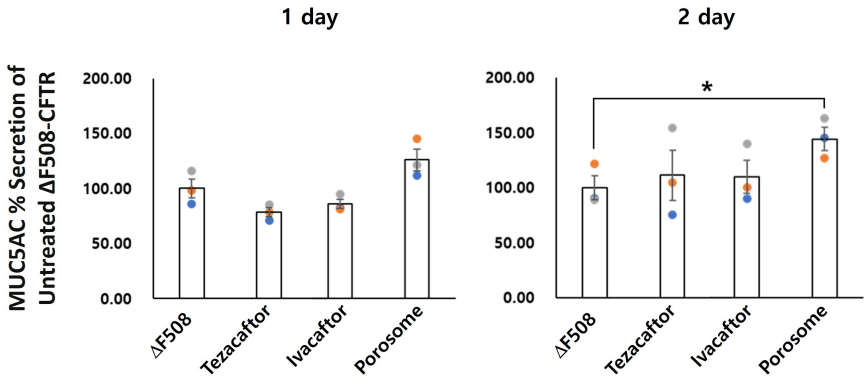
RATIONALE: Human airway epithelium is coated with a thin film of mucus, composed primarily of mucin MUC5AC and MUC5B. Sputum from patients with CF, show a 70% decrease in MUC5B and a 93% decrease in MUC5AC. Our studies using differentiated Calu-3 3D cultures of human airway epithelial, also demonstrate loss of both chloride and mucus secretion following exposure to both the thiazolidinone CFTR inhibitor 172 and the hydrazide CFTR inhibitor GlyH101. Mass spectrometry and Western Blot analysis of porosomes isolated from WT-CFTR Human Bronchial Epithelial (HBE) Cells and ΔF508-CFTR CF HBE cells, demonstrate a varying loss or gain of several porosome proteins in the ΔF508-CFTR CF HBE cells, including a decrease in the t-SNARE protein SNAP-23 and undetectable levels of the Ras GTPase activating like-protein IQGAP1. These results suggested that mutation in porosome-associated CFTR protein additionally affects other proteins within the porosome secretory machinery, negatively impacting mucus secretion. Hence, to ameliorate defects in mucus secretion in CF, the reconstitution of functional porosomes obtained from WT-CFTR HBE cells into the plasma membrane of ΔF508-CFTR mutant cells was performed.

RESULTS: Air liquid interface (ALI) 3D differentiated WT-CFTR and ΔF508-CFTR CF HBE cell cultures were established that mimick normal lung physiology, responding to CFTR inhibitors and CF corrector and modulator drugs Tezacaftor and Ivacaftor. Reconstitution of functional porosome complexes obtained from WT-CFTR HBE cells into the plasma membrane of ΔF508-CFTR CF cells demonstrate that porosome reconstitution restores mucin MUC5B and MUC5AC secretion approximately four-fold more effectively than the currently available CF drugs Tezacaftor and Ivacaftor. On average, porosome reconstitution was able to restore by >40% the secretion of MUC5B in ΔF508-CFTR cells. Similarly, in ΔF508-CFTR CF cells, a 9% and 11% increase in MUC5AC secretion is observed in the presence of Lvacaftor and Tezacaftor respectively, only on day 2 following exposure to the drugs, while porosome-reconstitution in the same period demonstrated a significant 43.8% increase ($P<0.05$), a near four-fold greater than the two CF drugs. These results show great promise of the porosome-reconstitution therapy in treating CF including all forms of CFTR mutations.

CONCLUSION: Our results reveal that porosome-reconstitution therapy is a powerful approach in treating all forms of CFTR mutations resulting in CF. Functional reconstitution of the porosome in live cells holds great promise for future therapeutic applications. Cellular

secretory defects resulting from impaired porosome functions could be overcome by reconstituting isolated porosomes from healthy tissue. Since the porosome is a nanoscale cellular structure present in all secretory cells, its reconstitution is unlikely to elicit an immune response and therefore in various secretory diseases could be used for therapeutic applications. Porosome reconstitution could be advantageous in optimizing the secretory capability of various tissue transplants. For example, a major issue in treating Type 1 Diabetes (T1D), using beta cells derived from induced pluripotent stem cells (iPSC), is the inability of these beta cells to optimally secrete insulin in response to a glucose challenge. Consequently, a large number of such iPSC-derived beta cells is required for a successful transplant in the treatment of T1D, which poses a significant clinical problem. This problem can now be overcome by reconstituting insulin-secreting porosomes into the cell plasma membrane of iPSC-derived beta cells prior to their transplant in patients. Porosome reconstitution holds promise in restoring normal secretion capability to secretion compromised cells.





Porosome-reconstitution therapy for cystic fibrosis. We functionally reconstituted normal CFTR porosomes obtained from WT-CFTR human bronchial epithelial (HBE) cells into differentiated 3D ΔF508-CFTR CF HBE cells in culture, potentiating mucus secretion and rescuing from cystic fibrosis. In ΔF508-CFTR CF cells, a 9% and 11% increase in MUC5AC secretion is observed in the presence of Lvacaftor and Tezacaftor respectively, only on day 2 following exposure to the drugs, while porosome-reconstitution in the same period demonstrated a significant 43.8% increase ($P<0.05$), a near four-fold greater than the two CF drugs.