Cystic fibrosis rescued using a reprogrammed porosome secretory machinery

Won Jin, Cho[1,2], Thomas J. Kelley[3], Bhanu P. Jena,[2,4*]

[1]Porosome Therapeutics, Inc., Boston, MA 02201, USA; [2]Viron Molecular Medicine Institute, Boston, MA 02201, USA; [3]Department of Genetics and Genome Sciences, Case Western Reserve University, Cleveland, OH 44106, USA, [4]Department of Physiology, School of Medicine, Wayne State University, Detroit, MI 48201, USA

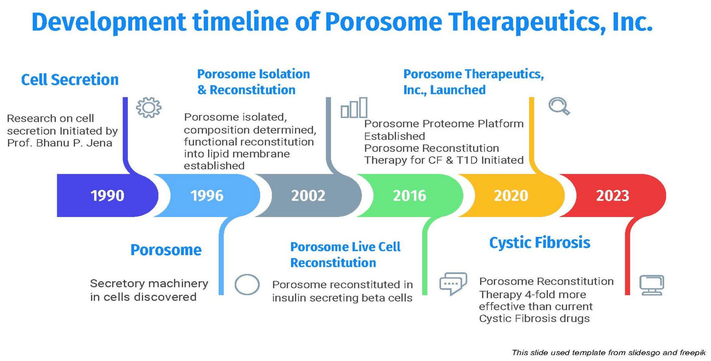
Introduction

Porosomes are cup-shaped lipoprotein structures at the cell plasma membrane, where secretory vesicles transiently dock and fuse to release intra-vesicular contents during secretion. Porosomes measure around 100-180 nm in endocrine and exocrine cells and 15 nm at the nerve terminal. Porosomes are composed of nearly 30 proteins. The porosome has been isolated and functionally reconstituted into lipid membrane and live cells. The principles discovered and described by Prof. Jena turned out to be universal, operating similarly in all animal cells. A number of human diseases are caused by mutations in some of the 30+ proteins composing the porosome complex. Jena's discovery of the porosome, in addition to providing a deep understanding of cell secretion, has contributed to the establishment of a drug development platform for the treatment of a wide range of diseases. Among the few examples of the therapeutic application of the porosome discovery, is the reconstitution of the porosome complex into CFTR-mutated cells that restores normal mucin secretion, and porosome reconstitution into stem cell derived insulin secreting beta cells, in the treatment of Type 1 diabetes. A recent Editorial elegantly summarizes the pioneering contributions of Prof. Jena, including his discovery of the porosome [https://static.s123-cdn-static-d.com/uploads/5744411/secure/norm al_6539c9b2a5bef.pdf].

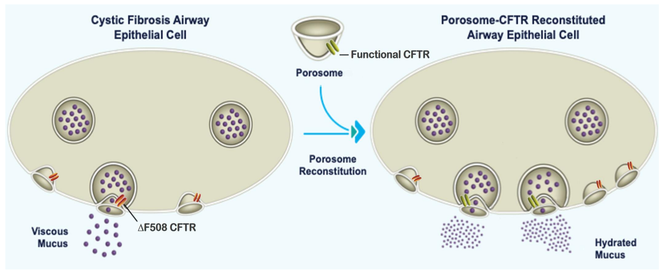
Cystic fibrosis (CF) is a genetic disorder resulting from mutations in the CF Transmembrane Conductance Regulator (CFTR) gene that codes for a chloride transporting channel at the cell plasma membrane. In CF, highly viscous mucus is secreted in the airways, leading to lung infections and respiratory 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.

*Correspondence: bjena@med.wayne.edu (B.P.J)

Porosome Therapeutics Inc., History



Porosome Reconstitution



Porosome Therapeutics, Inc.

Methods

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 cells, 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

Studies Using Human Bronchial Epithelial 3D Cell Cultures: Air liquid interface (ALI) 3D differentiated WT-CFTR and ΔF508-CFTR CF HBE cell cultures were established that mimics 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 Ivacaftor 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.

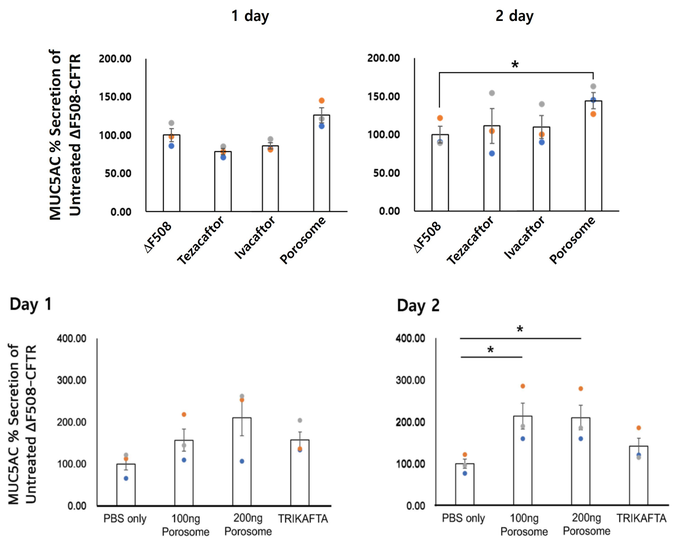


Figure (Above): Porosome-reconstitution therapy for cystic fibrosis. We functionally reconstituted normal CFTR porosomes obtained from WT-CFTR human bronchial epithelial (HBE) cells into differentiated 3D ΔF508-CFTR CF HBE cells in culture, potentiating mucus secretion and rescuing from cystic fibrosis. In ΔF508-CFTR CF cells, a 9% and 11% increase in MUC5AC secretion is observed in the presence of Ivacaftor 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. In ΔF508-CFTR CF cells, a significant ($P<0.05$) and much greater increase (>2-fold over TRIKAFTA) in MUC5AC secretion is observed following porosome-reconstitution, compared to TRIKAFTA in the same period.

Animal Studies: Porosome reconstitution experiments were conducted using a B6.129 *Cftr*^{tm1Kth}Tg(FABPCFTR)1Jaw/Cwr mice. They are a ΔF508 line backcrossed onto a C57bl/6 background strain. The mice strain express CFTR in the gut driven by the FABP promoter to avoid intestinal blockage. Porosomes obtained from WT-CFTR HBE cells were used to treat the nasal passage of the CF mice and the vehicle (PBS pH7.5) alone was used as control. Experimental animals were treated using four micrograms of the isolated porosome complexes in PBS pH 7.5 using a nasal catheter and nasal potential difference (PD) was measured after 24h post porosome reconstitution, to determine CFTR ion channel activity. In this experiment, nasal potential difference is used to measure the voltage across the nasal epithelium, which results from trans-epithelial ion transport reflecting in part, CFTR function. Results from even this cross-species reconstitution experiment are promising, showing a clear positive effect following reconstitution of porosomes with functional CFTR.

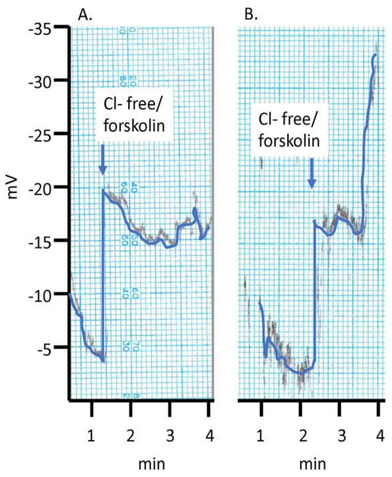


Figure (Left): Nasal potential difference assay following **porosome reconstitution in nasal passage of CF mice.** Porosome obtained from WT-CFTR HBE cells rescues chloride secretion in nasal passage of CF mice**.** Nasal potential difference assay of mice treated with carrier **(A)** or 4 µg porosome **(B)** for two consecutive days. Mice were analyzed 24 hours after the last treatment. Mice are begun in amiloride containing Ringer's to block sodium transport. Mice are then switched to chloride-free Ringer's with forskolin (10 µM) and amiloride (10 µM) to drive chloride secretion. Upward inflection indicates CFTR function.

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 could be used in therapeutic applications for various secretory diseases. This is evident from our studies in mice, where even in a cross-species experiment, porosome reconstitution was successful. 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.

References

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