29 Attempts of facilitated trafficking ΔF508-CFTR to the plasma membrane

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Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations of the Cystic Fibrosis transmembrane conductance regulator protein (CFTR), a cAMP-regulated chloride channel. One of the most common mutation of CFTR is the deletion of phenylalanine in 508 position (ΔF508-CFTR). This mutation induces small conformational change hence CFTR trafficking is no more effective due to its rapid degradation by means of chaperon machinery. In CF airways, abnormal epithelial ion transport mainly initiates mucus stasis resulting in infections. In this study we tried to (1) understand intracellular trafficking of CFTR and ΔF508-CFTR, (2) facilitate transport of ΔF508-CFTR by means of relieving it from degradation and (3) create assay for the robotized high-throughput drugs screening. Using broad spectrum of methods from recombinant DNA and immunocitochemistry to electron microscopy, we identified main ethiologic mechanisms of cystic fibrosis. Our data demonstrate what we believe is possible to find a small molecule (adaptors and potentiators) for facilitation of ΔF508-CFTR trafficking to the plasma membrane.
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31 Rescue of deltaF508 CFTR in CFBE41o- cells is dependent on actin cytoskeleton interaction with ezrin and NHERF1

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Confocal microscopy demonstrated that polarized monolayers of the normal 16HBE14o- airway cells displayed well-organized actin stress fibers at the apical surface, while the CFBE41o- cell monolayers displayed substantial disassembly of actin filaments. This result was confirmed by an actin polymerization assay in which F-actin content is higher in normal cells than in CF cells. Moreover, the disruption of actin filament organization by cytochalasin D treatment, strongly decreased both F-actin content and CFTR activity in 16HBE14o- cells while having no effect in CFBE41o- cells demonstrating that CF cells have a non-organized actin cytoskeleton and suggests that reduced CFTR activity in CF cells could be linked to their reduced F-actin content.

To understand if NHERF1, a scaffolding protein organizing apical membrane proteins into regulatory complexes, is involved in organizing the actin cytoskeleton, we transfected both cell lines with NHERF1. NHERF1 over-expression increased F-actin levels in both cell lines. Interestingly, confocal analysis of the CF cells over-expressing NHERF1 showed a reorganized actin cytoskeleton. In addition, in 16HBE14o- cells transfection with either NHERF1 truncated of ERM domain or with the dominant negative of ezrin caused reduction of both the CFTR-dependent chloride efflux and the F-actin content suggesting that an organized cytoskeleton is critical for apical membrane CFTR expression and/or CFTR activity and that NHERF1 and ezrin interactions have an essential role in regulating these mechanisms through the cytoskeleton.
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32* Pro-inflammatory effects of sodium 4-phenylbutyrate in CF lung epithelial cells containing F508del-CFTR

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Sodium 4-phenylbutyrate (4-PBA) is a butyrate analogue, a chemical chaperone used in cancers and cystic fibrosis (CF) disease. In CF, 4-PBA has been reported to correct the F508del-CFTR trafficking defect and to restore CFTR function at the plasma membrane of CF lung epithelial cells in vitro and in vivo. The aim of our study was to gain insights into the potential effects of 4-PBA on the inflammatory response in CF lung epithelial cells. With two CF bronchial epithelial cell types (CFBE41o- and IB3–1 cell lines with F508del-homozygous and heterozygous genotype, respectively), we clearly demonstrated that 4-PBA induced a strong increase of two pro-inflammatory cytokines IL-8 and IL-6 (at both the levels of mRNA and protein expression) whereas no significant variation of IL-1β and TNF-α was found. Surprisingly, the NF-κB transcriptional activity was decreased after treatment of the two CF epithelial cell lines with 4-PBA alone or in combination with 10 ng/ml TNF-α. These data prompted us to investigate other potential pathways controlling the level of IL-8 production after 4-PBA treatment. In presence of 4-PBA, IL-8 secretion is blocked by two different inhibitors of ERK1/2 MAPK signalling pathway contrary to the specific inhibitor of p38 MAPK signalling.

These results suggested that 4-PBA mediated IL-8 expression is controlled by ERK1/2 pathway in an NF-κB-independent manner. Our data suggest that, in CF, treatment with 4-PBA aimed at restoring CFTR function should be combined with ERK inhibitors to prevent increased inflammation.
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33* Optical imaging of calcium-evoked fluid secretion by murine airway submucosal gland serous acinar cells

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Airway submucosal glands express CFTR and contribute to lung fluid homeostasis, but the mechanisms of gland ion and fluid transport are poorly defined. Submucosal gland serous acinar cells were isolated from murine airway and used in physiology studies. Stimulation of isolated cells with carbachol (CCh), histamine or ATP caused marked decreases in cell volume (20±2%) that were tightly correlated with increased cytoplasmic calcium concentration ($[Ca^{2+}]_i$) as revealed by simultaneous DIC and fluorescent indicator dye microscopy. Simultaneous imaging of cell volume and the Cl^--sensitive fluorophore SPQ showed that shrinkage was associated with a fall of $[Cl^-]_i$ from 65 to 28 mM, reflecting loss of 67% of cell Cl^- content, accompanied by parallel efflux of K^+. Upon agonist removal, $[Ca^{2+}]_i$ relaxed and the cells swelled back to resting volume via a bumetanide-sensitive Cl^- influx pathway, likely NKCC1. Accordingly, agonist-induced serous acinar cell shrinkage and swelling are caused by activation of solute efflux and influx pathways, respectively, and cell volume reflects the secretory state of these cells. In contrast, elevation of cAMP failed to elicit volume responses, or enhance those induced by sub-maximal [CCh]. Cells from mice that lacked CFTR, as well as wild-type cells treated with a CFTR inhibitor, exhibited identical rates and magnitudes of shrinkage and Cl^- efflux compared with control cells. These results provide insights into the mechanisms of fluid secretion by submucosal glands, and suggest that while murine gland fluid secretion in response to cholinergic stimulation can originate from CFTR-expressing acinar cells, it is not dependent upon CFTR function.
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