53 SiRNA glucosidase promotes endogenous F508del-CFTR trafficking

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The most common mutation in cystic fibrosis (CF), F508del, results in CFTR (CF transmembrane conductance regulator) protein that is retained in the endoplasmic reticulum and degraded via the proteasome. Previously, we have shown that miglustat, an inhibitor of the α-1,2-glucosidase, corrects the trafficking of F508del-CFTR and hypothesized that by inhibiting the interaction of F508del-CFTR with calnexin, miglustat prevents its retention and degradation (Norez et al., 2006). The purpose of this study was to verify our hypothesis by i) determining the effect of Small interfering RNA (SiRNA) glucosidase transfection on endogenous F508del-CFTR trafficking and ii) studing the effect of siRNA glucosidase transfection on the interaction of F508del-CFTR with calnexin.

SiRNA glucosidase transfection was done on the CF-KM4 tracheal cell line to inhibit expression of glucosidase. The level of glucosidase expression was verified by biochemical technique and consequences on CFTR and ENaC activities were assessed using single-cell fluorescence imaging. The time-dependence effect of transfection revealed a maximum transfection efficiency for 0.5 μg/ml of siRNA at 72 hours. We demonstrated that transfection of siRNA glucosidase significantly knocked down glucosidase expression and resulted in a rescue of F508del-CFTR activity at the plasma membrane and a decrease of ENaC activity.

In conclusion, this work shows that siRNA glucosidase rescues F508del-CFTR activity at the plasma membrane and decreased ENaC activity. Next step will be to verify if transfection with siRNA glucosidase inhibits the interaction of F508del-CFTR with calnexin.

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54* Pharmacological stabilization of ΔF508-CFTR at the cell surface: design of CAL-selective PDZ inhibitors

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The chloride channel mutated in cystic fibrosis, CFTR, suffers from several major defects that reduce its activity including a shortened surface half-life. Several PDZ domain-containing proteins appear to regulate the trafficking and activity of CFTR, including Na^+/H^+ Exchanger-3 Regulatory Factors 1, 2 and 3 (NHERF1,2,3), which increase CFTR-mediated chloride efflux whereas association with CFTR-associated ligand (CAL) limits cell-surface levels of the disease-associated mutant ΔF508-CFTR. RNAi-mediated knockdown of CAL increases CFTR surface expression and chloride efflux by enhancing the channel's stability at the apical surface, demonstrating that CAL is a novel target for enhancing the CFTR half-life. However, given the bi-directional promiscuity of the CAL:CFTR interaction, a more direct test of this hypothesis requires inhibitors that can block the CAL:CFTR interaction specifically and with high affinity. Using fluorescence polarization assays and peptide array technology, we have identified a peptide inhibitor that is selective for CAL over NHERF1, 2 and 3. *In vitro* pull-down assays and mass spectrometry analysis indicate this peptide inhibitor is potent and selective for endogenous CAL. Treatment of CFBE41o− airway epithelial cells with this peptide results in an increase in ΔF508-CFTR activity. To identify pharmacological reagents that recapitulate the effects of peptide inhibitors, we have now developed a high-throughput screening platform utilizing fluorescence detection and NMR heteronuclear single quantum coherence (HSQC) spectroscopy.

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55* ΔF508-CFTR and ENaC association

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An imbalanced ion transport in several epithelia is a feature of CF, an inherited disease as a consequence of mutations in the *cftr* gene. The *cftr* gene codes for a Cl^- channel. With some mutations in this gene, the balance between Cl^- secretion and Na^+ absorption is disturbed in airways; Cl^- secretion is impaired while Na^+ absorption is elevated. In this study we have examined the association of ENaC subunits with mutated ΔF508-CFTR, the most common mutation in CF. ΔF508 mutation prevents proper processing and targeting of CFTR to the plasma membrane. When ΔF508 and ENaC subunits were coexpressed, we found that ENaC subunits could be co-immunoprecipitated with ΔF508. Additionally, we evaluated the ΔF508 and ENaC association by fluorescence resonance energy transfer imaging (FRET). FRET exploits the exquisite sensitivity of fluorescence measurements to detect molecular complexes with near angstrom resolution. FRET efficiencies were not significantly different from negative controls; these FRET results do not place ΔF508 and ENaC in close proximity to each other. Even though our biochemical findings are suggestive of an interaction (that could be direct or indirect) between the mutated CFTR with ENaC subunits, these FRET data speak to the possibility that the ΔF508 mutation might prevent a functional link between ΔF508 and ENaC. This association most likely takes place in the ER; however, ENaC on the cell surface may not be affected by the regulatory influence of mutated CFTR, and thus exhibits an excessive activity.

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56 Molecular characterization of a CAVD-causing mutation − V1108L *CFTR*

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The aim of this study was to analyze the mechanism by which the new *CFTR* V1108L mutation identified in a CAVD patient, disrupt CFTR function and therefore cause male infertility. The V1108L mutation was generated by site directed mutagenesis in a eukaryotic expression vector containing the wt CFTR cDNA. HEK 293 cell line was transiently transfected with the CFTR vector construct containing the V1108L mutation, as well as with the pCMVCFTRNot6.2 (wt CFTR) and pCMVCFTRNot6.2DF508 (F508del CFTR) plasmids as controls. To assess the effect of the V1108L mutation in CFTR processing and intracellular trafficking, V1108L-CFTR protein was analyzed by Western blotting and immunofluorescence, respectively. Our results suggested that V1108L mutation, leads to a CFTR protein that is correctly processed and trafficked to the cell surface and hence is likely not to cause a drastic defect on CFTR biosynthetic pathway. However, as indicated by densitometric analysis, the ratio of mature fully-glycosylated to immature core-glycosylated bands detected for V1108L-CFTR appeared to be lower than those obtained for wt CFTR, indicating that V1108L mutation may cause dysfunction of CFTR by decreasing the total amount of mature protein that reach the cell surface. As the relative impact of CFTR genotype on clinical phenotype is organ specific, the reduced amount of V1108L-CFTR that reach the cell, though small, as suggested by the immunofluorescence analysis, may affect the development of the vas deferens, leading the CAVD phenotype observed in our patient. Thus, based in our results and bioinformatic tools, the V1108L mutation may be classified as being associated with CFTR-related disorders.