

Correction of the CFTR Chloride channel defect in Cystic Fibrosis Airway Cells

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Introduction: Cystic Fibrosis is a genetic disease that affects approximately 1 in 2000 newborns in the Caucasian population. It is a lethal disorder caused by mutations in the CF Transmembrane Conductance Regulator (CFTR) gene. The most common mutation (known as $\Delta F508$) is a three base pair deletion that results in the loss of phenylalanine at position 508 of the CFTR protein. In this mutation, the epithelial cells from places such as the lungs, sweat glands, intestines, reproductive tract and pancreas have impaired chloride transport that results in thick, sticky mucus secretions. Normally the CFTR protein would function as a cyclic AMP regulated chloride ion channel that controls movement of fluid and salts into the airways. In cells with this $\Delta F508$ mutation the trafficking of the $\Delta F508$ -CFTR protein from the endoplasmic reticulum to the plasma membrane is impaired when compared to cells containing the wild-type (WT) CFTR. **Objective:** Our studies attempt to identify how the degree of CFTR expression correlates to CFTR chloride ion transport. Furthermore, we are looking at the differences between cell lines in which the 4.7kB open reading frame (ORF) of the CFTR gene was introduced, and those in which the 6.2kB entire reading frame was introduced which is comprised of not only the ORF, but also the 3' and 5' untranslated regions. **Methods:** For experimentation CF cell lines have been complemented with a WT-CFTR and $\Delta F508$ -CFTR cDNA using an episomal expression vector (pCEP4) in an attempt to reverse the chloride ion transport defect. WT-CFTR cDNA containing either the open reading frame (ORF) or the entire CFTR cDNA (4.7 or 6.2 Kb, respectively) were introduced in the pCEP4 vector and transfected into CF airway cell lines carrying mutations in the CFTR gene with genotypes of $\Delta F508/\Delta F508$ or $\Delta F508/2QX$. Two cell lines were used: the CFSMEo- ($\Delta F508.Q2X$) derived from airway submucosal gland tissue isolates and the CFBE41o- ($\Delta F508/\Delta F508$) derived from bronchial tissue isolates. We used the Ussing chamber assay to monitor the degree of cyclic-AMP dependent chloride secretion across the apical membrane. The magnitudes of the cyclic AMP stimulated and the glibenclamide-blocked chloride currents were measured. With this, we can then compare the degree of CFTR-mediated Cl⁻ chloride transport in each of the different cell lines to see what cell lines have been successfully transfected. **Results:** Two of five cultures taken from the 6.2kB construct of the CFBE41o- cell line demonstrated a significant cyclic-AMP dependent response. The forskolin-stimulated short circuit current averaged $16 \pm 3 \mu A/cm^2$ (n=8) and $33 \pm 4 \mu A/cm^2$ (n=6) for clone #7 and #10 respectively. A small, but detectable response was found for the $\Delta F508$ construct, which averaged $5 \pm 5 \mu A/cm^2$ (n=11). The experiments on the 4.7kB construct of CFSMEo- cells showed a smaller response. **Conclusion:** The data from this project suggests that the 6.2kB construct does generate a higher chloride secretory response than the 4.7kB construct. Over-expression of $\Delta F508$ restores mutant CFTR function to 25% of chloride secretion observed in normal human airways.