INVESTIGATING CFTR CDNA MODIFICATIONS AS TOOLS FOR GENE THERAPY

Woodall, M.1; Tarran, R.2; Hart, S.3; Baines, D.L.1 1. St. George’s University, London, London, United Kingdom; 2. University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; 3. UCL, Great Ormond Street Institute of Child Health, London, London, United Kingdom

Class I CFTR mutations cause severe CF lung disease and occur in ~10% of CF patients. These types of CFTR mutations are untreatable with pharmaceuticals. However, genetic therapy strategies offer a potential cure for all patients. Currently these techniques are low efficiency and have not translated into improved lung function in vivo. CFTR cDNA employed in CF gene therapy clinical trials has been modified by both CpG deletion, to reduce immunogenicity, and codon optimisation, to increase CFTR protein production. Other modifications of CFTR, including E1371Q, K978C and P335A, have been characterised in cell lines and shown to have superior channel open probability to wild-type (WT)-CFTR. To date, functionality of these modified forms of CFTR has not been studied in a human primary epithelial cell model. In this study, I investigated the function of different CFTR cDNAs as candidates for gene therapy: codon-optimised CFTR (hCAI), increased open-probability CFTR mutant (K978C) and codon-opti-mised CFTR with K978C mutation (h^K978C). The hypothesis is that lenti-viral transduction of CFTR cDNAs that increase protein abundance and/or activity will more effectively restore function to CF bronchial epithelia (CFBE) than WT-CFTR. Transient transfection in HEK293T cells showed hCAI and h^K978C produced more (10-fold) CFTR protein than WT or K978C (p0.05; n=3). Use of the halide-sensitive YFP quenching assay in HEK293T cells showed hCAI and h^K978C also displayed greater anion transport (>4-fold) than WT (p<0.05; n=3). Potentiation of hCAI with VX-770 increased transport to levels observed in h^K978C, indicating a similar mechanism of potenti-ation. However, functionality of the modified CFTR cDNAs expressed in CFBE were profoundly different. K978C produced greater (>2-fold) airway surface liquid heights and CFTR-dependent Cl- transport than WT, whilst hCAI and h^K978C had limited impact on functional readouts (p>0.05; n=6-8 from 3 CF donors). Further investigation provided evidence that hCAI localised to the basolateral membrane as well as the apical membrane in CFBE, preventing vectoral Cl- transport, whilst h^K978C protein production and transport was inhibited. These findings highlight the importance of measuring function in physiologically relevant models to determine efficacy of modified CFTR cDNAs for gene therapy. For the first time, these data provide evidence that codon-optimised CFTR cDNA may be unsuitable for some gene therapy practices that employ high-activity promoters. However, the use of K978C cDNA in genetic therapies may allow for more potent recovery of function in CF lung disease than WT cDNA.