Aims: 1. To optimise lipid/peptide-based delivery system for CF cells
2. To deliver CFTR IVT mRNA for therapy in CF

Hypothesis: CFTR IVT mRNA transfections induce CFTR protein expression in CF cells

Introduction

Cystic Fibrosis (CF) is an autosomal recessive disease, which affects approximately 10,000 people in the UK. CF is caused by mutations in the gene encoding CFTR (the cystic fibrosis transmembrane conductance regulator). Mutations on the gene result in absent or non-functional CFTR, which leads to imbalance of sodium, chloride and bicarbonate ion movement. The mucus becomes very sticky and cilia stop beating. Consequently, it causes chronic bacterial infection and inflammation with loss of lung functions.

mRNA and plasmid DNA or viral vector

In vitro transcribed mRNA (IVT mRNA) has emerged as an alternative nucleic acid to plDNA for protein replacement. It is required in the cytoplasm for expression, not nucleus, therefore, expression is more efficient than DNA. Furthermore, it induces less inflammation, and is safer as it is transient and cannot integrate.

Methods

-cRNA: CleanCap Cy5-GFP mRNA (5Umol) and CleanCap Fluc mRNA (5Umol) (Trilink) were transfected into Human Cystic Fibrosis Bronchial Epithelial (CFBE) BMI-1 cells (F508 mutation) using three cationic liposomes and 5 cationic receptor-targeting peptides

Result 1

Luciferase and GFP mRNA transfection (1921 nucleotides) to CFBE BMI-1 at submerged culture

- Peptide E achieved higher transfection efficiency among the three liposomes
- The transfection efficiency of the three liposomes were similar to each other
- The peak of the luciferase activity was at 8-24 hour time point

Result 3

- Co-delivery of CFTR mRNA and VX809 in CFBE BMI-1
  - VX809 was packaged in C18DOPE or C14DOPE/E-CFTR mRNA
  - Compared with mRNA nanocomplexes with VX809 (VX809 was added into transfection medium)

Discussion & Further study

- IVT mRNA was efficiently transfected to CFBE BMI-1 cells
- The protein expression by transfected IVT mRNA reached 8-24 hours, and decreased during 24 to 48 hours. (dependent on the IVT mRNA and the protein)
- CFTR IVT mRNA successfully induced CFTR protein in CFBE BMI-1 cells in both submerged and ALI culture
- CFTR mRNA were also transfected in mouse lungs
- Co-delivery of VX809 and CFTR mRNA enhanced Cor stabilised CFTR mRNA protein expression
- It is necessary to assess the function of CFTR induced by the IVT mRNA

References

Tajer et al. 2015 Cystic Fibrosis
Yu Wai Man et al. 2016 Receptor targeted liposome peptide mRNA nanoparticles represent an efficient delivery system for MRST silencing in conjunctival fibrosis