Development of *In Vitro* Transcribed mRNA Therapeutics for Cystic Fibrosis

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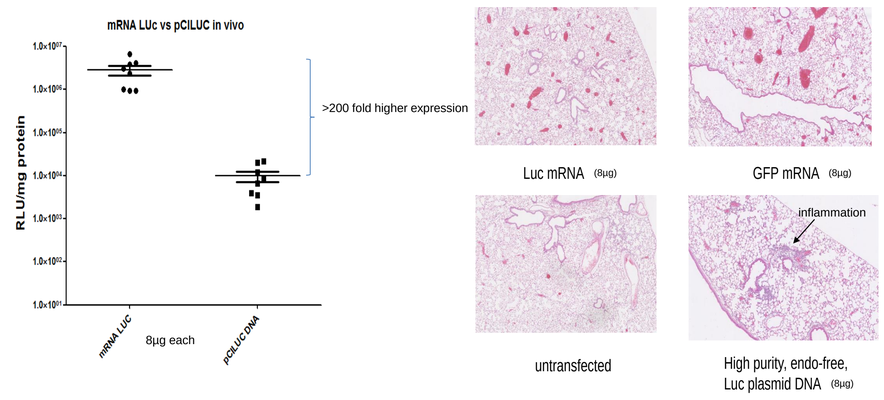
Aims: 1.To optimise lipid/peptide-based delivery system for CF cells
2.To deliver CFTR IVT mRNA for therapy in CF
Hypothesis: CFTR IVT mRNA transfections induce CFTR protein expression in CF cells

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 **c**ystic **f**ibrosis **t**ransmembrane conductance **r**egulator). 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.

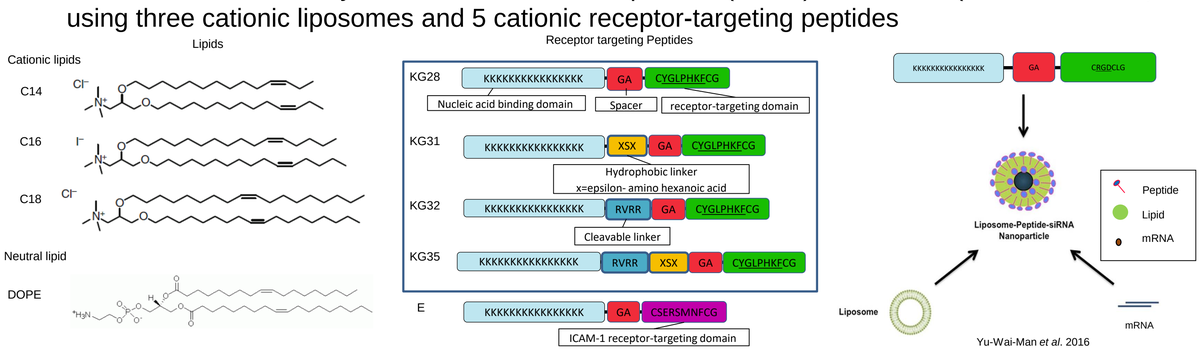
mRNA and plasmid DNA or viral vector

In vitro transcribed mRNA (IVT mRNA) has emerged as an alternative nucleic acid to pDNA 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

-mRNA: CleanCap Cy5-GFP mRNA (5Umol) and CleanCap Fluc mRNA (5Umol) (TriLink) were transfected to Human Cystic Fibrosis Bronchial Epithelial (CFBE) BMI-1 cells (ΔF508 mutation) using three cationic liposomes and 5 cationic receptor-targeting peptides

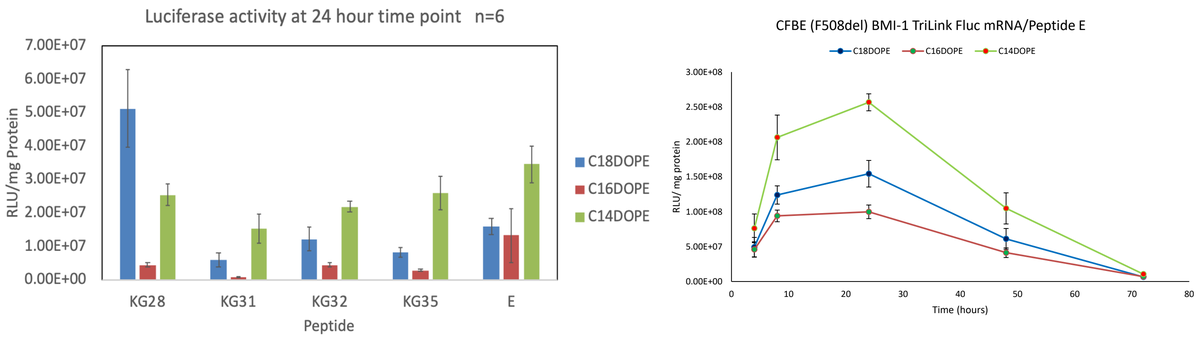


C14, C16 or C18:DOPE=1:1 molecular ratio

-CFTR mRNA were transfected using the optimised nanocomplexes into CFBE cells at Air-Liquid interface (ALI) culture
-Luciferase mRNA were transfected to mouse lungs

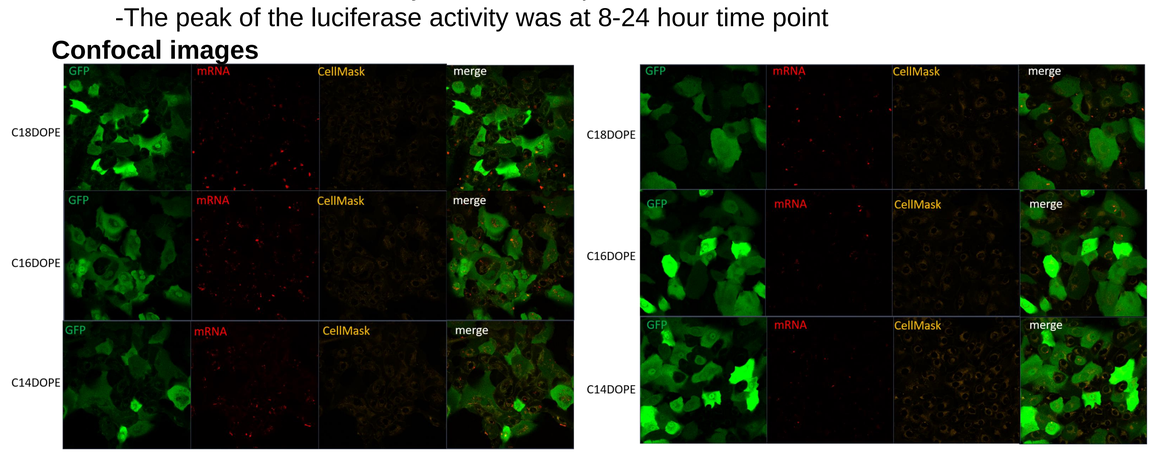
Result 1

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

Confocal images



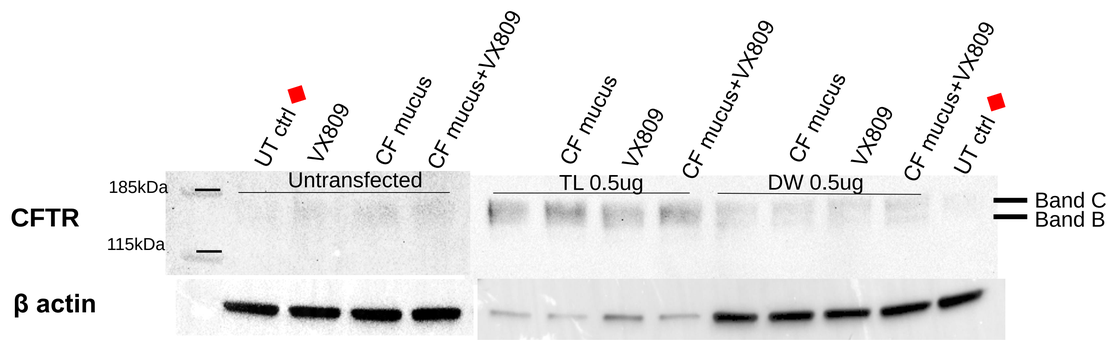
4 hour time point **24 hour time point**

C18DOPE/KG28 and C14DOPE/E

Result 2

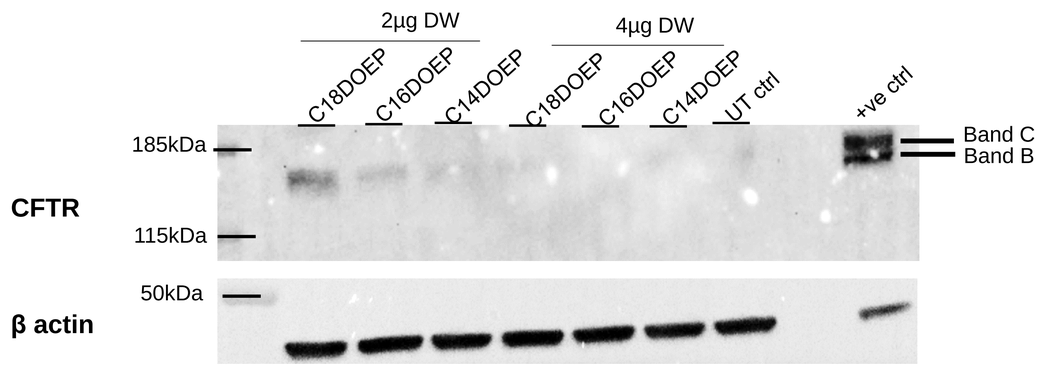
- **CFTR mRNA transfection In CFBE BMI-1 cells at submerged culture**

-CFTR mRNA approximately 4500 nucleotides)
-TL: CFTR mRNA TriLink, DW: CFTR mRNA by Drew Weissman lab
-VX809: Lumacaftor, helping CFTR protein trafficked and mature (Band C)



◆: the same untransfected/untreated sample In a 12 well plate

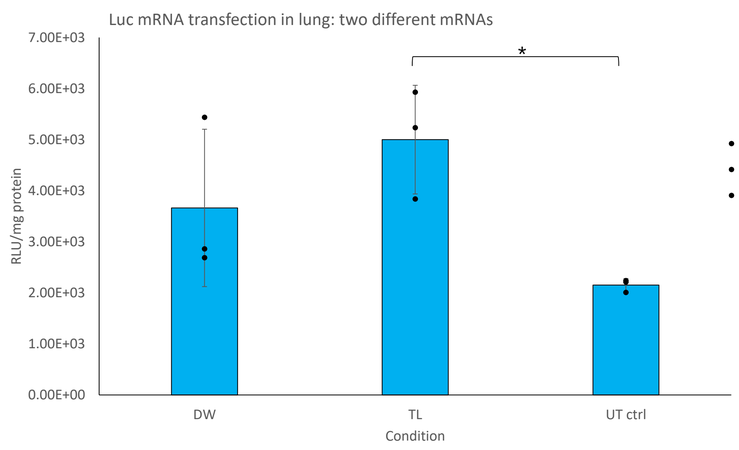
- **CFTR mRNA transfection in CFBE BMI-1 cells at ALI culture**



+ve ctrl: CFBE41o- transfected with DW CFTRmRNA 24hr

- **Luciferase mRNA transfection in mouse lungs**

-Lung transfection via Oropharyngeal route



- 8µg Luciferase mRNA/mouse
- C18DOPE/Peptide Y
- n=3

Luciferase mRNA from TriLink or DW lab

Result 3

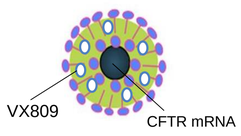
- **Co-delivery of CFTR mRNA and VX809 in CFBE BMI-1**

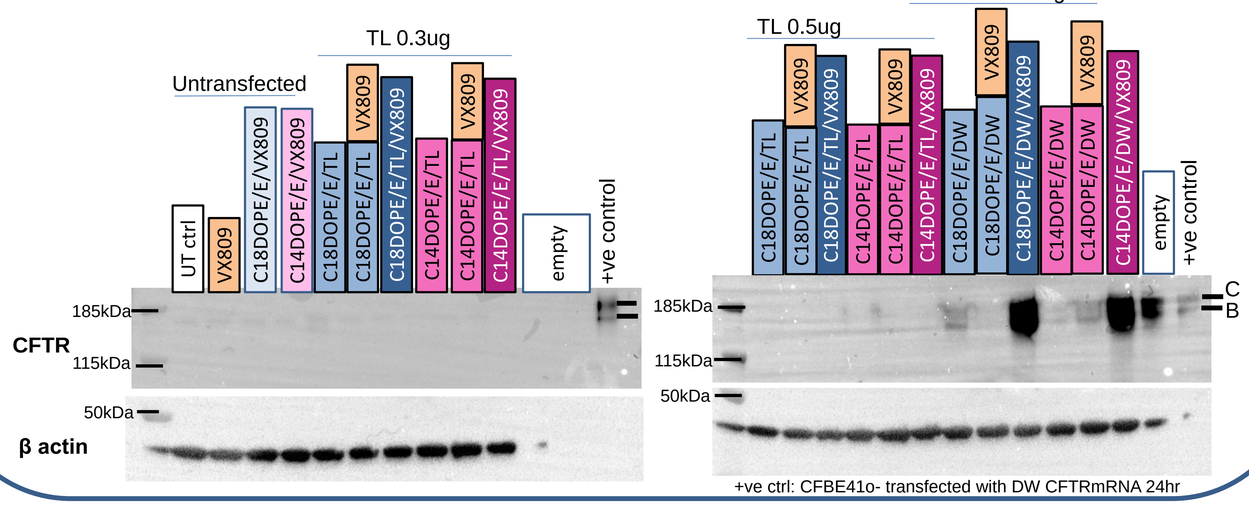
-VX809 was packaged in C18DOPE or C14DOPE/E/CFTR mRNA

C18DOPE/E/TL/VX809 | C14DOPE/E/TL/VX809 | C18DOPE/E/DW/VX809 | C14DOPE/E/DW/VX809

-Compared with mRNA nanocomplexes with VX809 treatment (VX809 was added into transfection medium)

C18DOPE/E/TL | VX809 | C14DOPE/E/TL | VX809 | C18DOPE/E/DW | VX809 | C14DOPE/E/DW | VX809





+ve ctrl: CFBE41o- transfected with DW CFTRmRNA 24hr

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 sub-merged and ALI culture

•IVT 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
Tatjen *et al.* 2015 'Cystic fibrosis'
Yu-Wai-Man *et al.* 2016 'Receptor-targeted liposome-peptide-siRNA nanoparticles represent an efficient delivery system for MRTF silencing in conjunctival fibrosis'