SELF-AMPLIFYING RNA FOR LONG TERM RESTORATION AND ENHANCEMENT OF CFTR FUNCTION

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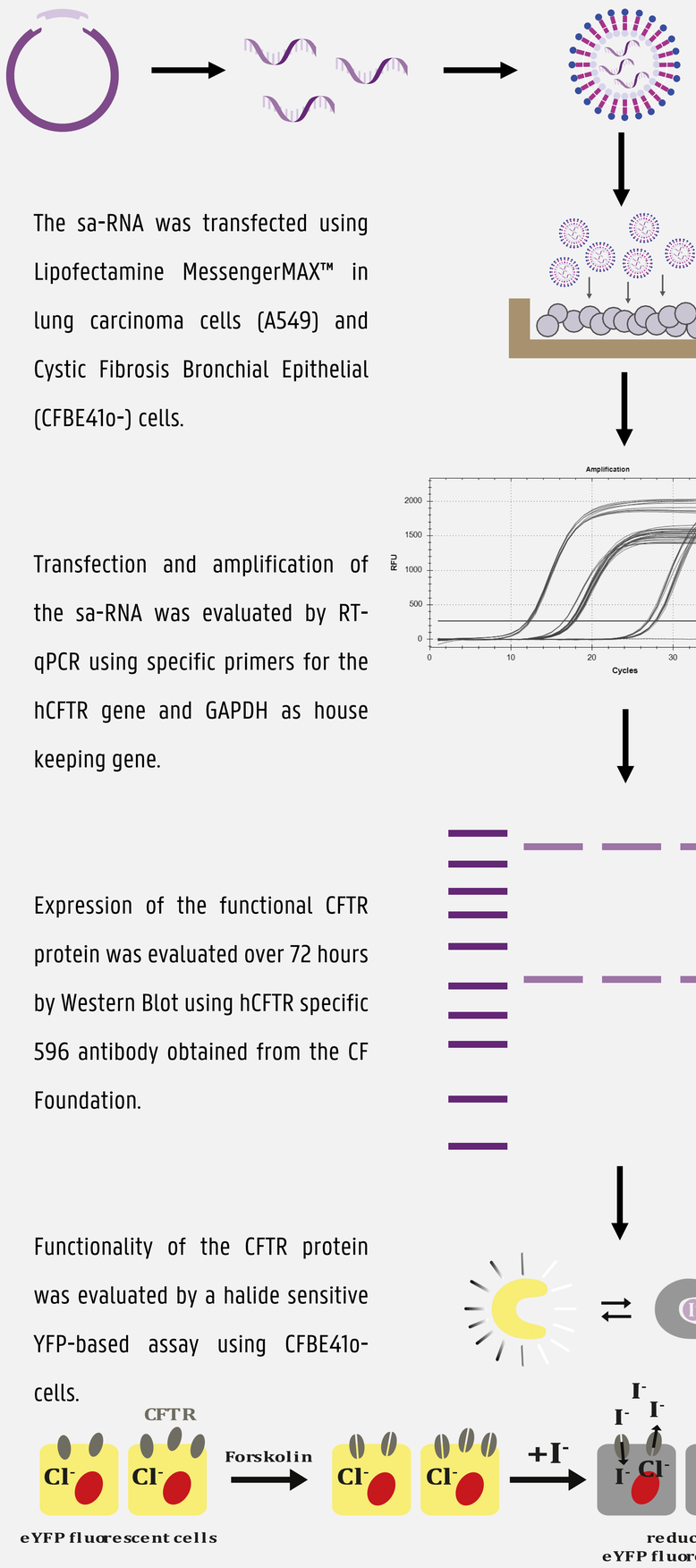
INTRODUCTION & RESEARCH AIM

Cystic Fibrosis (CF) is an autosomal recessive disorder affecting approximately 80,000 people worldwide. CF is a multiorgan, multifactorial disease with gastrointestinal (GI) impairment and airway infection, being the main cause of death in CF patients. CF is caused by a mutation in the cystic fibrosis transmembrane regulator (CFTR) gene leading to no or malfunctioning CFTR protein. Up until now, there is no effective therapy that tackles all CFTR mutations. In this project, we aimed to develop a self-amplifying RNA (sa-RNA) that encodes the wild-type hCFTR for supplementation of the CFTR protein in the lungs of CF patients.

MATERIAL & METHODS

- The gene sequence of hCFTR was cloned into a VEEV based sa-RNA expression vector by HiFi DNA Assembly.
- hCFTR sa-RNA was produced by *in vitro t*ranscription (IVT).



- The sa-RNA was transfected using Lipofectamine MessengerMAX™ in lung carcinoma cells (A549) and Cystic Fibrosis Bronchial Epithelial (CFBE41o-) cells.
- Transfection and amplification of the sa-RNA was evaluated by RT-qPCR using specific primers for the hCFTR gene and GAPDH as house keeping gene.
- Expression of the functional CFTR protein was evaluated over 72 hours by Western Blot using hCFTR specific 596 antibody obtained from the CF Foundation.
- Functionality of the CFTR protein was evaluated by a halide sensitive YFP-based assay using CFBE41o- cells.

RESULTS

RT-qPCR and Western Blot

A549 cell transfection

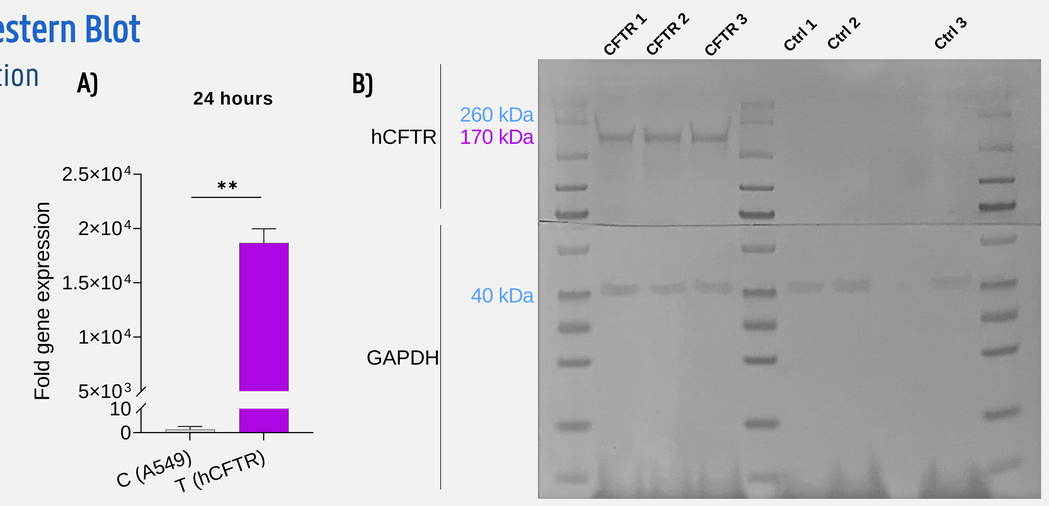


Fig 1. A) Fold gene expression of hCFTR sa-RNA 24 hours after transfection in A549 cells. C = Control, T = Treatment **B)** Western Blot of functional CFTR protein (170 kDa) 24 hours after transfection in A549 cells.

CFBE41o- cell transfection

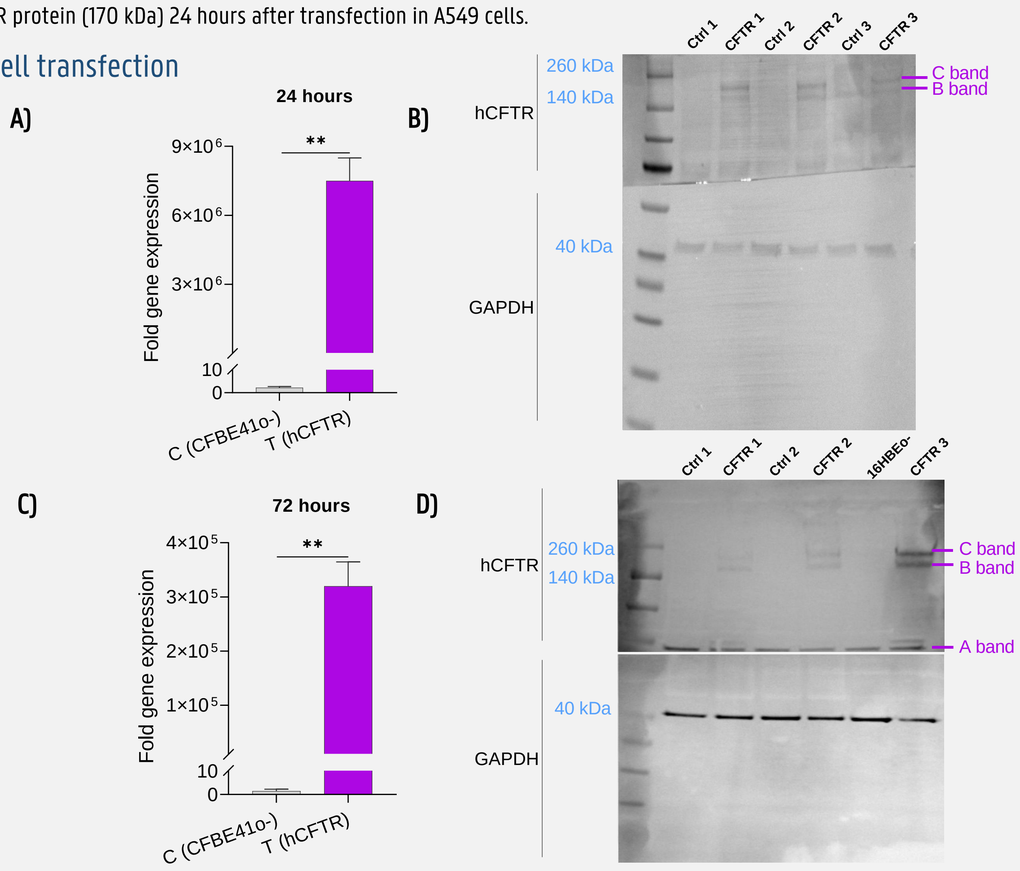


Fig 2. A, C) Fold gene expression of hCFTR sa-RNA respectively 24 and 72 hours after transfection in CFBE41o- cells. C = Control, T = Treatment **B, D)** Western Blot of functional CFTR protein (C band, glycosylated form) and non-functional hCFTR protein (B band, core-glycosylated form) respectively 24 and 72 hours after transfection in CFBE41o- cells.

Halide sensitive YFP-based functionality assay in CFBE41o- cells

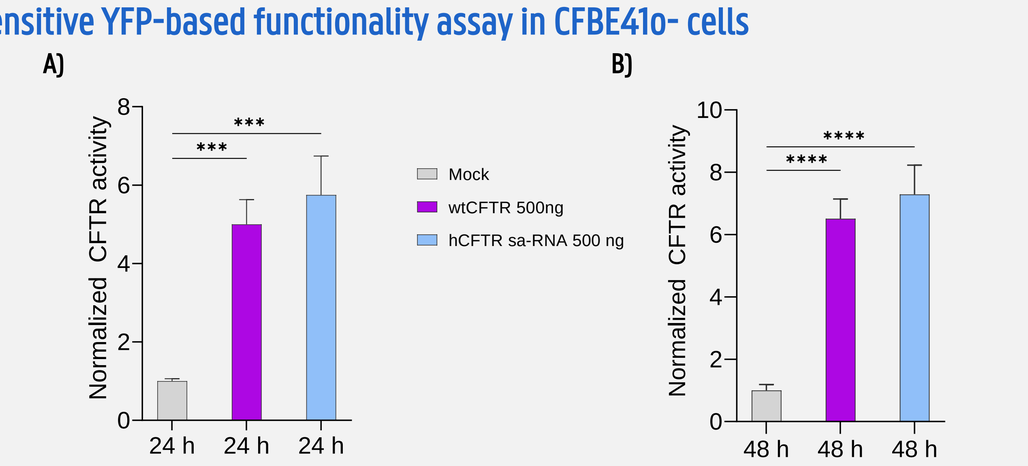


Fig 3. A, B) Normalized activity of CFTR protein respectively 24 and 48 hours after transfection of stably expressing YFP protein CFBE41o- cells with hCFTR sa-RNA and wtCFTR as positive control.

CONCLUSION

- *In vitro* results show high sa-RNA expression declining over time and presence of the mature hCFTR protein up until 72 hours.
- CFTR functionality was up to 7 folds higher after 24-48 hours incubation compared to non-transfected cells.
- Follow-up experiments will be performed using a suitable LNP as delivery vector in epithelial cells and *in vivo* experiments will be performed in mice and pigs to rectify GI and lung impairment.

Abbreviations: VEEV = Venezuelan Equine Encephalitis Virus, YFP = yellow fluorescent protein, LNP = lipid nanoparticle

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