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Combining Different Techniques for Cystic Fibrosis Treatment

Introduction

Cystic fibrosis and gene therapy

Cystic fibrosis is a life-threatening genetic disease affecting 90,000 individuals worldwide, mainly caucasians. This disorder is inherited in an autosomal recessive manner and is caused by mutations in the *CF gene* that encodes for a protein called cystic fibrosis transmembrane conductance regulator (CFTR) which is a transmembrane protein responsible for chloride and bicarbonate transportation across epithelial cells. The p.F508del mutation (phenylalanine deletion at position 508) it's the most common mutation being responsible for up to 75% of the patients worldwide. Malfunctions or low levels of CFTR disrupt the salt and fluid homeostasis causing dysfunctions in mucociliary clearance in the lungs, intestine obstruction and pancreatic hormone regulation [1,2].

Many strategies have been tested to cure cystic fibrosis, from drugs like albuterol, guaifenesin or triamcinolone for lung inflammation and mucus clearance, to CFTR modulators and genome editing such as CRISPR-Cas9, however, none of then have already solve the problem. Conventional treatments given to CF patients reveal to treat only the symptoms, being costly and ineffective at long term due to antibiotics resistance [3]. CFTR modulators, which are proteins that improve the tracking of p.F508del-CFTR protein, demonstrated to be effective in patients with homozygous mutations, specifically the elexacaftor–tezacaftor–ivacaftor treatment [4]. Finally, genome editing techniques such as CRISPR-Cas9, although promising, showed limited success in both animal models and cells [5]. Therefore, we propose a combined approach composed of the CFTR modulator triple treatment and the CRISPR tool, as an efficient way to combat cystic fibrosis on a more genetic basis, targeting the p.F508del mutation, and not only the symptoms.

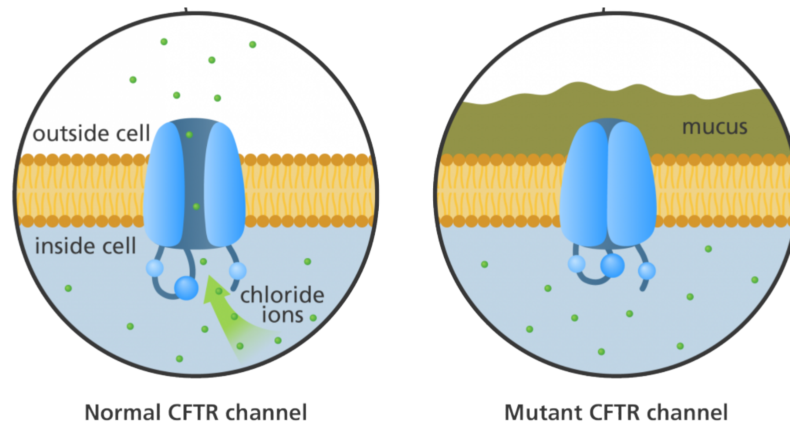

Figure1. Comparison between a normal and a mutant CFTR protein channel. Adapted from https://www.yourgenome.org/facts/what-is-cystic-fibrosis.

Experimental Design

Material and controls

The target cells for the gene editing procedures are ferret induced pluripotent stem cells. These cells were picked based on previous articles that show that they are supported for the intended experiments. There will be healthy ferrets and ferrets with the p.F508del mutation, the most common cystic fibrosis mutation that causes CFTR protein misfolding. The healthy ferrets will serve as the negative controls. There will be an additional assay where we administer a proven combination of small-molecule correctors (elexacaftor–tezacaftor–ivacaftor) [4] to half of the ferrets with the mutation to assess if combining these drugs with gene editing is beneficial.

CRISPR-Cas9 treatment of the p.F508del mutation

Previous to our work, some ferrets will already have the p.F508del mutation. The induced pluripotent stem cells from the ferrets with the mutation will then be extracted and treated using CRISPR-Cas9 delivered as purified Cas9 protein pre-loaded with gRNA in a ribonucleoprotein (RNP) complex [6]. RNPs may be transfected into cells via electroporation as described in [7]. This allows transient expression of the nuclease and limited off-target activity [8]. The treated cells will be re-inserted on the ferrets with the mutation.

Administering the triple combination drugs and blood extraction

A combination of elexacaftor-tezacaftor-ivacaftor will be administered intravenously to only one half of the mutated ferrets. Previous phase II clinical trials in humans using these drugs were able to notice an increase in the respiratory function at the 4th week [4]. Given that, we will collect blood samples every week for a maximum of 8 weeks from all ferrets.

Gene expression assay

After obtaining weekly blood samples, we will proceed with RNA isolation to avoid interferences from unwanted whole blood components like enzymes or red blood cells, using the protocol described in [9]. Afterwards, we will use RT-qPCR to compare CFTR gene expression between samples with both treatments (CRISPR-Cas9 and Triple Drugs) as well as single treatment. The gene expression from all sample groups will be compared week by week to assess which treatment provided the higher fold in CFTR expression.

Fluorescence Microscopy

To observe the amount of CFTR proteins after the treatment, fluorescence microscopy will be used. This will give us an insight on how protein expression changed with high sensibility and specificity, allowing us to compare the efficiency of the combined treatments with the single treatment.

Immunofluorescence

The CFTR proteins will be analysed through immunofluorescence where a rat anti-HA antibody was applied to stain surface HA-tagged CFTR as in [10] allowing us to observe the cells where it is present. Further quantification can be obtained using ImageJ software.

Expected Results and concluding remarks

With this work we expect to analyse two different approaches to treat cystic fibrosis using one or two combined techniques in order to verify if combining different methods is more efficient to tackle this disease. New projects should continue to work on targets for new drugs that can help to increase CFTR function in cystic fibrosis patients. Genome editing seems to be a good technique for some of the

issues caused by this disease enabling the appearance of novel therapeutic solutions and experimental models. Future studies could also focus on higher efficacy of CRISPR delivery as well as a method of packaging both of these treatments in one single insertion.

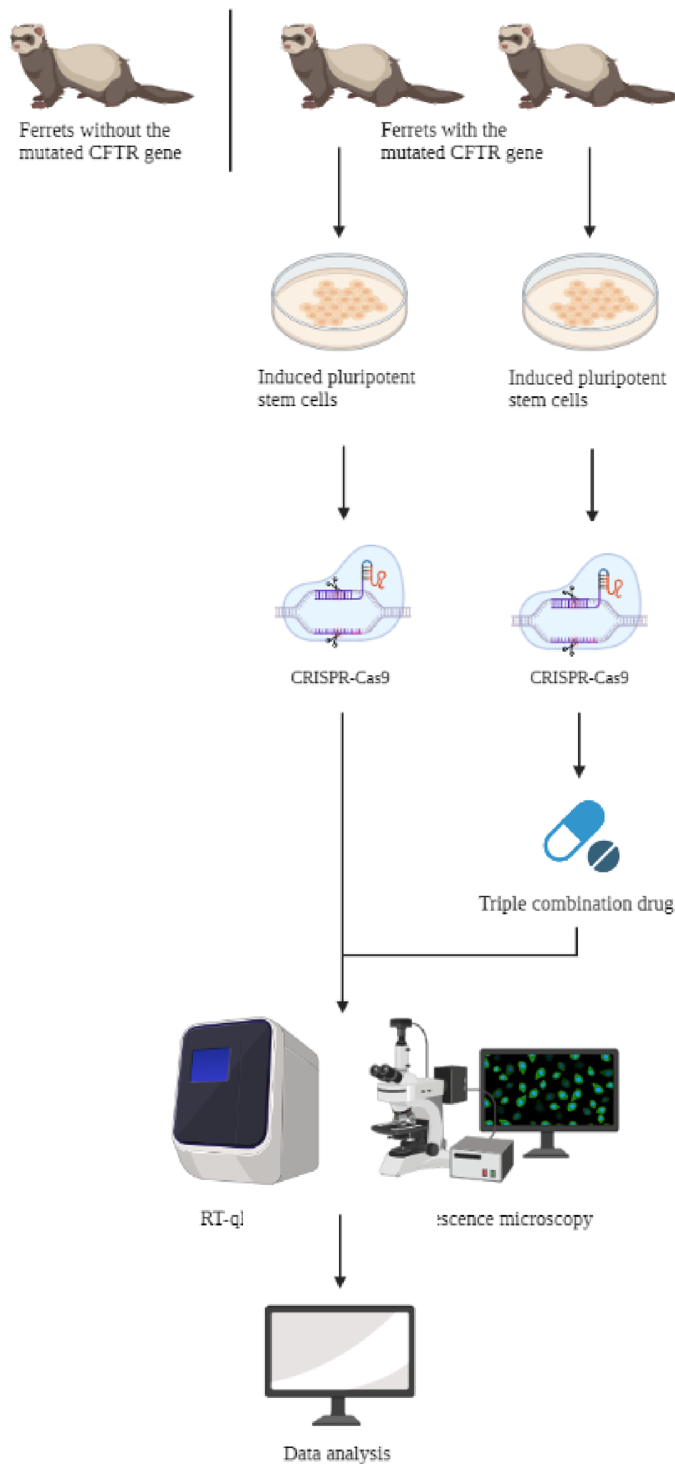

Figure 2. General workflow of the experiment. Diagram created with BioRender.

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