RADICHAL RARE DISEASE CHALLENGE
Project Preliminary Report

Project Name:

CORRECTION OF ΔF508 MUTATION IN IONOCYTES BY PRIME EDITING

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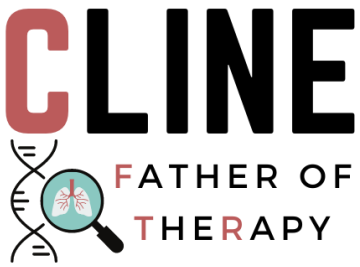
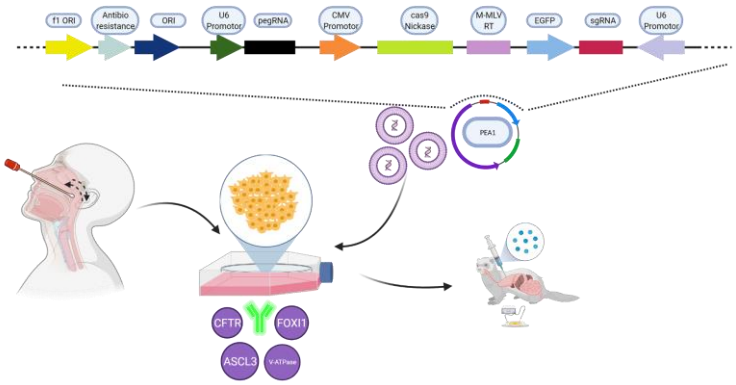


Cystic Fibrosis (CF) is the most common and fatal of rare diseases and is caused by mutations in the CFTR gene, which encodes a chloride channel configuration protein. It occurs with many symptoms such as dehydration, thickening of secretions, and salty sweat due to dysfunction of Cl- channels. While the respiratory system becomes vulnerable to infections, especially when the secretions fail to function, the digestive system progresses with poor absorption of food, fatty stools, and growth retardation. CFTR is expressed on chromosome 7, in the gene-specific long arm region 3 (q), band 1, subband 2 (7q31.2). Transcription of this gene results in CFTR mRNA which is translated into the 1480 amino acid CFTR protein. A single mutation, ΔF508, accounts for 70% of mutant CFTR genes in the world; It corresponds to the deletion of phenylalanine at position 508 of the CFTR protein. However, there are more than 1500 Cystic Fibrosis mutations reported, although most of them are rare. These are evaluated under 6 classes of mutation types. The protein encoded by the CFTR gene is essential for maintaining osmotic balance and the normal transport of electrolytes on the apical surface of epithelial cells from the lungs, upper respiratory tract, pancreas, liver, and gallbladder, intestines, and other exocrine glands such as sweat glands, male reproductive system, and epididymis. Due to the CFTR dysfunction, the ionic imbalance, hyperviscosity of respiratory mucus reduces mucociliary clearance, promotes bacterial colonization, and creates cycles of infection or inflammation. Cystic fibrosis occurs with many symptoms such as dehydration, thickening of secretions, and salty sweat due to dysfunction of Cl- channels. While the respiratory system becomes vulnerable to infections, especially with the secretions failing to function, the digestive system progresses with poor absorption of food, fatty stools, and growth retardation.

This project aims to correct the ΔF508 mutation, which occurs with a 3-base deletion (CTT) in the CFTR gene, which is the most common mutation in Cystic Fibrosis (CF), in ionocyte cells with the Prime Editing method. Because of this mutation, which is the most common in the world, the expression of the CFTR gene does not occur and this affects many systems in the body, especially the lungs. For this reason, the cells used mainly in cystic fibrosis studies are airway epithelial cells. Epithelial cells in the airway until the last lung cell mapping; were classified as ciliated cells, goblet cells, club cells, and airway basal cells. However, in recent mapping studies, "ionocyte cells" have been defined in addition to these cells. Ionocytes are the cells that makeup only 1 to 2% of airway epithelial cells and provide fluid regulation at the epithelial interface, and although they are few, they are thought to be the main source of CFTR activity in the airway epithelium, as they account for approximately 60% of all CFTR expression. Targeting the lungs, the organ most affected by this deadly and common mutation, the genetic treatment plan of this project is based on ionocytes, an airway epithelial cell. Ionocytes are cells that alone account for approximately 60% of CFTR gene expression in the bronchial epithelium, the main site of involvement of CF, and will be functional in genetic therapy designed for CF. These cells will be isolated from the nasal epithelium by non-invasive brushing and cultured in the ALI medium. Control experiments will be provided by monitoring the development and differentiation steps of ionocyte cells differentiated from human airway basal cells with various markers. These ionocyte cells will be inserted into Prime Editing All-in-One plasmids following the procedure outlined in the literature for the pegRNA and sgRNA designs.

To increase the efficiency of Prime Editing, a guide RNA library was created with three different PE2 pegRNA designs and 3 different PE3 and 1 PE3b guide RNA designs prepared for the second notch. Prime Editing All-in-One (PEA1) vectors were selected for insertion of guide RNAs. PEA1 plasmids contain all the necessary components for PE3 regulation, as well as a puromycin or GFP (green fluorescent protein) selection marker that binds to Cas9 using a T2A self-clearing peptide. PEA1 also contains three BbsI golden gate cloning sites to allow simultaneous insertion of oligonucleotides encoding the sgRNA protospacer, RT template, and second-notch guide RNA protospacer. BbsI sequences were added to the beginning and end of the pegRNA sequences to be prepared for transfer to PEA1 plasmid vectors containing GFP with different combinations. After the supply of these plasmids, which will contain the pegRNA sequences, the plasmids will be packaged into liposomes, which is a non-viral delivery method, and transferred to ionocyte cells isolated from cystic fibrosis patients in the laboratory environment.

With the appropriate response in culturing the cells, the next step is to model animals in ferrets, where CF has the highest compatibility with humans. Following these steps, especially when using in newborns where the feasibility of our isolation method is high, the fact that mucus accumulation is at a minimum level in the newborn compared to adults and is at the beginning of the progression will ensure that our gene therapy is transferred more effectively both inhaled corrected ionocytes and liposome containing the corrective plasmid. In addition to all these advantages, the transfer to be performed after the routine lung cleaning protocols of the patients in adults and patients with advanced lung contamination will apply to all segments.



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