RaDiChal'21
FINAL

2021 Rare Disease Challenge

CORRECTION OF CFTR ΔF508 MUTATION IN IONOCYTES BY PRIME EDITING

CLINE

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Abstract

Our project aims to correct the ΔF508 mutation and is based on prime editing (1). The pegRNAs prepared to correct DeltaF508 will be placed into Prime Editing All-in-One (2) plasmid vectors containing all the necessary components for prime editing, including Cas9n and M-MLV reverse transcriptase and transferred to the cells in vitro. The cells we will target in the project will be ionocytes that can be isolated from cystic fibrosis patients along with other airway epithelial cells (3). While ionocyte cells provide a very large part of both CFTR expression, the high efficiency of the treatment development we will perform here will give us an advantage. Liposomes, a non-viral and chemical method, will be used to transfer the sequence to ionocytes.

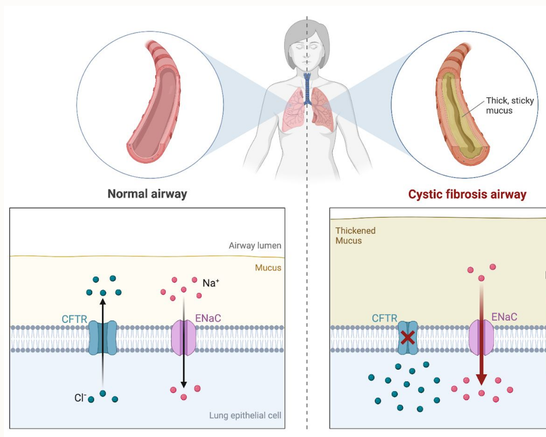


Figure 1. CFTR lung, airway and channel diagram with normal vs. cystic fibrosis. Cystic fibrosis is the most common rare disease in Europe, is caused by mutations in the CFTR gene and presents with many symptoms such as increased concentration of secretions, thickening at airways due to dysfunction of Cl- channels.

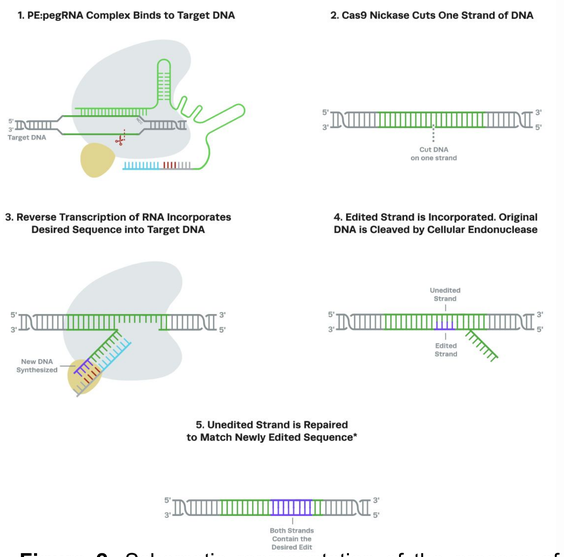
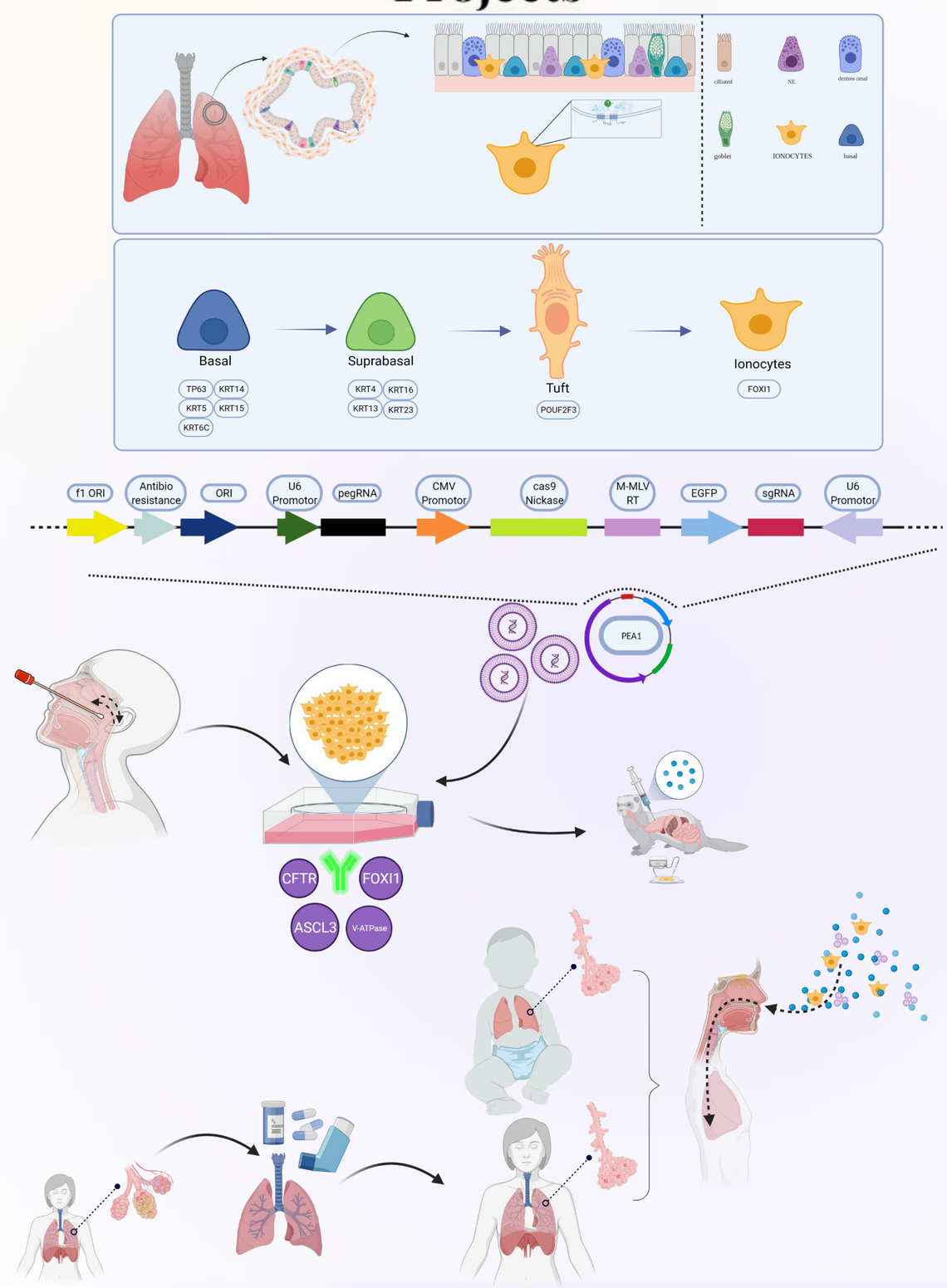


Figure 2. Schematic representation of the process of prime editing (1).

Projects



Social Impact

Conclusion

Since it is known that ionocyte cells targeted in the project alone meet approximately 60% of the CFTR gene expression, it is thought that providing wtCFTR expression in these cells can greatly improve the patient's lung pathology and eliminate the lethal aspect of the disease. Also, Prime Editing All-in-One plasmid vectors are expected to both increase the efficiency of gene editing and facilitate experiments. In addition, cholesterol has been shown to increase the stability of liposomes, which is a non-viral delivery method. For this reason, it is estimated that the transfer will be carried out more efficiently by using cholesterol-containing liposomes in the project. The effectiveness of applied gene editing will be tested by methods such as gene sequencing methods, flow cytometry, and short-circuit current (Isc) measurements.

References

1. Anzalone AV, et al. Search-and-replace genome editing without double-strand breaks or donor DNA. Nature. Online October 21, 2019.
2. Adikusuma, F., Lushington, C., Arudkumar, J., Godahewa, G. I., Chey, Y., Gierus, L., Piltz, S., Geiger, A., Jain, Y., Reti, D., Wilson, L., Bauer, D. C., & Thomas, P. Q. (2021). Optimized nickase- and nuclease-based prime editing in human and mouse cells. Nucleic acids research, gkab792. Advance online publication. https://doi.org/10.1093/nar/gkab792
3. Plasschaert, L. W., Žilionis, R., Choo-Wing, R., Savova, V., Knehr, J., Roma, G., Klein, A. M., & Jaffe, A. B. (2018). A single-cell atlas of the airway epithelium reveals the CFTR-rich pulmonary ionocyte. *Nature*, *560*(7718), 377–381. https://doi.org/10.1038/s41586-018-0394-6

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