RaDiChal 21
Semi Final

2021 Rare Disease Challenge

GENE EDITING, REHYDRATION OF DEHYDRATED MUCUS PHENOTYPE IN CYSTIC FIBROSIS, TARGETING PROGENITOR EPITHELIAL CELLS WITH MODIFIED MRNA TECHNOLOGIES AND CRISPR PRIME EDITING.

RE-HYDRA

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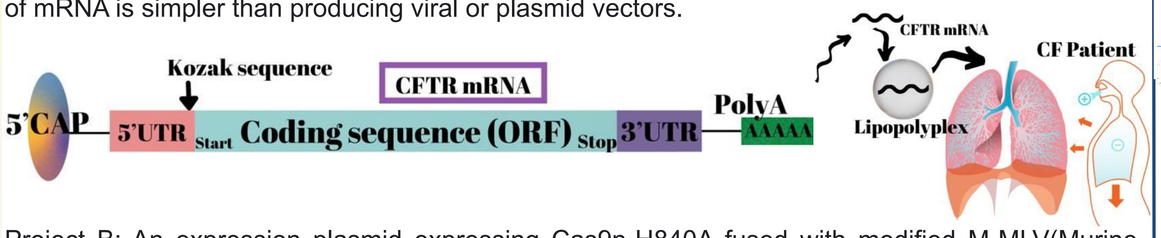
Abstract

Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene.CFTR encodes for a cAMP-regulated chloride channel located in the apical membrane of epithelial cells that catalyze the passage of small ions through the membrane, inducing anion transport.[1] CF is a progressive,life-shortening and -threatening genetic multiorgan disease caused by loss of protein quantity and/or function due to nearly 2000 mutations in the CFTR gene.[2,3] Dysregulation of the mechanism causes severe impairment of lung function,serious pathology of the pancreas and gut, male infertility, and reduced growth.The disease affects about 70,000 individuals worldwide.[1] The absence of the anion channel CFTR causes electrolyte and transepithelial fluid imbalance resulting in viscous mucus along the epithelial lining of organs impairing both the pulmonary and gastrointestinal systems.The difficult-to-clear,thick sticky airway mucus in patients with cystic fibrosis contributes to lung infections and inflammation eventually leading to lung failure. [1]

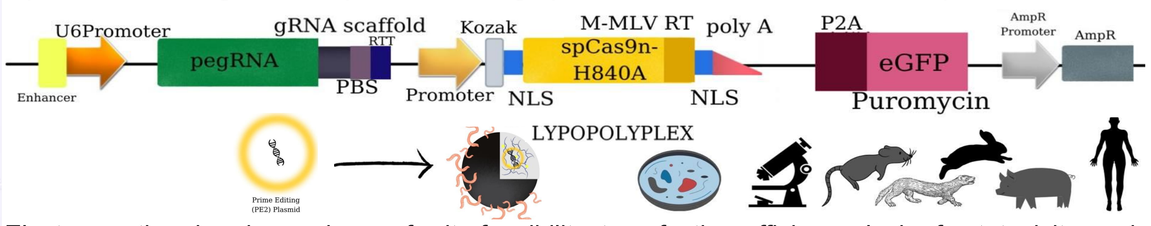
ReHydras' goal is rehydrating the dehydrated mucus in CF pathology which is a monogenic disorder and that makes it more suitable for mRNA based therapies. In this direction, we designed a multiple genetic treatment project which targets nearly all the mutations in CFTR,a novel modified exogenous CFTR-mRNA(modmRNA) delivery to lung epithelial progenitor cells using a hybrid system non-viral vector, Lipopolyplexes. Besides, targeting all CFTR-caused mutations, we aim to use the Prime Editing(PE2) system,which is an improved version of the CRISPR method, in a part of our Cystic Fibrosis genetic treatment project. The homozygous ΔF508 mutation which occurs in exon 11, has the highest incidence of CFTR mutations worldwide, chosen to keep our target population at maximum capacity.Therefore, an expression plasmid, expressing Cas9n-H840A (which is efficacious in insertions, in the PE system) and containing optimized pegRNA suitable for genetic therapy and the necessary marker-selection genes designed for prime editing will be used during the transfer of genetic therapy to the lung epithelial progenitor cells via LPP.

Project

Project A; CFTR is a low level expressed gene, and mature protein can be long-term stable, after reaching the plasma membrane with a half-life>15 hours. Taking this into consideration, 10% of CFTR mRNA (or 20-35% protein) will be adequate for the function of the CFTR channel. Aiming to restore CFTR-mRNA successfully, we used modifications such as codon optimization, artificial 5'CAP, 5' and 3' UTRs of long expressed genes, optimum length PolyA tail, and Kozak sequence. The key mRNA stabilizing, translation efficiency increasing, and in vivo immunogenicity reducing modification used is the replacement of uridine with N1methylpseudouridine(m1ψ). Furthermore, encapsulation of mRNA with LPP, protect it from degradation during the delivery stages. Encapsulated modmRNA will be delivered via inhalation or endotracheal after the in vitro and pre-clinical trials. Same delivery and confirmation methods will be used at both projects. The potential therapeutic advantages of using m1ψ-mRNA, are numerous such as improved safety because RNA is inherently incapable of integrating into the genome, efficient transduction of primary cells, rapid protein expression, mRNAs are translated within minutes following entry into the cytoplasm, the extent and duration of the encoded protein expression can be closely controlled due to mRNAs have shorter half-lives and, unlike other vectors, do not replicate and manufacturing of mRNA is simpler than producing viral or plasmid vectors.



Project B; An expression plasmid expressing Cas9n-H840A fused with modified M-MLV(Murine Leukemia virus) RTase, containing pegRNA with desired edit 'CTT' which has optimized PBS and RTT length and the required marker-selection genes designed for prime editing will be used during the transfer of genetic therapy to the lung epithelial progenitor cells. With this method, we aim to transfer our Prime Editing components to a specific expression plasmid (which we designed by modifying pegRNA expression plasmids) to perform Genome Editing in individuals with Cystic Fibrosis and delivery of it with tissue-specific Lipoplyplexes (LPP), containing lung tropic polycation and cationic lipid components. Combining the advantages of both polyplexes and lipoplexes, Lipopolyplexes, a new approach for CF, shows low toxicity thanks to their compatibility with body fluids and do not trigger immune responses. Modifications were made for LPP could be specific to the target cell, tissue, and effectively endocytose into the cell by passing through the mucus. Also, a promoter showing tissue tropism to the lung-epithelial cells was selected for the plasmid.

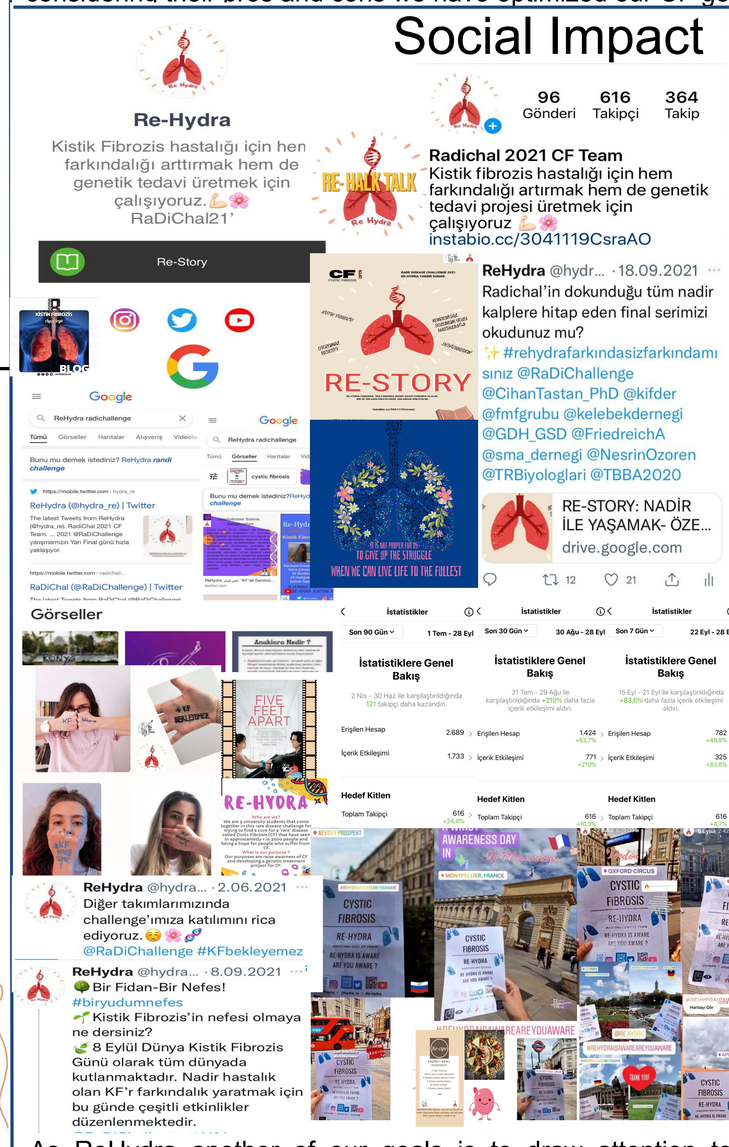


Electroporation, has been chosen for its feasibility, transfection efficiency, lack of cytotoxicity, and as a convenience of our plasmid to be tested on the CF bronchial epithelial cell line for the first preclinical trials.To make the selection,plasmid contains eGFP/Puromycin markers,which are frequently used and easily found for CF studies. For animal and clinical trials, inhaler,endotracheal delivery methods have been chosen for the CF phenotype. As transgenic animal models, it is crucial to mimic the CF phenotype, especially F508del for PE2 and lung findings for mRNA, henceforth; rat, rabbit, ferret, and pig models for larger human-like lungs are selected.For the proof of concept and feasibility of two of the projects genome-wide association studies(GWAS), qRTPCR and Sanger sequencing methods will be used for DNA and RNA level of detection. WesternBlot technique will be used for the CFTR protein existence, lastly, Patch-Clamp to measure the CFTR ion channel capability and apical membrane potential. All the data of the project will be considered as significant using the Mann-Whitney U test and student t-test (P<0,05).

Conclusion

Cystic fibrosis (CF) is a lethal rare disease with a prevalence of 1/3000.CFTR located on chromosome 7 contains a multiverse of mutations leading to mucus build-up and implicitly respiratory, digestive, and reproductive problems.[2] As ReHydra,we have presented derived strategies as a path forward from conventional gene therapy based on the delivery of therapeutic nucleic acids toward gene correction of CFTR mutations.Genome editing is at the forefront by offering novel therapeutic solutions, as PE2-ΔF508 correction.Although the concerns about in-vivo delivery of modified mRNA and CRISPR genome editing, hurdles and limitations seem well-defined and manageable.To conclude,our novel mRNA approach is based on the COVID19 mRNA vaccine modification successes,particularly Biontechs' and considering their pros and cons we have optimized our CF gene therapy persistency[1].

Social Impact



As ReHydra another of our goals is to draw attention to rare diseases,notably CF. In this context,informative posts,videos,films,and stories about CF and rare diseases were shared on our social media accounts(Twitter,Instagram,Youtube,Facebook). For the evaluation and increment of CF awareness,we have output different series both in Turkish and English such as ReView;broadcasts with experts in the CF field, ReHalkTalk;interviews, Re-cipe; diet list for CF patients, Re-ad;blog writings, ReWatch;CF-related movie suggestions, ReStory; Living with CF journal,RePhysio;physio techniques for CF patients and ReMotivation;motivational post for our followers. Rare disease awareness day posts have been published and fellowships with other teams have been made. Furthermore, a hashtag was created on the behalf of enhancement of our profile interactions #ReHydraIsAwareAreYouAware and tagged in our every post.Sponsorships have been made for our "Be Aware CF" brochures which are hanged to the most crowded places and distributed in Turkey and abroad, at 8 countries e.g. GR-Komotini, EN-London, FR-Montpellier, NL-Harderveijk. Moreover, ReHalkTalk is carried out in 6 countries,afterward,brochures were distributed to our ReHalkTalk participants. Also,our sponsors shared every post using our hashtag.To extend our target group and support the cooperation between Radichal21 teams, our team challenged other CF teams to support the #CFcantwait campaign by tagging our accounts and using our hashtag.Overall,ReHydra has reached out to more people (over 5000) than excepted.To leave a mark behind and make people wonder what is CF, we have donated 3 saplings to TEMA on 8 September World Cystic Fibrosis Awareness Day.

References

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