DEVELOPMENT OF RECEPTOR-TARGETED NANOCOMPLEXES FOR IN VIVO DELIVERY OF CRISPR/CAS9 AS A POTENTIAL THERAPY FOR CYSTIC FIBROSIS

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Background: Cystic Fibrosis (CF) is recessively inherited, multi-organ disease, however morbidity and mortality is caused mostly by progressive respiratory impairment due to mucus retention and chronic bacterial infection in the lungs. Gene therapy is an attractive therapeutic option for CF, targeting the underlying cause of the disease, rather than treating symptoms. We aim to develop a novel gene therapy for the respiratory manifestations of the disease based on gene editing with CRISPR/Cas9. This allows for the precise introduction of double strand breaks in the DNA followed, in the absence of a DNA template, by non-homologous end joining (NHEJ), an error-prone but efficient DNA repair pathway functioning in both dividing and terminally differentiated cells.

Methods: For treatment of CF, CRISPR/Cas9 must be delivered with sufficient efficiency to the lung. Our approach is to deliver Cas9/gRNA ribonucleoprotein (RNP) complexes with a non-viral, receptor-targeted nanoparticle (RTN), previously described for in vivo DNA and siRNA delivery to the lung. RTNs comprise a peptide component, mediating targeting of epithelial cells, and lipid components, enabling endosomal escape.

Results: To provide a model, we engineered primary, human bronchial epithelial cells to stably express GFP by lentiviral transduction and used this model to compare nanoparticle formulations for knockout of GFP by CRISPR/Cas9. We achieved levels of up to 75% GFP knockout, as measured by flow cytometry. Complexes had a desirable size (90 nm), charge + 40 mV and polydispersity index (~0.2) appropriate for delivery to the CF lung, where mucus accumulation prevents penetration of larger particles.

We next used RTNs to deliver RNP formulations to primary CF basal epithelial cells to delete a deep intronic CFTR mutation that creates a cryptic splice site (3849+10kb C>T). Following repeat delivery of RTNs, indel frequency was more than 80% by ICE analysis of DNA sequencing. Correct CFTR splicing was restored, while chloride ion transport was shown to be partially restored in Ussing chamber measurements.

Finally, to evaluate the efficiency of in vivo editing, we delivered the RNP nanoparticles to the lungs of Ai9 TdTomato reporter mice, where a loxP-flanked STOP cassette prevents transcription of TdTomato. By using two gRNAs targeting upstream and downstream of the STOP cassette, we aimed to excise the cassette, restoring TdTomato fluorescence. Following oropharyngeal instillation of RTNs, TdTomato expression was evident in the airway epithelium, with no evidence of an inflammatory response.

Conclusions: This work advances potential therapeutic avenues for nanoparticle-mediated Cas9 RNP delivery to the airways for treatment of CF.