SiRNA and CRISPR/Cas9 Mediated Knockout of αENAC

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Abstract:
Cystic fibrosis (CF) is caused by mutations in the cystic fibrosis conductance regulator (CFTR) gene, which encodes for a chloride channel. Loss of CFTR upregulates the epithelial sodium channel (ENaC) causing hyperabsorption of sodium, reducing the watery lining of the lung, leading to impaired mucociliary clearance and enabling inflammatory lung damage. Therefore, inhibition of ENaC has been proposed as a treatment in CF. In this study we are assessing the use of siRNA and CRISPR/Cas9 system as complementary methods of achieving disruption of ENaC expression in airway epithelial cells using receptor-targeted nanocomplexes. We sought to compare the efficiency and persistence of silencing, repeatability of delivery and its toxicity. We assessed silencing of the αENaC subunit by transfecting primary CFBE cells growing in Air-Liquid Interface cultures with siRNA-bearing nanocomplexes and achieved 30% silencing at the mRNA level. We then assessed their silencing efficiency in lungs of normal mice (C57BL/6) delivered by oropharyngeal instillation. Following a single dose, the siRNA formulations achieved ~30% knockdown of mouse αENaC. Following repeated delivery (3 doses at 48h intervals) we found out that 48h after the last administration ~50% silencing was achieved, with no adverse effects (as judged by body weight and histology), whilst following a single administration there was still ~30% silencing 7 days later. An algorithm was used to design 6 different guide RNA targets for αENaC. These were used to transfect cells along with Cas9 and showed varied levels of indel mutation rates in the αENaC gene by the T7 endonuclease I assay although two, T3 and T4, were optimal. In order to increase the amount of indels created we subjected the cells (CFTE and HBE) to sequential transfections of the same target at 48h intervals. This showed an accumulation of indels in cells targeted with T3 at a rate of 33.3% in HBE cells. This correlated with a 60% decrease in ENaC mRNA compared to controls and with 40% after a single dose to 65% decrease in protein levels after three doses. The siRNA-mediated silencing showed that we can repeatedly deliver the formulations with no adverse effects and that the effect of a single dose, although transient, can last for at least 1 week. CRISPR/Cas9 also mediated efficient gene disruption of αENaC, but with the advantage that this knockdown will be permanent in cells where the life span is quite long. Another encouraging finding was the accumulation of gene disruption with both systems when delivered by a non-immunogenic vector. This is very important as CF therapy would need repeated administrations. It is reasonable to anticipate that these systems offer new prospects to CF gene therapy.