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Abstract

TITLE: RESCUE OF CFTR FUNCTION IMPAIRED BY MUTATIONS IN EXON 15 IN CHILDREN WITH CYSTIC FIBROSIS
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ABSTRACT BODY:
Abstract Body: Introduction: Over 2000 different mutations have been reported in patients with CF and found to occur in all CFTR exons and introns. Of these, 168 are nonsense mutations, 295 are frameshift mutations that are not amenable to current therapies, and therefore new drugs must be developed. Antisense oligonucleotides (AOs) are synthetic RNA analogues that can be designed to anneal to selected splice motifs within pre-mRNAs. AO binding alters the recognition of the splice site by the spliceosome and therefore modulates exon selection. Exon 15 has been selected as an initial target since it has been reported to harbour ~40 mutations and exclusion of this exon will not disrupt the mRNA reading frame. We hypothesis by skipping exon 15 in patients with amenable mutations such as p.Phe861Leufsx3, studied here, the disease-causing mutation can be by-passed and the induced isoform may retain some residual function, therefore alter the course of disease.
Methods: AO sequences were initially optimised using 2'-O-Methyl modified bases on a phosphorothioate backbone (2OMe) and transfected into monolayer primary non-CF (2.6 yrs M) and p.Phe861Leufsx3/p.Phe508del CF airway epithelial cells (4.1yrs M). The ratio of the AO induced, CFTR RT-PCR transcript product missing the target exon, relative to the full length product provides an estimate of AO exon skipping efficiency. The most effective 2OMe AO sequence was identified and re-synthesised as the clinically validated phosphorodiamidate morpholino (PMO) chemistry. Monolayer transfections were repeated. AO mediated modification of protein was shown by western blot analysis and comparison to size standards. CFTR function before and after PMO application was measured using Ussing Chamber studies.
Results: 2OMe AOs were designed, evaluated and further optimised by micro-walking around sequences shown to be capable of modifying splicing. The 2OMe sequence that was most efficient induced an estimated 50% skipping in p.Phe861Leufsx3/p.Phe508del CF airway epithelial cells The PMO induced efficient skipping of exon 15 from non-CF (49%) and p.Phe861Leufsx3/p.Phe508del CF (88%) cells after 7 days in culture. Western blot was used to determine the effect on the induced CFTR protein. Airway epithelial cells from children with CF were also grown at Air-liquid interface 28 days to become mucocillary differentiated then assessed for CFTR function using an Ussing chamber.

Conclusion: Exon 15 can be efficiently skipped from the CFTR transcript in both non-CF and CF-derived airway epithelial cells. We propose that exon skipping to remove disease causing mutations in selected in-frame exons can improve function in amenable CF patients, either alone or in combination with current therapeutics.
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