Plan of Work - MSc Thesis

Characterization and correction of Cystic Fibrosis Splicing Mutations by Antisense Oligonucleotides

Background: Cystic Fibrosis (CF) is the most common, lethal autosomal recessive disease. Clinically, CF is dominated by the severe impairment of the respiratory tract, the main cause of morbidity and mortality, with airway obstruction by thick mucus and chronic infections, eventually leading to progressive loss of the respiratory function. Other symptoms include pancreatic insufficiency, elevated sweat electrolytes and male infertility. CF is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene, which is a typical large, multi-exon gene consisting of 27 exons encoding the CFTR protein. CFTR mutations lead to aberrant chloride (Cl^-) transport in epithelial tissues resulting in altered hydration and pH of airway surface fluid, and viscous mucus [1]. Up to date, there are ~2,000 CFTR gene variants reported, a significant fraction (13%) comprises pathogenic mutations that affect mRNA splicing [2]. Such mutations can lead to a significant reduction or total absence of normal CFTR mRNA and thus also of protein expression, being associated with a mild to severe clinical phenotype. Despite novel drugs that correct the defective CFTR protein patients carrying splicing mutations still do not benefit from these therapies. As such, there is an unmet need for novel therapeutic strategies to treat these patients.

Objectives: To characterize four splicing mutations identified in CF patients located in the same splicing consensus (711+1G>T, 711+3A>T, 711+3A>G and 711+5G>A) and correct them using antisense oligonucleotides (AONs).

Methodology: The current proposal comprises the following specific tasks:

1) To generate stable cells lines expressing CFTR minigenes carrying each of the above splicing mutations, 711+1G>T, 711+3A>G, 711+3A>T and 711+5G>A;
2) To characterize each mutation using cell models and patient-derived tissues at the mRNA, protein and functional levels;
3) To use an RNA-based AON strategy to correct the deleterious effect of these mutations and assess its effect at the mRNA and protein levels.

The materials used in this project include: cellular systems and CF patient-derived tissues and the techniques are: quantitative RT-PCR to determine the levels of alternatively spliced mRNA, western blotting to determine CFTR protein levels, immunofluorescence to determine CFTR localization in the cell and ion transport measurements in Ussing chamber to determine CFTR function. In addition, AONs will be designed as previously [3] to explore whether we are able to correct the splicing defect caused by each mutation.

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References

1. Strug LJ, Stephenson AL, Panjwani N, Harris A (2018) Recent advances in developing therapeutics for cystic fibrosis. *Hum Mol Genet* **27**:R173-R186.
2. CFTR Mutation Database: http://www.genet.sickkids.on.ca/app
3. Igreja S, Clarke LA, Botelho HM, Marques L, Amaral MD (2016) Correction of a Cystic Fibrosis Splicing Mutation by Antisense Oligonucleotides. *Hum Mutat* **37**: 209-15.