Plan of Work - MSc Thesis in Biochemistry

Correction of Cystic Fibrosis-causing Mutations with a Traffic Defect by FDA-approved Drugs

Background: Mutations in the *CFTR* gene cause Cystic Fibrosis (CF) – the most common life-threatening inherited disease in Caucasians. Although over 2,000 mutations have been reported, the deletion of a phenylalanine at position 508 (F508del) is the most common CF-causing mutation, affecting ~85% of CF population worldwide [1,2]. This mutation leads to CFTR protein misfolding (*i.e.,* class II CFTR mutation) that is retained by the endoplasmic reticulum quality control (ERQC), thereby precluding its processing and trafficking to the cell surface, being instead targeted for proteasomal degradation [1,2]. Recently, two combinations of CFTR modulators (corrector/potentiator) have been approved as drugs for patients F508del-homozygous [3,4]. However, the excessive cost of these drugs has rendered their access rather limited for patients globally. Based on this limitation, repurposing drugs that are already approved for the treatment of other (unrelated) diseases can unravel effective therapies for CF in an expedite way and at a feasible cost for national healthcare systems. Moreover, there are several CFTR mutations classified in the same defect group as F508del-CFTR [5-8], i.e., having a trafficking defect (class II) but which do not respond to the treatment with the current available CFTR modulators approved for F508del, leaving an unmet need for these patients.

Objectives: To investigate the effects of FDA-approved drugs in rescuing trafficking and function of other class II (non-F508del) CFTR mutations to the cell surface, namely: G85E, I507del, A561E and N1303K.

Methodology: This proposal comprises the following specific tasks:

1) To generate stable CFBE41o- cell lines expressing each of the above CFTR mutations (G85E, I507del, A561E and N1303K);

2) To evaluate the effects of FDA-approved drug in each mutation using cell lines and patients-derived tissues at the protein and functional levels.

The techniques/assays used in this project include: mutagenesis to generate the cell lines, Western blotting to determine CFTR protein levels, fluorescence microscopy to determine CFTR localization in the cell, ion transport measurements in Ussing chamber and forskolin-induced swelling assay in organoids to determine CFTR function.

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