BioISI - Biosystems & Integrative Sciences Institute

Identification of Corrector Drugs for the A561E-CFTR Traffic Mutant

Place of work: BioISI/DQB-FCUL, C8 building (labs 8.3.68 and 8.4.62), (http://bioisi.pt/fungp)

Supervisor: Dr. Miquéias Lopes-Pacheco (mlpacheco@fc.ul.pt), BioISI/FCUL (ORCID ID: 0000-0002-7444-9359)

Co-Supervisor: Professor Margarida D. Amaral (mdamaral@fc.ul.pt), BioISI/FCUL (ORCID ID: 0000-0002-0828-8630)

Background: Cystic fibrosis (CF) is a lethal recessive monogenic disease caused by mutations in the gene encoding the CF transmembrane conductance regulator (CFTR) protein, an anion channel expressed at the plasma membrane (PM) of secretory epithelia [**1**]. Despite recent progress in developing CFTR corrector drugs for F508del-CFTR, the most prevalent CF-causing mutation, there is still a significant number of individuals with CF carrying rare CFTR mutations (so-called 'orphan' mutations) who remain without any effective causative therapy [**1**]. This includes the A561E mutation that, although rare worldwide, has a relatively high prevalence in the Portuguese CF population [**2**]. Similar to F508del, the A561E mutation causes CFTR protein misfolding (*i.e.,* class II CFTR mutation) that leads to its retention by the endoplasmic reticulum quality control (ERQC), thereby precluding its trafficking and processing to the PM, being instead targeted for proteasomal degradation [**2,3**]. Although mutations in the same class are expected to be treated by the same approach (*i.e.,* correctors for class II CFTR mutations), they might not be efficiently rescued by the same chemical compound [**1,4**]. We have recently identified ~50 compounds that are able to rescue F508del-CFTR trafficking and processing to the PM, among experimental and clinically approved drugs for unrelated disease indications. However, the ability of these compounds to rescue A561E-CFTR remains unknown.

Objectives: To investigate the potential ability of F508del-CFTR correctors identified by our group in rescuing A561E-CFTR trafficking, processing and function.

Methodology: The techniques/assays used in this project will include:

1) Site-directed mutagenesis and cloning to generate cell lines expressing A561E-CFTR [**4**];
2) Halide sensitive-yellow fluorescence protein (HS-YFP) assay performed on a plate reader for high-throughput to determine CFTR function [**5,6**];
3) Western blotting to determine CFTR protein levels and processing [**5,7**];
4) Immunofluorescence microscopy to determine CFTR subcellular localization [**5,8**];
5) Ion transport measurements in the Ussing chamber for CFTR functional studies [**4,5**].

Bibliography:

1. Lopes-Pacheco M (2020) CFTR Modulators: The Changing Face of Cystic Fibrosis in the Era of Precision Medicine. *Front Pharmacol* 10: 1662.
2. Mendes F, *et al* (2003) Unusually Common Cystic Fibrosis Mutation in Portugal Encodes a Misprocessed Protein. *Biochem Biophys Res Commun* 311: 665-671.
3. Jensen TJ, *et al* (1995) Multiple Proteolytic Systems, Including the Proteasome, Contribute to CFTR Processing. *Cell* 83: 129-135.
4. Awatade NT, *et al* (2019) R560S: A Class II CFTR Mutation That Is Not Rescued by Current Modulators. *J Cyst Fibros* 18: 182-189.
5. Lopes-Pacheco M, *et al* (2020) Characterization of the Mechanism of Action of RDR01752, a Novel Corrector of F508del-CFTR. *Biochem Pharmacol* 180: 114133.
6. Pedemonte N, *et al* (2011) High-throughput Screening of Libraries of Compounds to Identify CFTR Modulators. *Methods Mol Biol* 741: 13-21.
7. Lopes-Pacheco M, *et al* (2017) Combination of Correctors Rescues CFTR Transmembrane-Domain Mutants by Mitigating their Interactions with Proteostasis. *Cell Physiol Biochem* 41: 2194-2210.
8. Botelho HM, *et al* (2015) Protein Traffic Disorders: An Effective High-throughput Fluorescence Microscopy Pipeline for Drug Discovery. *Sci Rep* 5: 9038.

"Students selected for this project, after thesis registration, are eligible to apply to the BioISI Junior Programme (supporting 8 students with a 6-month Scholarship(BII)), being the selection criterium the academic merit of the candidates."