Characterizing the regulation of the CFTR protein by the kinase GRK5 in Cystic Fibrosis models

Place of work: Functional Genomics and Proteostasis Lab, BioISI (FCUL, C8)

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Bacground: Cystic Fibrosis (CF) is the most common life limiting rare disease in the Caucasian population (~90,000 individuals worldwide). CF is caused by mutations in the CFTR gene, which encodes an anioinc channel located at the apical plasma membrane (PM) of several epithelial cell types. CFTR mutations impair transepithelial chloride transport and lead to a progressive lung disease, the foremost cause of morbidity and mortality in CF. One single mutation– p.Phe508del – accounts for 85% of all CF cases. In the cell, p.Phe508del-CFTR is retained in the endoplasmic reticulum (ER), thereby failing to reach its physiological location at the PM and leading to defective transepithelial anionic transport, which negatively affects the epithelium in the lungs and other organs. Drugs which correct, to some degree, the molecular defects of selected CFTR mutants – such as the Kaftrio cocktail – are already available to most people with CF. Nevertheless, this pharmacotherapy is not available to many individuals and healthcare systems due to its high cost and lifelong need. The supervisor laboratory has recently discovered that the efficacy of the drug cocktail is maximized in CF cellular models when the kinase GRK5 is inhibited with a specific inhibitor known as 9g. The mechanism whereby GRK5 participates in recovering the activity of mutant CFTR is not known and may hold important implications towards improving CF therapy, which this proposal will aim at leveraging.

Objective: Identify the composition of the signaling pathway responsible for regulating p.Phe508del-CFTR traffic and activity through GRK5.

Work plan: This proposal includes the following laboratorial work:

1) Culturing human lung epithelial cell lines expressing the wild type or p.Phe508del CFTR variants, with and without tags for fluorescence microscopy (mCherry-Flag);
2) Performing a genetic screen through automated fluorescence microscopy to identify genes whose expression is essential for the rescue of p.Phe508del-CFTR by the inhibitor 9g. This task will make use of siRNA libraries to silence individual genes;
3) Validation of screen hits through Western blot (biochemical detection of mutant CFTR release from the ER) and HS-YFP (quantification of anionic transport through CFTR);
4) Proposal of a model for the signaling pathway coupling GRK5 inhibition to p.Phe508del-CFTR ER release and activation.

References:

1) Amaral MD et al (2020) CFTR processing, trafficking and interactions. J Cyst Fibros (2020) 19(Suppl 1): S33-S36
2) Botelho HM et al (2015) Protein Traffic Disorders: an Effective High-Throughput Fluorescence Microscopy Pipeline for Drug Discovery. Sci Rep. 5: 9038