

Plan of Work – MSc thesis

Factors Affecting Traffic of Plasma Membrane Proteins: Implications for Cystic Fibrosis and Cancer

Background: The Cystic Fibrosis Transmembrane Conductance Regulator (CFTR, ABC7) and the permeability glycoprotein (P-gp, ABCB1) are both members of the ABC (ATP binding cassette) transporters superfamily, one of the largest in the human genome. CFTR is a chloride/bicarbonate channel expressed at apical membrane of epithelia and a master regulator of epithelial ion and water homeostasis which when mutated causes Cystic Fibrosis (CF), the most common life shortening, genetic disease in Europe [1]. P-gp, also known as multidrug resistance protein 1 (MDR1) is an ATP-dependent efflux pump with broad substrate specificity which pumps many foreign substances out of cells, namely drugs and which plays a significant role in cancer because it confers multiple drug resistance to cancerous cells [2]. It has been widely studied, namely with the goal of identifying effective inhibitors. However, using P-gp inhibitors with the aim of enhancing the therapeutic efficacy of anti-cancer drugs has led to disappointing outcomes [2].

The most frequent CF-causing mutation, F508del – leads to protein misfolding, its retention at the endoplasmic reticulum (ER) by the ER Quality Control (ERQC) that targets it for premature degradation [3,4]. In P-gp, the equivalent mutation to F508del-CFTR is Y490del, which is also misfolded but can be rescued with drug substrates [5]. Another P-gp mutant (G268V), also caused misprocessing of P-gp to even a higher extent than Y490del [6].

We have previously used a double-tagged CFTR construct (mCherry-Flag-F508del-CFTR) to develop a cell-based fluorescence microscopy plasma membrane (PM) traffic assay and automated pipeline to study F508del-CFTR traffic [7]. Using this assay/pipeline, we have performed a loss-of function screen (by knocking-down ~half of the genes in the human genome) so as to identify those that by modulating the ERQC can release F508del-CFTR from its ER retention and thus become potential drug targets for CF (HM Botelho and MD Amaral, unpublished data).

This primary screen identified 227 hit genes which, when inhibited, lead to increased levels of F508del-CFTR in the PM of respiratory epithelial cells. These genes thus deserve being further studied as novel potential drug targets for CF patients bearing the F508del-CFTR mutation, as they hold the potential of correcting the primary cause of CF disease. One of the key questions to be addressed is the specificity of the observed effects on F508del-CFTR. Moreover, the above screen also identified 91 genes which, when knocked-down, further decreased the PM traffic of F508del-CFTR. It is possible that these genes may also affect the traffic of other PM proteins, namely of P-gp.

Objectives: 1) To characterize the specificity of the genes which when knocked down rescue F508del-CFTR PM traffic by testing their effects on P-gp traffic mutants (Y490del and G268V); 2) to determine whether the genes which when knocked down further decrease PM F508del-CFTR traffic also produce the same phenotype on the traffic of wild-type P-gp.

Methodology: The current proposal comprises the following specific tasks:

- 1) To introduce by mutagenesis double tags on wt- and mutant (Y490del and G268V) P-gp constructs (already existing in the lab) and cloning into lentiviral pLVX-puro vector under an inducible promoter;
- 2) To produce lentiviral particles and use them to transduce human cells so as to create stable CFBE41o- cell lines expressing either wt- or mutant double tagged P-gp;
- 3) To use the above mutant P-gp expressing cell lines to determine the intracellular localization of mutant P-gp after transfection with the siRNAs that rescue PM expression of F508del-CFTR by high-throughput fluorescence microscopy, as previously for CFTR [7];
- 4) To use the above wt P-gp expressing cell line to determine the intracellular localization of wt-P-gp after transfection with the siRNAs which further decreased the PM traffic of F508del-CFTR by high-throughput fluorescence microscopy, as previously for CFTR [7];
- 5) To validate the effects of the hit siRNAs from the two above screens (in 3 and 4) on total and processed levels of wt- and mutant P-gp by Western blot.

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Work Place BioISI, DQB-FCUL (C8 bdg)

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