BioISI - Biosystems & Integrative Sciences Institute

A combined PTC read-through and NMD inhibition approach for PTC mutations in CFTR.

Place of Work: Functional Genomics and Proteostasis (FunGP) lab, BioISI - Biosystems & Integrative Sciences Institute, FCUL (C8 Bldg)
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MSc Thesis Project Proposal

A major current challenge in cystic fibrosis (CF) research is developing therapies specific to CF transmembrane conductance regulator (CFTR) mutation classes. Six classes of mutations that may be susceptible to CFTR drug-based rescue are recognized. A significant proportion of the ~2000 potentially disease causing CFTR variants identified to date are Class I mutations, the majority of which introduce an in-frame premature termination codon (PTC). Translation of mRNA transcripts bearing PTCs produces truncated and potentially deleterious proteins, but this is largely avoided by nonsense mediated decay (NMD), a cellular surveillance mechanism. One potential treatment for PTC mutations is the use of read-through agents, which can promote insertion of near cognate amino acids in place of the PTC and allow translation of full-length and potentially functional proteins. However, NMD might lower the potential effectiveness of PTC read-through by removing its mRNA substrate, and additional inhibition of NMD might therefore represent a useful strategy for correction of PTC mutations. We have determined that many PTC mutations in CFTR are subject to similar levels of NMD, which reduce but do not abolish PTC bearing mRNAs (Clarke *et al*, 2019).

This proposal for an MSc project aims to investigate the effectiveness of combining PTC read-through agents and NMD inhibitors, some of which are novel compounds recently identified in our lab, on increasing the stability and abundance of PTC-bearing CFTR mRNA and protein. This approach will be tested in cellular models CF expressing PTC mutations, and will provide a basis for targeted design of read-through approaches to CFTR Class I mutation correction.

We will use several cellular models of CF airway epithelium. Firstly, we will characterize a novel Cystic Fibrosis Bronchial Epithelium (CFBE) cell line stably expressing a CFTR minigene with one of the most common PTC mutations, G542X. This CFTR minigene model will be used alongside human bronchial epithelial (HBE) cells expressing three different PTC mutations (Y122X, G542X, W1282X), which will be used to assess mutation specificity of combined treatments.

Experiments using transcriptional shutdown with Actinomycin-D will also be used to study the stability (ie, rate of degradation) of CFTR mRNA under different conditions and in different cell models. In the HBE cell lines, Western blot will be used to confirm whether stable full length CFTR protein is produced following optimised treatments. For the CFBE cells expressing the G542X-CFTR minigene, incorporated fluorescent (5'-mCherry, 3'-GFP) and tag (Flag tag in intracellular loop) elements will be used to assess the differential effect of treatments on PTC read-through, NMD inhibition and expression of mature CFTR protein.

Several different combinations of known and novel PTC read-through agents and NMD inhibitors will be used to stimulate optimal production of functional CFTR from PTC bearing CFTR mRNA. We expect this work to provide crucial data for the design of rescue strategies specific for this as yet untreated class of mutations.

Bibliography

Clarke LA, Awatade N, Felicio V, Silva IA, Calucho M, Pereira L, Azevedo P, Cavaco J, Barreto C, Bertuzzo C, Gartner S, Beekman J Amaral MD (2019). The effect of PTC mutations on CFTR mRNA abundance in human nasal epithelium and intestinal organoids: a basis for read-through therapies in Cystic Fibrosis. Human Mutation 40:326-334.