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Trafficking defects in Cystic Fibrosis – protein interactions and membrane insertion

Place of work: Functional Genomics & Proteostasis Unit – FCUL/BioISI - Lab 8.3.63/8.4.62

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Background: Cystic Fibrosis is caused by mutations in the gene encoding the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein. To this date, more than 2,100 variants have been described in the CFTR gene, presumably leading to CF. Variants are generally grouped into different classes, to better understand the molecular defect caused by each specific variant. Class II mutations (among which most frequent disease-causing mutant protein F508del-CFTR) lead to CFTR misfolding, retention in the endoplasmic reticulum (ER) and premature degradation [1, 2].
Targeting of CFTR bearing class II mutations to degradation takes place at the ER as a result of an intricate series of events that constitute a quality control surveillance system within the secretory pathway. Several studies have characterized different checkpoints assessing CFTR biogenesis in the ER [2-4], but the mechanistic basis for this surveillance, which includes a large number of protein partners, is still not fully understood. Among these potentially relevant partners is the ER membrane complex (EMC) [5, 6], which seems to be involved in the detection of protein misfolding at the earliest stages of protein synthesis biogenesis. Previous results suggest that the EMC may positively influence the biogenesis of CFTR folding/trafficking mutants.
Characterization of mechanism of disease for different mutations is of special relevance, as response to the currently available drugs (termed correctors) is diverse, even for mutations that cause similar cellular defects.

Objective: The objective of this project is to explore the role of critical protein interactions, in particular the EMC, in the biogenesis of CFTR bearing different class II mutations.

Methodology:
Human bronchial epithelial cell lines (CFBE) expressing wt-, F508del- and other class II mutant -CFTR will be used. Most of these cell lines are already available in the host lab.
Knockdown (KD) of the components of the EMC complex will be achieved by transfection with small interference RNA (siRNA). KD will be confirmed by reverse transcriptase (RT)-PCR and Western Blot. Impact of the KD upon CFTR processing (abundance/presence of the immature and mature forms) will be assessed by WB.
Interactions of CFTR variants with components of the EMC (and the Sec61 translocon, as a control) will be assessed by co-immunoprecipitation of CFTR under low stringency conditions, followed by WB for EMC components.
The effect of EMC KD and the strength of the interactions will be correlated with the response of each variant to CFTR correctors and to its specific domain localization within CFTR structure.

Bibliography:

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