Targeted CFTR Expression in Specific Airway Cell Types

Place of work/: BioISI, DQB-FCUL (C8 building)

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Background: *Cystic Fibrosis (CF) is caused by variants in the CF transmembrane conductance regulator (CFTR) gene encoding a Cl^-/HCO_3^- channel expressed at the apical plasma membrane (PM) of epithelial cells. The variant p.Phe508del is the most common CF-causing variant which leads to defective PM traffic of CFTR. While CF affects multiple organs, lung disease represents the primary cause of morbidity and mortality [1,2]. The airway epithelium is mainly composed of basal, secretory club and goblet, ciliated, neuroendocrine and tuft cells which all play specific roles. The cell types expressing CFTR are still being discussed. For a long time it was believed that ciliated cells are the predominant CFTR-expressing cells. However, pulmonary ionocytes were recently discovered as a novel rare cell type with unexpected high CFTR expression levels. Other reports also describe a CFTR expression in secretory club and basal cells. However, it is necessary to understand which cell types express CFTR under physiological conditions in order to directly target CFTR by possible novel therapeutic approaches. In our previous studies we used a recently described human respiratory multipotent basal cell line from large airways (BCi-NS cells) [3,4] and generated a basal CF cell line by introducing p.Phe508del /p.Phe508del (BCi-CF cells) using CRISPR/Cas9. Both, BCi-NS and BCi-CF cell lines differentiate into the various airway epithelial cell types when cultured at air-liquid interface (ALI). These two novel cell lines are unique models which allow us to investigate CFTR expression in different cell types.*

Objective: *The aim of the MSc work is to identify in which specific cell type(s) CFTR expression contribute(s) to physiological functioning of the airway epithelium.*

Methodology: *The MSc project proposal comprises the following tasks:*

1) *Cloning of cell type-specific promoters into lentiviral CFTR expression vector;*

2) *To generate stable BCi-CF cell lines expressing wildtype-CFTR under cell type-specific promoters.*

The techniques applied within this project include: Mammalian cell culture, PCR based cloning, Western blot, Immunofluorescence microscopy and Ussing Chamber.

References

1. De Boeck K, Amaral MD (2016) Lancet Respir Med 4: 662–674 [PMID:27053340]
2. Riordan et al. (1989) Science, 245(4922), 1066–1073. [PMID: 2475911]
3. Walters et al. (2013) Respir Res 14:135 [PMID: 24298994]
4. Simoes et al. (2019) Life Sci Alliance 2(6):e201900462. [PMID: 31732694]

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.