Characterization of a Small Airway Derived Basal Cell Line to Study the Role of CFTR in Epithelial Differentiation

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Background: Cystic Fibrosis (CF) is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene encoding a Cl^-/HCO_3^- channel expressed at the apical plasma membrane (PM) of epithelial cells. F508del, the most common CF-causing mutation, leads to defective PM traffic of CFTR [1,2].
Besides its function as anion channel, CFTR has also been associated to other cellular processes such as, epithelial differentiation/polarization, regeneration, development, proliferation and when dysfunctional, epithelial-to-mesenchymal transition (EMT) and cancer [3,4]. In differentiation, the presence of CFTR at the apical PM was found to be essential to maintain the normal organization and function of tight junctions [5]. CF airway epithelia also exhibit an overall delay in the differentiation process compared to non-CF [6]. In our previous studies we used a recently described human respiratory multipotent basal cell line from **large** airways (BCi-NS1.1 cells) [7,8] and generated a basal CF cell line by introducing F508del/F508del using CRISPR/Cas9. Both, BCi-NS1.1 and BCi-CF1.1 cell lines differentiate into the various airway epithelial cell types when cultured at air-liquid interface (ALI). Our data show that wt-CFTR expression increases as BCi-NS1.1 cells differentiate, suggesting the importance of CFTR in maintaining a full epithelial differentiation state. In contrast, BCi-CF1.1 cells showed a differentiation defect towards ciliated cells. However, as this can be region-specific, the role of CFTR in the differentiation of **small** airways is still to be investigated.

Objective: The aim of the MSc work is to study the role of CFTR in **small** airway epithelial cell differentiation and determining how F508del-CFTR affects the differentiation into the different airway epithelial cell types.

Methodology: The MSc project proposal comprises the following tasks:

1) Characterization of the small airway basal cells (hSABCi-NS1.1) [9] regarding their differentiation (over 30 days) into different cell types with focus on CFTR and airway specific basal and epithelial cell markers using qRT-PCR, Western Blot (WB) and immunofluorescence (IF);
2) Generation of a CF-hSABCi-NS1.1 cell line by introducing F508del/F508del using CRISPR/Cas9 and investigating the effect on epithelial cell differentiation in comparison to the wt-CFTR expressing cells using methods as in task 1).

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