



[Project CFTR-DIFF-EMT - Supervisor: Margarida Amaral, \(BioISI\) | Co-supervisor 1: Ines Pankonien \(BioISI\) | Co-supervisor 2: Emanuel Gonçalves \(Ophiomics\)](#)

**Title:** Role of CFTR in Epithelial Differentiation and EMT by functional genomics

**Objectives:** Elucidating the mechanisms how dysfunctional CFTR triggers EMT and defective differentiation in Cystic Fibrosis (CF)

**Background:** Cystic Fibrosis (CF), the most common life-threatening, monogenic disorder among Caucasians, is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene, encoding a chloride and bicarbonate channel protein expressed at the apical plasma membrane (PM) of epithelial cells [1,2]. The absence of functional CFTR causes a severe imbalance in ion and water transport affecting mainly the airways and thus being respiratory failure the main cause of morbidity and mortality in CF [1]. The most common CF-causing mutation, F508del, leads to defective CFTR PM traffic [3]. Other CFTR mutations cause decreased protein production, defective channel gating/conductance (e.g., G551D), decreased PM stability or total absence of CFTR protein [1].

CFTR has been associated to other cellular processes than its major function as an anion channel which include epithelial differentiation, polarization, tissue regeneration, proliferation but also cancer [4]. The Amaral group has recently generated a novel unique multipotent basal cell model of airway epithelial differentiation by introducing the F508del mutation into the endogenous CFTR gene of human airway basal cells (BCi-NS1.1), previously shown to be able to differentiate into the multiple airway epithelial cell types [5]. Using this novel cell model (BCi-CF1.1) the group showed that impairment of airway epithelial cell differentiation in CF is a direct consequence of dysfunctional CFTR, as it occurs in the absence of 'secondary CF events', like bacterial infection or inflammation [unpublished data]. However, the mechanisms by which CFTR regulates epithelial differentiation and regeneration are poorly understood.

Moreover, Amaral's group has also recently identified that epithelial-to-mesenchymal transition (EMT), a cellular process during which epithelial cells acquire mesenchymal features, is active in CF. It was found to be mediated through the EMT-associated transcription factor (EMTa-TF) TWIST1 [6]. Additionally, YAP1, another EMTa-TF, has been identified as an important player linking CFTR with EMT and epithelial differentiation (unpublished). However, how the TFs relate to CFTR and which pathways are involved is still not clear.

**Methodology:** Within this PhD project a new human respiratory multipotent basal cell line expressing G551D-CFTR (termed BCi-CF2.1), which traffics to the PM but has no function, will be generated using CRISPR/Cas9 in order to clarify whether it is CFTR PM correct localization and/or CFTR function that stimulate correct epithelial differentiation. Comparing BCi-CF1.1 and BCi-CF2.1 the epithelial/mesenchymal status over a 30-day differentiation period will be assessed by Western blot (WB) and/or immunofluorescence (IF). In parallel, experiments will be performed under CFTR-modulator drugs to correct its localization (VX-445/661) and/or function (VX-770) [7,8].

To further identify the airway cell types which most contribute to CFTR function/expression and related differentiation single-cell RNAseq will be performed on fully-differentiated BCi cell lines (wt, F508del-, G551D-CFTR). This will be complemented with experiments applying different compounds on those cells that lead the epithelium to differentiate towards specific cell types. Differentiated cells will then be analysed by WB, quantitative real-time PCR (qRT-PCR), IF, and Ussing chamber to assess CFTR expression and function.

In order to identify which are the key proteins within the CFTR-YAP1 network that have most influence in the differentiation status (causing EMT) in CF, YAP1 immunoprecipitation in CF bronchial epithelial (CFBE) cells expressing wt- vs F508del-CFTR followed by mass spectrometry (MS) will be performed. Differential wt- vs F508del-CFTR/YAP1-interactors identified by MS and further characterization of CFBE/BCi (wt- and F508del-CFTR) cells will determine their influence over the course of epithelial cell differentiation in the presence of either wt- or F508del-CFTR.

Cell types and states of differentiated BCi cell lines will be identified by processing and analysing the single-cell RNAseq data proposed here using established computational approaches, including methods for clustering and differential expression [9]. Additionally, cell differentiation trajectory and the integration of existing complementary single-cell RNAseq datasets will be explored [10–12]. The transcriptional regulatory network involved in EMT and CFTR will be defined by using the expression of targets of transcription factors (TFs) to estimate *in silico* TFs activities, i.e., a TF is considered active if its targets are consistently upregulated over the rest and vice versa [13]. Systems biology approaches will be applied to represent and model YAP1 protein interactions in CF to determine nodes in CFTR/YAP1 protein network linking CFTR to epithelial differentiation and when dysfunctional to EMT. These will be potential drug targets essential to design precise molecular strategies to correct the differentiation defect in CF.

#### References:

1. De Boeck, K, Amaral, MD. (2016) Progress in therapies for cystic fibrosis. *The Lancet Respiratory Medicine* [PMID:27053340].
2. Riordan, JR, Rommens, JM, Kerem, B *et al.* (1989) Identification of the cystic fibrosis gene: Cloning and characterization of complementary DNA. *Science* (80- ). [PMID:2475911].
3. Amaral, MD. (2004) CFTR and chaperones: Processing and degradation. *Journal of Molecular Neuroscience* [PMID:15126691].
4. Amaral, MD, Quaresma, MC, Pankonien, I. (2020) What role does cftr play in development, differentiation, regeneration and cancer? *International Journal of Molecular Sciences* [PMID:32365523].
5. Walters, MS, Gomi, K, Ashbridge, B *et al.* (2013) Generation of a human airway epithelium derived basal cell line with multipotent differentiation capacity. *Respir. Res.* [PMID:24298994].
6. Quaresma, MC, Pankonien, I, Clarke, LA *et al.* (2020) Mutant CFTR Drives TWIST1 mediated epithelial–mesenchymal transition. *Cell Death Dis.* [PMID:33106471].
7. Middleton, PG, Mall, MA, Dřevínek, P *et al.* (2019) Elexacaftor–Tezacaftor–Ivacaftor for Cystic Fibrosis with a Single Phe508del Allele. *N. Engl. J. Med.* [PMID:31697873].
8. Sermet-Gaudelus, I. (2013) Ivacaftor treatment in patients with cystic fibrosis and the G551D-CFTR mutation. *European Respiratory Review* [PMID:23457167].
9. Wolf, FA, Angerer, P, Theis, FJ. (2018) SCANPY: Large-scale single-cell gene expression data analysis. *Genome Biol.* [PMID:29409532].
10. La Manno, G, Soldatov, R, Zeisel, A *et al.* (2018) RNA velocity of single cells. *Nature* [PMID:30089906].
11. Montoro, DT, Haber, AL, Biton, M *et al.* (2018) A revised airway epithelial hierarchy includes CFTR-expressing ionocytes. *Nature* [PMID:30069044].
12. Plasschaert, LW, Žilionis, R, Choo-Wing, R *et al.* (2018) A single-cell atlas of the airway epithelium reveals the CFTR-rich pulmonary ionocyte. *Nature* [PMID:30069046].
13. Holland, CH, Tanevski, J, Perales-Patón, J *et al.* (2020) Robustness and applicability of transcription factor and pathway analysis tools on single-cell RNA-seq data. *Genome Biol.* [PMID:32051003].



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**Co-Supervisor 2:** Emanuel Gonçalves

**Co-Supervisor 1:** Ines Pankonien

The proposal of 2 co-supervisors is essential here because co-supervisor 1 will keep up with daily tasks in the lab, namely in advanced lab approaches; the second co-supervisor is justified given his unique expertise in Bioinformatics and Systems Biology, which neither supervisor nor co-supervisor 1 have.

**Type of fellowship** (select the correct option)

National

Mixed (Portugal and abroad:)