BioISI - Biosystems & Integrative Sciences Institute

Role of CFTR in Epithelial Differentiation, Regeneration and Cancer

Place of work/: BioISI, DQB-FCUL (C8 building)
Supervisor: Dr. Ines Pankonien (ipankonien@fc.ul.pt) BioISI/FCUL; ORCID 0000-0002-4622-7521
Co-Supervisor**:** Prof. Margarida D. Amaral (mdamaral@fc.ul.pt) BioISI/FCUL; ORCID 0000-0002-0828-8630

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, foetal 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]. Moreover, functional CFTR is required for the rapid regeneration of human airway surface epithelium after injury, shown by a delay in wound healing of CF epithelia [6-7].

Recent studies from the Amaral lab revealed that EMT, a process where epithelial cells acquire mesenchymal features, is indeed active in CF native airways tissue and CF cells. It included 1) destructured epithelial proteins; 2) defective cell junctions; 3) hyper-proliferation; and 4) impaired wound healing [5]. We also recently showed that impairment of airway epithelial cell differentiation in CF is a direct consequence of defective CFTR, as it occurs without 'secondary CF events', as bacterial infection or inflammation (Amaral, unpublished). However, the mechanistic relationship between CFTR and wound healing, differentiation and cancer is not established.

To this end, an automated siRNA high-throughput wound healing live-cell microscopy screen using CFBE cells expressing wt-CFTR or F508del-CFTR has been performed to determine which genes among those rescuing F508del-CFTR traffic also affect wound closure and those that act differently on wt- and or F508del-CFTR expressing cells. We have identified >10 hit genes that either accelerate or delay the wound closure with a differential effect on wt- and F508del-CFTR expressing cells. This knowledge sheds new light on the coupling of these mechanisms of which further investigation will contribute to our understanding of CFTR involvement in epithelial differentiation and regeneration in CF and to identify novel therapeutic targets.

Objective: To investigate genes identified as affecting wound healing and rescuing F508del-CFTR traffic and explore their role on epithelial differentiation and regeneration.

Methodology: The MSc project proposal comprises the following tasks:

1) Characterization of hit genes in CFBE wt- and F508del-CFTR expressing cells using qRT-PCR, Western Blot (WB) and immunofluorescence (IF);
2) Investigation of the effect of the selected gene(s) on epithelial differentiation (over 30 days) in wt- and F508del-CFTR BCi cells measured by qRT-PCR, WB, and/or IF at different time points, looking at specific basal and epithelial cell markers during the differentiation process;
3) Using shRNA for specific genes (selected from task 1 and 2) and lentiviral transfection to generate new BCi cell lines on the background of either wt- or F508del-CFTR and investigate their effect on differentiation (as in task 2).

Bibliography:

1. De Boeck K, Amaral MD (2016) *Lancet Respir Med* 4: 662–674 [PMID:27053340]
2. Riordan, JR et al. (1989) *Science*, 245(4922), 1066–1073. [PMID: 2475911]
3. Amaral MD et al. (2020) *Int J Mol Sci* 21: 3133 [PMID: 32365523]
4. Quaresma MC et al. (2020) *Cell Death & Dis* 11: 920. [PMID: 33106471]
5. LeSimple P et al. (2010) *J Physiol* 588: 1195–1209 [PMID:20156845]
6. Hajj R et al. (2007) *J Pathol* 211: 340–350 [PMID:17186573]
7. Schiller KR et al. (2010) *Am J Physiol Physiol* 299: C912–C921 [PMID:20686068]