The Role of CFTR in Intestinal Epithelial Differentiation

Place of work/: BioISI, DQB-FCUL (C8 building)
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Background: Cystic Fibrosis (CF), the most common genetic disease among Caucasians is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene encoding a $Cl^-/HCO3^-$ channel expressed at the apical membrane of epithelial cells. F508del, the most common CF-causing mutation, leads to defective PM traffic of CFTR. CF affects mostly the lungs, but also pancreas and the intestine with thick mucus clogging the organs [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]. Our recent data from the lab indicate that dysfunctional CFTR impairs airway epithelial differentiation, in particular towards ciliated cells. However, there is not much known about the differentiation in the intestine, another organ that is affected in CF. In our lab, we have established a protocol/assay to generate intestinal organoids from native tissue (rectal biopsies) of individuals with CF in order to measure CFTR-dependent fluid secretion to the interior of the organoid with the consequent organoid swelling induced by forskolin (FIS) [5]. This assay is being used so far on organoids from individuals with CF to assess the rescue of CFTR function by CFTR modulator drugs in a personalized medicine approach [5,6]. Moreover, as the organoids contain stem cells they can be further differentiated from 3D into 2D-monolayers when grown on permeable supports [6]. Preliminary data from the lab show that such cultures express CFTR and that its function can be measured. We here propose the following aim.

Objective: The aim of the MSc work is to investigate the role of CFTR in intestinal epithelial differentiation and to determine if this process is affected when CFTR is dysfunctional.

Methodology: The MSc project proposal comprises the following tasks:

1) Establish and characterize the differentiation of intestinal 3D-organoids into 2D-monolayers over time, assessing CFTR and other intestinal-specific epithelial cell type markers using qRT-PCR, Western Blot (WB), and immunofluorescence (IF);
2) Assess the CFTR function on those 2D-monolayers and further the CF drug response using the Ussing Chamber technique.

Bibliography

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. Amaral *et al.* (2020) *Int J Mol Sci* 21: 3133 [PMID: 32365523]
4. Quaresma *et al.* (2020) *Cell Death & Dis* 11: 920. [PMID: 33106471]
5. Dekkers *et al.*(2013) *Nat Med* 19: 939-45. [23727931]
6. Zomer-van Ommen DD *et al.* (2018) *J Cyst Fibros.* 17: 316-324. [29544685]