BioISI - Biosystems & Integrative Sciences Institute

Characterization of Models to Study CFTR-dependent Ion Channel Activity in Cystic Fibrosis

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Abstract / MSc thesis project proposal:
Cystic Fibrosis (CF), the most common life-shortening genetic disease in Europe is caused by >2,000 different mutations in the CFTR gene, encoding a Cl^- channel expressed at the apical membrane of epithelia and a master regulator of epithelial ion and water homeostasis [1]. Classical CF is usually diagnosed early in life, through a combination of clinical evaluation and laboratory analyses, including a positive sweat test and CFTR mutation analysis [2]. However, in individuals without two CFTR mutations identified or with rare mutations of unknown prognosis, additional laboratory tests should be performed like measurements of CFTR-mediated Cl^-/HCO_3^- secretion in rectal biopsies by micro-Ussing chambers [3,4].
The same biopsies can be used to generate intestinal organoids by isolating the crypts (which contain stem cells) and growing them in the appropriate culture medium with matrigel [5]. This approach, in current use in our lab, allows the measurement of CFTR-dependent fluid secretion by stimulation with forskolin and the CFTR-dependent fluid secretion to the interior of the organoid with the consequent organoid swelling. This is the forskolin-induced swelling (FIS) assay which is performed by confocal microscopy [5]. Moreover, organoids contain stem cells that can further differentiate into 2D-monolayers of epithelial polarized cells [6]. Furthermore, organoids from individuals with CF have been widely used to assess the rescue of CFTR function by CFTR modulator drugs, and as predictors of the individual's response to these drugs, in a personalized medicine approach [5,6]. Previous work from our lab showed that these organoid-derived 2D-cultures express CFTR and can be used to measure basal CFTR function, as well as CFTR rescue by CFTR modulators in Ussing chamber assays. Here, we aim to:

1. Characterize the differentiation status of organoid-derived 2D-monolayers of epithelial polarized cells

2. Assess their potential to evaluate patient drug response in a personalized medicine approach, especially in individuals with rare or unknown mutations, similarly to the FIS assay in 3D-organoids.

In the end of this project we expect to develop a new diagnosis tool that could also be used to assess each individual response to CFTR modulators, allowing for a better prediction of clinical benefit in a personalized fashion.

Bibliography:
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