Plan of Work – MSc thesis

Role of CFTR in cell migration and epithelial wound repair

Background: Cystic Fibrosis (CF), the most common genetic disease among Caucasians, CF is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene encoding a Cl^-/HCO_3^- channel expressed at the apical membrane of epithelial cells. CF affects mostly the lungs and is characterized by mucus stasis, poor mucociliary clearance (MCC), recurrent bacterial infections and chronic inflammation in the airways that promote progressive lung destruction, and eventually lung failure. Upon injury (by pollutants, pathogens, etc), a healthy lung is able to rapidly regenerate and restore epithelial integrity in order to maintain epithelial integrity for an effective lung function. However, in patients with CF these wound healing mechanisms are impaired [1-3]. Several studies have shown that wound healing (a combination of both migration and proliferation) and cell differentiation are both altered in CF [3,4]. Moreover, it was shown that CFTR itself, which is defective in CF, plays a role in airway epithelial repair and vice versa, i.e., its traffic is regulated by epithelial differentiation [4]. However, the mechanistic relationship between CFTR and epithelial wound healing /differentiation has not been established. We have previously characterized the wound healing process in CF and our data indicate that it is mainly migration which is affected in CF. Cells migrating into the wound gap require lamellipodia protrusion at the cell front and the force that directs the plasma membrane is provided by actin polymerization at the leading edge [5,6]. Moreover, our data suggest that it is the efficiency of lamellipodia protrusion (determined by the extent of cytosolic actin assembly [5,6]) that is defective in CF (Pankonien, Amaral, unpublished data). It is known that CFTR is indirectly anchored to actin and this interaction may be needed for actin rearrangements at the plasma membrane [7]. It is also known that the formation of lamellipodia is controlled by Rac (a member of the Rho small GTPases [8]) and that manipulation of Rac1 signalling affects CFTR traffic [7].

Objective: To characterize how CFTR affects the wound healing in human bronchial epithelial (HBE) cells expressing either normal (wt-) CFTR or F508del-CFTR, namely to further explore the relationship between CFTR and lamellipodia protrusion.

Methodology: The current MSc project will explore the role of CFTR in epithelial wound repair and comprise the following specific tasks:

1) Wound healing experiments using live cell microscopy performed in HBE cells with CFTR knocked-down/inhibited or overexpressed/activated;
2) Investigation of the relation between CFTR and the lamellipodia protrusions at the wound edge by immunocytochemistry and confocal (live-cell) microscopy;
3) Assessment of the possible interaction between CFTR and components of the lamellipodia (tropomyosin, myosin and Arp2/3 II, besides actin) by CO-IP and Western Blot.

This study will contribute to our understanding of CFTR involvement in epithelial wound repair and differentiation in CF.

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