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MAPK Activation in Airway Epithelial Cells Expressing Cystic Fibrosis Causing Variants

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MAPK Activation in Airway Epithelial Cells Expressing Cystic Fibrosis Causing Variants

Proposed by Kaitlin Bryce* under the guidance of Dr. Ahmed Lawan

Background

Genetic analysis in a population of patients with the autosomal recessive disorder cystic fibrosis (CF) revealed that up to 70-90% of CF patients have mutations in the human gene, cystic fibrosis transmembrane-conductance regulator (CFTR) [1]. The most common mutation of CFTR is an amino acid (phenylalanine) deletion at position 508, and it affects 1 in ~ 3500 newborns in the United States. CFTR belongs to the family of ATP binding cassette (ABC) transporters, that function to move substances needed for enzymes to bind like ions, drugs, peptides, and vitamins across biological membranes. While the molecular mechanisms underlying the activation of CFTR have been studied for several years, many questions remain unresolved. It is still unclear how protein kinases and phosphatases regulate CFTR activity through the addition and deletion of phosphate leading to the development of CF and related diseases. Dysfunction of the CFTR, in addition to other contributing factors, leads to a variety of clinical symptoms affecting the lungs and gastrointestinal system.

Increased inflammation is a significant feature of CF patient airways. The pathophysiology of CF-related inflammation and the role of CFTR in this process is not clear. Mitogen-activated protein kinases (MAPKs) are established mediators that can control inflammation [2]. We hypothesize that altered MAPK phosphorylation and hence downstream signaling is associated with changes in inflammatory status in human epithelial cell lines lacking the functional CFTR. In vitro cell model systems have been valuable in characterizing the association between CFTR gene and CF considering the limitations in using primary cells and the availability of tissue. There are cell-type specific differences in expression and interaction of CFTR mutants with other proteins such as kinases and phosphatases and ion channels. Recently, an epithelial cell line of human origin that is clinically relevant to CF and CFTR function in vivo has been characterized [3]. A balance between protein kinase and phosphatase activity is critical in regulating the CFTR phosphorylation and its channel activity. There is a need to investigate these mechanisms in a physiologically relevant system, the human epithelial cell line lacking the functional CFTR, to uncover signaling pathways involved in CF and related disorders.

Aims:

1. To determine MAPK phosphorylation in human airway epithelial cell line stably expressing CFTR variants
2. To determine the effect of modulating MAPK activity induced by inflammatory conditions in human epithelial cell line stably expressing CFTR variants.

Hypothesis: The cells lacking the functional CFTR will have altered MAPK phosphorylation and other signaling changes associated with changes in inflammatory status.

Research plan:

Over the first several weeks, CF8Flp-F508del-CFTR and CF8Flp-WT-CFTR will be grown in culture to confluence and starve overnight under low serum before being harvested. Cells will be lysed in the RIPA buffer (25 mM Tris. HCl pH 7.4, 150 mM NaCl, 5 mM EDTA, 1% NP-40, 0.1
% SDS, 1.0 % sodium deoxycholic acid), supplemented with protease and phosphatase inhibitors (5 μg/ml leupeptin, 5 μg/ml aprotinin, 1 μg/ml pepstatin A, 1 mM PMSF, 1 mM benzamidine, 1 mM Na₃VO₃, and 10 mM NaF)[4]. After this process and over the next few weeks, homogenates will be lysed for 30 min on the shaker at 4oC prior to clarification at 11,000 rpm for 15 min at 4oC. Protein concentrations will be determined by using a Pierce BCA Protein Assay kit. Lysates will be resolved by SDS-PAGE and transferred to nitrocellulose membranes, which will be incubated with phospho-specific antibodies followed by enhanced chemiluminescence or fluorescent detection. Lysates derived from CF8Flp-F508del-CFTR and CF8Flp-WT-CFTR cells will be examined for basal phosphorylation of p38 MAPK, JNK and ERK. Subsequently inflammatory conditions will be induced in these cells using LPS, TNFa or IL-1b over time followed by assessment of p38 MAPK, JNK and ERK phosphorylation.

Research outcomes:

These studies, with data collected ideally in ten weeks over the summer, will establish the activation status of p38 MAPK, JNK and ERK in human epithelial cell lines lacking the functional CFTR and will uncover the role played by CFTR in regulating inflammatory responses. Understanding the function of CFTR in inflammatory responses may provide mechanistic insight, promoting therapeutic application and improving treatment and patient care.

References: