

LTRC Concept Sheet # 07-99-0002**Functional characterization of cigarette smoke induced IL-8 signaling****ABSTRACT**

Cigarette smoke has long been accepted as a major causative factor in the development of diseases such as chronic obstructive pulmonary disease (COPD), chronic bronchitis (CB), emphysema and chronic sinusitis (CS). The sputum and bronchoalveolar lavage fluid of COPD, CB and CS patients have elevated levels of Interleukin (IL)-8, the C-X-C chemokine. IL-8 is known to be responsible for large influx of neutrophils in the lungs of these patients. Although NF- κ B mediated IL-8 signaling is well documented, we have preliminary data indicating the presence of alternative pathways/mechanisms of IL-8 induction. We observed PGE-2 mediated IL-8 signaling through the EP-2 receptor and CHOP (C/EBP homologous protein) transcription factor in bronchial epithelial cells. We also identified that proteasome (PS-341/MLN-273, Millenium Pharmaceuticals), histonedeacetylase (4-PBA), NF- κ B (Caffeic acid) and Cox-2 (NS-398) inhibitors can selectively down regulate TNF- α ; or IL-1 β ; induced IL-8 chemokine levels (Vij et al AJRCMB 2007, in press; JBC 2006). We propose that sequential deciphering of IL-8 regulation in inflammatory lung diseases may lead to identification of better and more specific therapeutic target(s), to improve the overall disease pathophysiology and lung function. The present project is designed with an aim to identify the mechanism(s) of cigarette smoke mediated IL-8 induction in COPD, CB and CS. The goal of this proposal is to apply the selective genetic screen, using an Affymetrix microarray and vector-based RNA interference library, to characterize the mechanism of cigarette smoke induced IL-8 secretion in human bronchial epithelial cells. The general hypothesis is that identification and selective silencing or induction of genes involved in cigarette smoke induced IL-8 secretion will lead to complete correction of smoke-mediated proinflammatory signaling and reduction in inflammatory phenotype. The Specific Aim 1 is to identify genes implicated in cigarette smoke extract (CSE) mediated IL-8 induction, we will use an Affymetrix based gene expression profiling followed by functional annotation and characterization of IL-8 secretion pathway. Specific Aim 2 is to identify the gene targets to correct the CSE mediated IL-8 hyper-secretion. We will use high throughput lentiviral vector-based gene targeting coupled to IL-8 chemokine assay. Specific Aim 3 is to examine the safety and efficacies of selective gene interference(s) to improve airway pathology of cigarette smoke induced chronic inflammation in mouse model, and validate the differential expression of these targets in lung tissues from COPD patients with FEV1% predicted of >80, 50-80% and <50%. The COPD samples with severe, moderate and mild emphysema will be compared to tissues from control group with minimal emphysema and normal lung function. We will measure IL-8 chemokine levels in patient serum to correlate IL-8 chemokine levels to the differential expression of selected target. The data from control and treated murine multiple cytokine profile and lung pathology, and human gene expression will be examined to quantify the therapeutic potential of selected target(s). The overall projected outcome is reduction in IL-8 signaling and baseline inflammation. The selected therapeutic targets can be developed for clinical trials in a future proposal.