

Title: Decrement of Nrf2 activity in COPD

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Abstract: Nrf2 is a redox-sensitive bZIP transcription factor that is involved in induction of many antioxidant and xenobiotic detoxification genes. In response to oxidative, Nrf2 detaches from its cytosolic inhibitor, Keap1, translocates to the nucleus, and binds to the antioxidant response element (ARE) in the promoter of target antioxidant and xenobiotic detoxification genes, leading to their transcriptional induction. Our previous studies have shown that (1) disruption of Nrf2 in mice causes early onset and more severe emphysema due to chronic cigarette smoke (CS). The responsiveness of the Nrf2 pathway acts as a major determinant of susceptibility to tobacco smoke-induced emphysema by upregulating antioxidant defenses and decreasing oxidative stress, lung alveolar cell apoptosis and inflammation. (2) Emphysema sensitive strains such as C57BL/6 have low Nrf2 response that contributes to greater oxidative stress relative to emphysema resistant CD-1 mice which has a robust Nrf2 response. (3) Emphysematous lung tissues from mice exposed to 6 months of chronic CS have severe decrement in Nrf2 activity. (4) We recently reported that decline in NRF2-regulated antioxidant genes and total glutathione declines with enhanced oxidative damage and severity of COPD in human tissues (AJRCCM 2008). This pilot study was carried out using limited number of samples from LTRC. (5) Significant decrease in NRF2 protein in advanced human COPD lungs with comparable NRF2 mRNA and KEAP1 protein levels as non-COPD lungs 6) Significant decrease in DJ-1 expression, a NRF2 protein stabilizer (7) miRNA profiling of lungs from Nrf2^{+/+} and Nrf2^{-/-} mice exposed to air and cigarette smoke identified several miRNA dependent miRNAs.

The proposed study will address these specific aims:

- (1) Does decline in lung function during progression of COPD correlate with decrement of Nrf2 activity? We will determine the expression of Nrf2 and its target antioxidant genes along with positive and negative co-regulators at the different stages of COPD.**
- (2) Does decrease in Nrf2 activity in advanced COPD correlate with increased oxidative stress and apoptosis?**
- (3) Global miRNA profiling of normal, mild and severe COPD lung tissue with and without carcinoma to identify novel miRNA/ pathways associated with pathogenesis of COPD.**

To assess Nrf2 pathway, we will measure i) Nrf2 and its target antioxidants (NQO1, GCLM, GPX2, HO-1 and GSTA1); ii) NRF2 co-regulators- a) NRF2 protein stability regulators-KEAP1 and DJ-1; b) co-repressors that affect Nrf2-ARE binding- ATF3 and BACH1; c) novel translational regulator-Nrf2 miRNA. We will measure a) total GSH b) lipid-peroxidation markers as biomarkers of oxidative damage. We will perform multivariate regression analysis to determine association of Nrf2 activity, oxidative damage with COPD severity and plausible role in COPD pathogenesis.

We propose to use the following samples from LTRC, controls non-smokers and smokers (n=8), mild COPD (n=67) and advanced COPD (n=119). To determine the expression of Nrf2 and its target antioxidant genes, we will use real time PCR, immunoblot and immunohistochemical assays. To quantitate oxidative stress and apoptosis in controls and advanced COPD, we will measure the apoptosis by Terminal Transferase dUTP Nick End Labeling (TUNEL) and oxidative stress by 4-Hydroxynonenal (4-HNE) and 8-oxo-7,8-dihydroguanine (8-oxodG) staining. In addition to these, we will perform miRNA microarray on these tissues using Taqman microRNA arrays from Applied Biosystems. Successful completion of this study will establish the importance of Nrf2 in COPD pathogenesis and will identify new pathways which can be targeted to improve the therapeutic outcome.