Probing molecular mechanisms of tobacco smoke-induced lung inflammation via cell-specific multi-omic analysis in a murine smoke exposure model

Andrew T. Rajczewski[1]; Jenna Fernandez[2]; Qiyuan Han[1]; Nicholas A. Weirath[2]; Pratik D. Jagtap[1]; Timothy J. Griffin[1]; Natalia Y. Tretyakova[2]

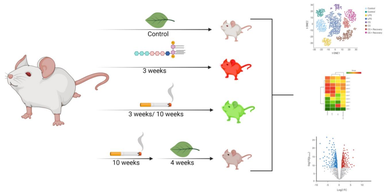
[1] Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, Minneapolis, MN

[2] Department of Medicinal Chemistry, University of Minnesota, MN

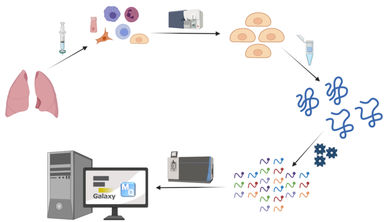
I. INTRODUCTION

- Tobacco consumption has been conclusively linked to lung cancer development
- Evidence suggests that tobacco smoke stimulate oncogenesis through the initiation of inflammation
- Research has shown that oncogenesis is preceded by epigenomic changes
- We performed multi-omic analyses on mouse models of chronic inflammation and tobacco smoke exposure
- Through our analyses, we obtained a comprehensive picture of a tobacco smoke-driven inflammation phenotype and how it contributed to oncogenesis

II. EXPERIMENTAL METHODS



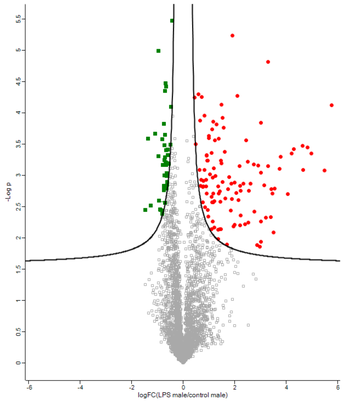
- Mice were kept in plastic holding cages where they were subjected to cigarette exposure for 4 hours a day, 5 days a week for extended periods
  - 3 weeks of exposure
  - 10 weeks of exposure
  - 10 weeks of exposure with a four-week recovery
- As a positive control for lung inflammation, mice were dosed intranasally with lipopolysaccharide (LPS) for 3 weeks
- Following exposure, mice were sacrificed, and tissues removed for further study



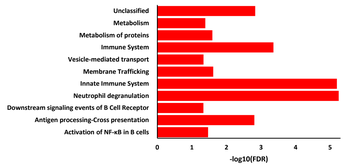
- Lungs were physically separated into their constituents, and type II pneumocytes were isolated via FACS
- Type II cells were disrupted, and the DNA, RNA, and protein isolated for omics analysis
- For proteomics, proteins were subjected to on-bead digestion and subsequently labelled with TMT-11plex isobaric tags
- Samples were fractionated and run on a Fusion Tribrid Orbitrap mass spectrometer before being analyzed in MaxQuant, Perseus, and the Galaxy MSI instance

III. EXPOSURE OF LUNGS TO STIMULI RESULTS IN ALTERED PROTEIN ABUNDANCES

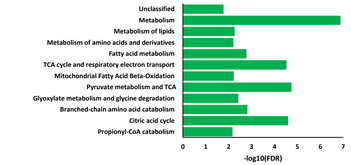
3-week LPS exposure



3-week LPS exposure: upregulated Reactome GO terms

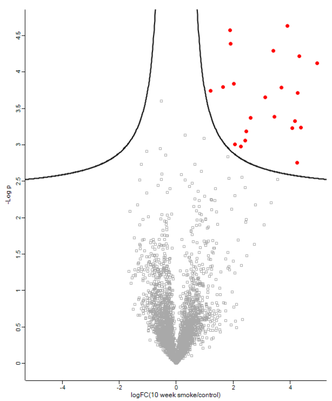


3-week LPS exposure: downregulated Reactome GO terms

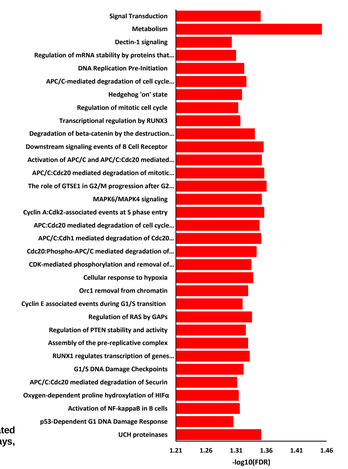


- 3-week LPS exposure results in the increased abundance of proteins associated with immune responses and inflammation as well as a decrease in metabolism-related proteins
- 3-week cigarette exposure results in the increased abundance of two proteins, Ras-related C3 botulinum toxin substrate 1 and Cytochrome b-c1 complex subunit Rieske, mitochondrial

10-week smoke exposure

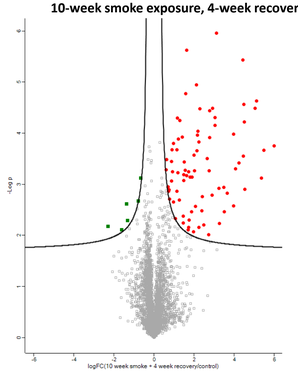


10-week smoke exposure: upregulated Reactome GO terms

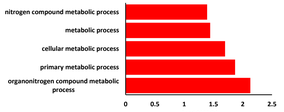


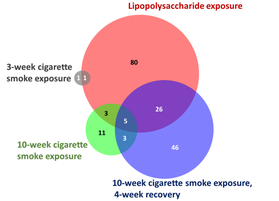
- 10-weeks of cigarette smoke exposure results in an increase of proteins associated with the hypoxia response as well as regulating the cell cycle, signaling pathways, DNA repair, etc.

10-week smoke exposure, 4-week recovery



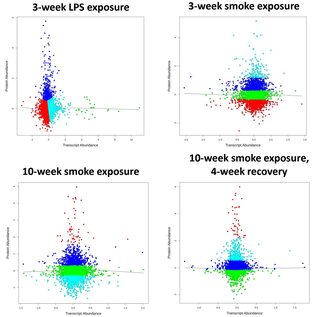
10-week exposure, 4 week recovery: upregulated GO terms





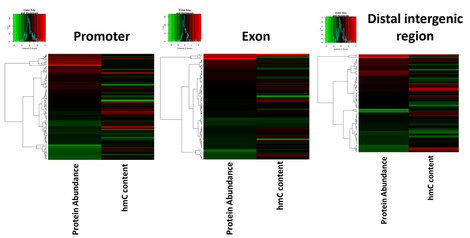
- Mice with a 4-week recovery following a 10-week smoke exposure show altered protein abundances
- The four conditions tested here show little overlap in their proteins showing increased abundance

IV. PROTEIN ABUNDANCE AND RNA EXPRESSION ANALYSES



- RNA transcription and protein expression show little correlation with added stimuli
- LPS shows the greatest disparity between the transcriptome and proteome

V. PROTEIN ABUNDANCE AND EPIGENOMIC CHANGE ANALYSIS



- Correlations between gene hydroxymethylation and protein abundance examined following 10-weeks of cigarette smoke exposure
- No patterns of correlations noted between changes in promotor hydroxymethylation and protein abundance in the promoter regions of genes
- Positive correlation between increased exon hydroxymethylation of Pre-MRNA Processing Factor 40 Homolog B and abundance of that protein
- Strong inverse correlation between the increased hydroxymethylation of associated intergenic distal region and the decreased abundance of Peroxiredoxin-6, SEC14-like protein 2, and cAMP-specific 3',5'-cyclic phosphodiesterase 4D

VI. SUMMARY

- Mice were subjected to tobacco smoke exposure to investigate inflammation-driven changes to Type II pneumocytes
- Proteomics was performed and integrated with transcriptomics and epigenomics to discern
- Short-term exposure of mice to cigarette smoke resulted in few changes at the protein level
- Extended exposure resulted in protein abundance changes related to hypoxia, regulation of DNA repair, signal transduction, and the cell cycle
- RNA transcription and DNA hydroxymethylation were largely decoupled from protein expression

VII. FUTURE DIRECTIONS

- Perform targeted LC-MS experiments and Western blots to validate those proteins observed to be increased in abundance with exposure
- Investigate changes to the microRNA landscape to account for the lack of correlations between the gene expression and observed protein expression
- Investigate the interactions between those proteins observed to be increased in abundance following exposure

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