Reviewer's report

Title: Induction of the interleukin 6/ signal transducer and activator of transcription pathway in the lungs of mice sub-chronically exposed to mainstream tobacco smoke.

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Reviewer: Avrum Spira

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In this manuscript, Halappanavar et al. report results of study characterizing the whole-genome gene expression changes in mouse lung exposed to tobacco smoke. The authors exposed mice to 6 or 12 weeks of mainstream tobacco smoke and then performed microarray profiling of lung tissue immediately post-exposure or 6 weeks after the last cigarette. They identified 79 genes that were differentially expressed between mice exposed to either 6 or 12 weeks of tobacco smoke vs. control mice. (FDR <.05), and found that these genes return to baseline levels 6 weeks after exposure was discontinued. Additionally, BAL on these mice found increases in mononuclear cells post-exposure, with no change in neutrophils. Given the increase in IL-6 expression post-exposure, the authors used ELISA to measure IL-6 in whole lung and found increases at 12 weeks of smoking. Finally, the authors explored downstream signaling events in the IL-6 pathway by measuring, via western blot, protein levels for STAT3, BCL-XL, MCL-1, JAK-1, Gp-30 and SOC3.

This manuscript represents an important advance in understanding the genomic changes in lung secondary to tobacco exposure. Strengths of this manuscript include the study design exploring both subacute and reversible consequences of tobacco exposure, the stringent statistical analysis of the microarray data and correction for multiple comparisons, and validation of select changes at both transcript and protein levels. The manuscript, however, could be strengthened by comparing findings in this study to previously published microarray studies exploring effects of tobacco smoke on human airway/lung tissue, providing clinical relevance to their findings in mice. Additionally, it is unclear whether the changes in gene expression are due to changing cell types within lung (i.e. due to inflammatory cell infiltrate) vs. changes in a given cell type's gene expression profile.

Major issues:

1. The authors have identified a set of genes that are differentially expressed after 6 or 12 weeks of tobacco smoke exposure, with return to baseline post-cessation. Comparison of these gene expression changes to previously published microarray datasets in human airway or lung tissue exposed to cigarette smoke would demonstrate the potential clinical relevance of their findings. While the previously published in vivo human datasets represent
chronic exposure and often involve a single cell type (e.g. airway epithelium), demonstrating overlap in gene expression changes would strengthen the physiological relevance of this smoking mouse model as it is unclear if the degree of exposure mimics that seen in human smokers (i.e. no carboxyhemoglobin measurements reported). Additionally, there are several microarray studies of human lung epithelial cell lines exposed acutely to smoke in vitro that could be used, although the physiological relevance of these datasets is less clear. The authors could use an approach like Gene Set Enrichment Analysis (GSEA) in order to provide statistical confidence for the enrichment of their gene set (79 genes) within the previously published studies.

2. The microarray studies were performed on whole lung tissue and it is unclear whether changes in gene expression with smoke exposure are due changing cell types (i.e. inflammatory cell infiltrates) within lung. The authors have shown a change in mononuclear cell counts (but not neutrophil counts) on BAL, but were there inflammatory cell changes seen histologically in the lung tissue post-exposure? The authors did validate select changes at the protein level by western, but no immunohistochemistry was performed to identify the cell type responsible for the changes. A description of the histological changes to the lung post-exposure and cessation from exposure would help strengthen the biological conclusions of this manuscript including their statement in the discussion that there is “an absence in inflammation in mouse lungs following 12 weeks of exposure to MTS” (p.20).

3. The authors report that samples group by their respective treatment groups when clustered across either all genes on the array or the 79 differentially expressed genes (p.12), but only show results for the 79 genes in Figure 1. The authors should also show, via PCA or via clustering dendogram, the more dramatic finding that samples cluster by treatment group across all genes on the array. Additionally, it is difficult to follow how samples cluster within Figure 1A and whether the mice studied 6 weeks post-cessation cluster with controls. The color scheme in their dendogram is difficult to follow as no color legend is provided.

Minor issues:
1. The authors should provide a rationale for the time points post-exposure (and post-cessation) that were selected for this study.
2. Were there any quality control metrics employed to filter out poor quality microarrays?
3. Figure 4 should include the results for the BAL neutrophil counts as the authors use this negative finding to support their conclusion regarding the absence of inflammation in lung.

Level of interest: An article of importance in its field
Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.