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.

**Version:** 3  **Date:** 15 May 2009

**Reviewer:** Gary M Hellmann

**Reviewer's report:**

Halappanavar et al. have employed gene expression profiling using Agilent oligonucleotide microarrays to investigate responses to sub-chronic cigarette smoke exposures in mouse lung, as well as recovery following a period of cessation. Exposures to the smoke from 2 cigarettes per day for 6 or 12 weeks resulted in 79 genes being differentially regulated. The authors focused on genes related to the IL-6/JAK/STAT signaling pathway for confirmation by RT-PCR and additional protein analysis by ELISA and Western blot. The authors concluded that their data indicates a lack of inflammation from the tested exposures, and propose that a decrease in proteins such as SOCS3 may protect against an inflammatory response at subchronic doses of cigarette smoke. This study adds incrementally to the body of literature that currently exists using similar techniques.

The authors of this manuscript have adequately addressed all of the issues that this reviewer noted in the initial evaluation. The manuscript could be further improved by addressing the following issues:

1) Additions to manuscript text: the new sections of the manuscript appear not to have been proof-read.

2) Figure 1: the changes to Figure 1 provide added clarity, but for Panel A, the hierarchical clustering tree is too crowded, the vertical lines are too thin to discern their color (indicating treatment), and the dense area representing differentially expressed genes is uninformative. In Panel B, additional explanation of the figure in the legend, the text, and the figure itself would make it easier to grasp the salient point- presumably that the treated samples cluster to a different branch than either the sham or recovery samples based on several functional categories of genes.

3) Figure 4: according to the figure legend, the lighter shaded bars represent the sham samples, and the darker bars represent the smoke-exposed samples. According to the figure, total mononuclear cells (Figure 4B) in the sham samples are 3-fold higher at 12 weeks of smoke exposure than in the smoke-exposed group. The manuscript text says the opposite. The authors may wish to verify if the symbols are correct.

4) Figure 6: Protein levels for each experimental group appear to have been
determined from a single lane on a gel (the methods sections says the values represent an average of three autoradiographic exposures, not three independent analyses). The statements regarding the levels of SOCS3 protein (as well as the others) should be interpreted with caution until more rigorously verified.

Overall, the study is still limited by the use of whole lung tissues, where it is not possible to distinguish between a transcriptional response and a shift in the population of cell types in the sample, especially since the observed changes in the levels of the most critical genes (IL-6 and SOCS3) were small.

Nonetheless, the subject of pulmonary response to low levels of cigarette smoke exposure is an important one that is not adequately addressed in the literature. The current manuscript helps address the need for this critical information.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

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

**Declaration of competing interests:**

I serve as a consultant on legal matters including smoking and health.