

# GENE EXPRESSION PROFILING IN LUNG TISSUES FROM MICE EXPOSED TO CIGARETTE SMOKE, LIPOPOLYSACCHARIDE, OR SMOKE PLUS LIPOPOLYSACCHARIDE BY INHALATION

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**Abstract**

The purpose of this study was to investigate whether the co-exposure to lipopolysaccharide (LPS) will heighten the inflammatory response and other pulmonary lesions in mice exposed to cigarette smoke, and thus to evaluate the potential use of this LPS-compromised mouse model as a model for COPD investigation. AKR/J male mice were exposed to HEPA-filtered air (sham control group), cigarette smoke (smoke group), LPS (LPS group), or smoke plus LPS (Smoke-LPS group) by nose-only inhalation. Lungs were collected at the end of the 3 wk exposure and processed for microarray analysis. Clustering and network analysis showed decreased heat shock response and chaperone activity, increased immune and inflammatory response, and increased mitosis in all three exposed groups. Two networks/function modules were exclusively found in the Smoke-LPS group, i.e., the down-regulated muscle development/muscle contraction process and the up-regulated reactive oxygen species production process. Notably, the number of genes and function modules/networks associated with inflammation was reduced in the Smoke-LPS group compared to the LPS group. The most up-regulated gene in the Smoke group, MMP12, is a matrix metalloproteinase that preferentially degrades elastin and has been implicated in COPD development. NOXO1, which was up-regulated in all three treatment groups, positively regulates the expression of a subunit of NADPH oxidase (NOX1), a major source of reactive oxygen species and may play an important role in the pathogenesis of COPD. Serum amyloid A1, which is an acute phase systemic inflammation marker and can be induced by LPS exposure, was significantly up-regulated in the LPS and Smoke-LPS groups. MARCO, a scavenger receptor expressed in macrophages that may play a significant role in LPS-induced inflammatory response, was up-regulated in the LPS group and the Smoke-LPS group, but not in the Smoke group. In conclusion, gene expression profiling identified genes and function modules that may be related to COPD pathogenesis and may be useful as biomarkers to monitor COPD progression. In addition, a LPS-compromised mouse model showed potential as a useful tool for studying cigarette smoke associated COPD (Sponsored by Battelle BSTI IR&D).

**Materials and Methods**

**Animal Treatment and Sample Collection:**

Groups of 6 male AKR/J mice were exposed via nose-only inhalation to HEPA-filtered air (sham control group), or to mainstream cigarette (2R4F) smoke at 250 µg/L wet total particulate matter (WTPM) for 5 hrs followed by 1 hr of HEPA filtered air per day, 5 days/week (smoke group), or to 0.5 µg LPS/mouse for 1 hr/day, twice per week (LPS group), or to cigarette smoke at 250 µg/L WTPM, 5 hrs/day, 5 days/week, plus 0.5 µg LPS/mouse for 1 hr/day, twice per week after smoke exposure (Smoke-LPS group) for 3 consecutive weeks. When not exposed to LPS or smoke, mice were exposed to HEPA-filtered air, so the exposure periods for each group were always 6 hrs per day. Mice were sacrificed in the following morning of the last day of exposure and the right lung lobes from each group of mice were collected into RNAlater®.

**Microarray and Data Analysis:**

The Affymetrix Mouse Genome 430 2.0 microarrays were used. The chip data was analyzed using Affymetrix GeneChip Operating Software (GCOS) version 1.2. The microarray data was imported into Genespring™ (version 7.2, Redwood City, CA) for further analysis. The quality of each sample was first evaluated by visually inspecting the distribution of genes in graphs and by calculating sample similarity values as correlation coefficients. All measurements less than 0.01 were set to 0.01 and gene values on each chip were normalized to 50% percentile. Samples were filtered by flag (marginal or present in at least half of samples), followed by raw intensity value (>50 in at least half of samples). Genes that changed 2-fold or greater in the treated groups compared to the sham control group were identified by filtering with fold change. These genes were subject to one-way ANOVA parametric test using Benjamini and Hochberg False Discovery Rate for multiple testing correction. A P value of <0.05 was considered significant (Fig. 1). The global relationships of individual samples were characterized by principal component analysis (PCA) using the genes that were changed in one or more treatment groups and displayed in a 3-D graph (Fig 2). Differentially expressed gene lists were imported into MetaCore™ (St. Joseph, MI) for functional and mechanistic analysis. A networks for each treatment group was constructed by a strict "direct interaction" algorithm, which connects objects in the differentially expressed gene list by experimentally confirmed physical interactions (Fig 3-5). Genes that were up- or down-regulated more than 10-fold in each group are listed in Tables 1-3. Networks/function modules unique to the Smoke-LPS group were obtained by performing logical operations, i.e., subtracting the Smoke and the LPS networks from the Smoke-LPS network (Fig 6).

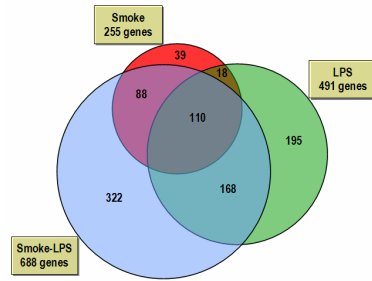


FIG.1. VennDiagram of differentially expressed genes in mice exposed to smoke, LPS, or smoke plus LPS.

The following networks (Fig 3-6) are generated by the direct interactions algorithm in MetaCore™ using lists of differentially expressed genes in each exposure group. Colored symbols represent genes (network nodes). Red solid circles represent up-regulated genes and blue solid circle represent down-regulated genes.

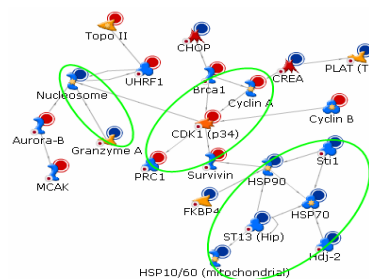


FIG. 3. Gene networks in the Smoke group. One single network was generated. Primary function modules include heat shock response (HSP70, HSP90, etc), mitotic process (CDK1 and Cyclin A, etc), DNA damage check point (Nuclosome, Granzyme A, etc).

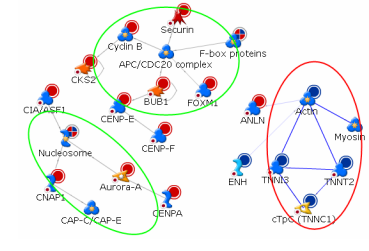


FIG. 6. Subtraction of the Smoke network and the LPS network from the Smoke-LPS network by logical operation. Three small networks unique to the Smoke-LPS were generated. Primary function modules include muscle development/muscle contraction (TNN3, Actin, etc), mitotic process and cell cycle regulation (APC/CDC20 complex, Aurora-A, etc).

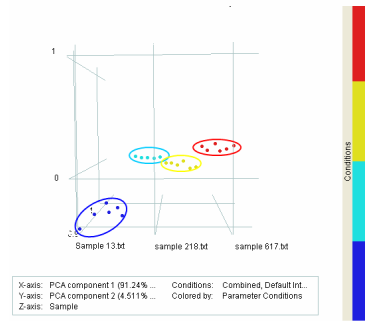


FIG.2. Principal component analysis on conditions of treatment. The sum of differentially expressed genes in three treatment groups (940 genes) were used for the analysis. Condition 1, Sham control group (dark blue); condition 2, LPS group (light blue); condition 3, Smoke group (yellow); condition 4, Smoke-LPS group (red).

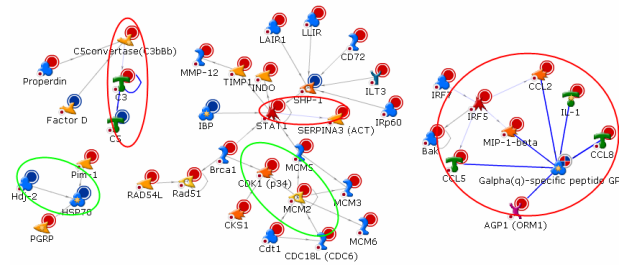


FIG. 4. Gene networks in the LPS group. Four networks were generated. Function modules related to inflammatory response are circled with red. Other primary function modules include heat shock response (HSP70 etc), mitotic process (CDK1, MCM2, etc).

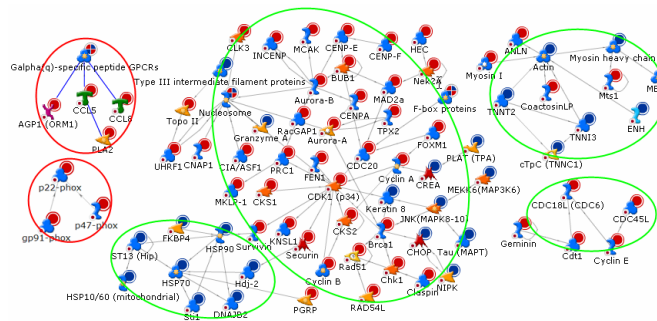


FIG. 5. Gene networks in the Smoke-LPS group. One large network and four smaller networks were generated. Function modules related to inflammatory response are circled with red. Other primary function modules include heat shock response (HSP70, HSP 90, etc), mitotic process and cell cycle regulation (CDK1, Aurora B, Cyclin A, etc), muscle development/muscle contraction (TNN3, Actin, etc), cell cycle regulation (Cyclin E, etc).

Table 1 Genes up- or down-regulated more than 10-fold in the Smoke group

Common Name	Fold Change	Description
Mmp12	18.2	matrix metalloproteinase 12
Noxo1	17.4	NADPH oxidase organizer 1
Saa3	16.3	serum amyloid A 3
Cxcl5	16.1	chemokine (C-X-C motif) ligand 5
Ly6i	16.0	lymphocyte antigen 6 complex, locus I
Slc26a4	12.0	solute carrier family 26, member 4
Hspa1b	-25.4	heat shock protein 1B
Nr1d1	-10.7	nuclear receptor subfamily 1, group D, member 1

Minus values indicate down-regulated genes.

Table 2 Genes up- or down-regulated more than 10-fold in the LPS group

Common Name	Fold Change	Description
Saa1	242.4	serum amyloid A 1
Gm1960	96.9	dendritic cell inflammatory protein 1
Cxcl9	66.3	chemokine (C-X-C motif) ligand 9
Irg1	62.7	immunoresponsive gene 1
Saa3	48.0	serum amyloid A 3
Cbln1	30.2	cerebellin 1 precursor protein
Marco	14.8	macrophage receptor with collagenous structure
Ly6i	19.2	lymphocyte antigen 6 complex, locus I
Bc018473	17.2	2 days neonate thymus thymic cells cDNA
Noxo1	16.9	NADPH oxidase organizer 1
Saa2	15.0	serum amyloid A 2
Orm2	14.2	orosomucoid 2
Cbln1	13.4	cerebellin 1 precursor protein
Cxcl2	11.9	chemokine (C-X-C motif) ligand 2
Cxcl9	10.7	chemokine (C-X-C motif) ligand 9
Hspa1b	-25.4	heat shock protein 1B
Fabp1	-15.2	fatty acid binding protein 1, liver
MGC29978	-14.6	3-ketocoyl-CoA thiolase B

Minus values indicate down-regulated genes.

Table 3 Genes up- or down-regulated more than 10-fold in the Smoke-LPS group

Common Name	Fold Change	Description
Saa1	167.7	serum amyloid A 1
Cxcl9	49.3	chemokine (C-X-C motif) ligand 9
Marco	47.3	macrophage receptor with collagenous structure
Saa3	38.0	serum amyloid A 3
Marco	25.5	macrophage receptor with collagenous structure
Orm2	19.4	orosomucoid 2
KIT22	16.5	kinesin family member 22
Melk	12.8	maternal embryonic leucine zipper kinase
Kntc2	12.0	kinetochore associated 2
Orm1	11.7	orosomucoid 1
Ly6i	11.6	lymphocyte antigen 6 complex, locus I
Ctsk	11.4	cathepsin K
Ccnb1	10.8	cyclin-dependent protein kinase
Hspa1b	-23.7	heat shock protein 1B
Nr1d1	-20.5	nuclear receptor subfamily 1, group D, member 1
Hsp105	-12.6	heat shock protein 105

Minus values indicate down-regulated genes.

**Summary**

- More genes were differentially expressed in Smoke/LPS group (688) than in Smoke (255) or LPS group (491), compared to sham controls.
- Decreased heat shock response and chaperone activity, increased inflammatory response and mitosis were the shared changes in all three groups, but the number of genes in corresponding clusters or function modules/networks varied among treatment groups.
- The number of genes and function modules associated with inflammation were reduced in the Smoke-LPS group compared to the LPS group. This reduction might be attributed to the decrease of inhaled smoke and LPS in the Smoke-LPS mice and the immune- and inflammation-suppressive effects of acute smoke exposure. On the other hand, the inflammatory response in the Smoke-LPS mice was more significant than in the Smoke mice.
- Two networks/function modules were exclusively found in the Smoke-LPS group. One was the down-regulated muscle development/muscle contraction process, and the other one was up-regulated reactive oxygen species production.
- Some highly up-regulated genes (10 to 167 fold), such as Mmp12, Noxo1, Saa1, Marco, Ctsk, are related to proposed mechanisms of COPD development and may represent potential biomarkers for monitoring COPD initiation and progression.
- With some modifications of the current exposure regimen a LPS-compromised mouse model is a potentially useful tool for studying cigarette smoke associated COPD.