Cross-study comparisons of gene expression changes in the lung, nasal epithelium, and liver tissues from C57BL/6 mice exposed to cigarette smoke via nose-only and whole-body exposure systems

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Abstract

Animal inhalation studies performed under different exposure systems may impact the biological responses to exposures. In this study we utilized the data from two chronic cigarette smoke mouse studies with different inhalation exposure systems (nose-only and whole-body exposure systems, NOES and WBES, respectively) and performed cross-study comparisons of transcriptomes from lung, respiratory nasal epithelium (RNE), and liver tissues in cigarette smoke (CS) or Sham (filtered air-exposed) groups. Differentially expressed genes (DEGs; fold change >2 or <-2, at false discovery rate corrected p value <0.05) identified using the Transcriptome Analysis Console, were evaluated for pathway enrichment using Ingenuity Pathway Analysis (IPA). When lung DEGs from Sham groups were compared across 3, 4, and 7 months, the NOES Sham group showed fewer predicted proinflammatory mediators in upstream regulators, as well as suppressed inflammation-related pathways compared to the WBES Sham group. In CS groups, about one half of the DEGs were common in the lung tissues from both exposure systems, resulting in similar top-enriched pathways, mainly involving activation of innate immunity. The unique genes from NOES and WBES reflect the different biological response to these two exposure systems. In NOES, the production of nitric oxide and reactive oxygen species, and atherosclerosis signaling are activated. In WBES, pathways in regulating tumor microenvironment and epithelial mesenchymal transition are activated. In summary, the exposure system-related differences were primarily observed in the RNE tissues from CS group, and both exposure systems induced significant changes in gene expression associated with CS-induced pulmonary diseases.

Introduction

Cigarette smoking causes chronic lung diseases such as chronic obstructive pulmonary disease (COPD). COPD is characterized by abnormal inflammatory responses in the airways and lung, including: (1) oedema which can contribute to increased airway resistance in large and small airways; (2) neutrophil infiltration that is related to increased oxidative stress, and cytokine and elastase release that is associated with airway wall fibrosis and mucus production/plugging; and (3) emphysema contributing to the loss of elastic recoil and small airway collapse at expiration.[1]

Cigarette smoke (CS)-induced COPD development was evaluated using C57BL/6 mice.[3,4] C57BL/6 mice have a moderate deficiency of the serine protease inhibitor Serpina-1 and are susceptible to oxidative stress,[2] leading to emphysema and goblet cell metaplasia upon smoke exposure. In these two studies, Philips et al. used whole body exposure system (WBES) with 3 air-breaks in the 4 h reference CS (3R4F) exposure.[3] Kumar et al. used nose-only exposure system (NOES) in the 4 h continuous CS (3R4F) exposure in the C57BL/6 mice.[4] The study designs and exposure parameters are shown in Table 1.

The objective of this study is to compare the gene expression profiles (transcriptomics) between the two (NOES and WBES) studies and evaluate potential impact of exposure system and schedule, focusing on the key biological and cellular processes related to COPD progression (Table 2). Additionally, Kogel et al. conducted exposure system comparison study on ApoE-/- mice.[5]

Table 1. NOES and WBES Study Design

Study Design	Nose-only Exposure System (NOES)	Whole-body Exposure System (WBES)
Group/Timepoint	1 m 2 m 3 m 4 m 5 m 6 m 7 m	1 m 2 m 3 m 4 m 5 m 6 m 7 m
Sham		
Sham		
Sham		
3R4F		
3R4F		
3R4F		

Daily Exposure regimen: 1h 1h 1h 1h — 3R4F (target 42.1 µg/L nicotine); 1h 0.5h 1h 1h 1h 1h 1h — 3R4F (target 34.4 µg/l nicotine); Fresh air

Exposure Parameters		NOES 7 m 3R4F	WBES 7 m 3R4F
Biomarkers	Plasma Nicotine (ng/ml)	121±41	6.4±0.5
	Plasma Cotinine (ng/ml)	374±106	35.7±6.4
	Blood COHb (%)	42.4±2.5	34.5±7.8
	Urinary Nicotine-N'-oxide (ng/ml)	3610.0±1033.3	7003±1212
Smoke Chemistry	Acrolein (ug/L)	4.81±0.098	3.50±0.24
	Acetaldehyde (ug/L)	32.39±1.15	40.22±2.52
	Formaldehyde (ug/L)	0.49±0.004	0.607±0.047

Table 2. Biological and Cellular Processes related to COPD

Biological processes	Cellular processes	Pathological changes
• Innate immunity • Acquired immunity • Stress response • Cellular activation • Extrapulmonary effects • Somatic mutation • Epigenetics	• Inflammatory cell recruitment/activation • T-cell activation • Mediator release • Autoimmunity • Tissue repair • Apoptosis/Cell proliferation • Cytoskeleton remodeling	• Mucous gland hyperplasia • Small airway obstruction • Emphysema • Atherosclerosis • Cardiovascular disease lung cancer

Methods

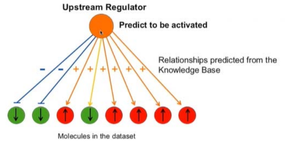
The microarray datasets (E-MTAB-9597, E-MTAB-3150) were dispersed to each treatment group in WBES and NOES. Differential expressed gene (DEG) is the gene expressed over 2-fold, FDR p value < 0.05. In direct comparison, ratio of DEG is NOES over WBES with the same treatment (sham or 3R4F). Sham groups ratio identifies base line difference and 3R4F groups ratio identifies difference from smoke exposure systems.

DEG in sham groups comparison = Nose-only sham / Whole-body sham DEG in 3R4F groups comparison = Nose-only 3R4F / Whole-body 3R4F

To compare potential differences in pathway enrichment analysis, we generated unique gene lists of NOES and WBES under each treatment group and the lists of common DEGs. The unique genes exclusively appear in one batch of WBES or NOES studies.

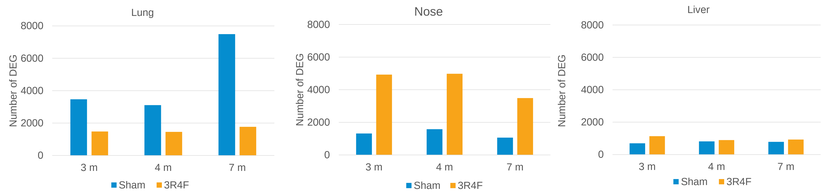
The p value measures whether there is a statistically significant overlap between the dataset genes and the annotated genes in a pathway. The p value was calculated by Fishers Exact Test and significance is generally attributed to p values < 0.01. The z score of pathway is the sum of activation and inhibition of transcriptional regulators in a pathway and is considered significant if >2 (-/+). The transcriptional factor z score calculation is from IPA Qiagen white paper.[5]

Regulator z score $z = \frac{x}{\sigma_x} = \frac{\sum_i x_i}{\sqrt{N}} = \frac{N_+ - N_-}{\sqrt{N}}$ Pathway z score $z = \frac{x}{\sigma_x} = \frac{\sum_i w_i x_i}{\sqrt{\sum_i w_i^2}}$



Results

Figure 1. DEGs in Lung, Nose and Liver Tissue at 3, 4, 7-months



The lung has more DEGs in Sham groups comparison (NOES/WBES), indicating that baseline is affected by exposure system difference. The nose has more DEGs in 3R4F groups comparison (NOES/WBES), indicating that nasal epithelium is more sensitive to different smoke exposure scenarios. The liver has the least DEGs and shows no difference in Sham and 3R4F comparisons.

Figure 2. Cell Stress Related Pathways Across Tissues of Common Genes

The average 3, 4, 7 months DEG number is calculated based on common genes in NOES and WBES. The cell stress-related pathway number indicated overall stress level in that treatment group across tissues. DEG ratio is NOES over WBES groups. The pathways shaded in orange in the heatmap indicated activation in NOES.

Table 3. Top Enriched Pathways of Common or Unique Genes in NOES and WBES Lung Tissue (4-month, CS)

Common genes

Ingenuity Canonical Pathways	-log(p-value)	zScore	Coverage%
Granulocyte Adhesion and Diapedesis	12.7		8.5%
Complement System	11.6	1.34	24.3%
Agranulocyte Adhesion and Diapedesis	9.66		6.5%
Phagosome Formation	9.58	4.8	3.3%
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	6.96	2.45	6.4%
IL-10 Signaling	5.04		8.3%
Acute Phase Response Signaling	4.42		4.3%
IL-17 Signaling	4.39	2.83	4.3%
MSP-RON Signaling Pathway	4.35		8.6%
Phagosome Maturation	3.98		4.4%

Unique genes in NOES

Ingenuity Canonical Pathways	-log(p-value)	zScore	Coverage%
Neuroinflammation Signaling Pathway	8.3	2.67	5.4%
IL-10 Signaling	7.74		12.5%
Granulocyte Adhesion and Diapedesis	7.7		6.9%
Agranulocyte Adhesion and Diapedesis	7.07		6.1%
Role of Macrophages, Fibroblasts and Endothelial Cells	5.75		4.3%
Atherosclerosis Signaling	5.52		6.9%
TREM1 Signaling	5.19	2.65	9.1%
Dendritic Cell Maturation	5.11	3.64	3.1%
Production of Nitric Oxide and Reactive Oxygen Species	4.99	1.9	5.2%
Leukocyte Extravasation Signaling	4.95	2.65	5.2%

Unique genes in WBES

Ingenuity Canonical Pathways	-log(p-value)	zScore	Coverage%
Tumor Microenvironment Pathway	4.82	1.51	6.2%
Circadian Rhythm Signaling	4.3		15.2%
HIF1a Signaling	4.22	0.9	5.3%
Hepatic Fibrosis Signaling Pathway	4.13	0.53	3.8%
Hepatic Fibrosis / Heptic Stellate Cell Activation	3.14		4.6%
D-myo-inositol (1,4,5)-triphosphate Biosythesis	2.88		16.7%
Inhition of Matrix Metalloproteases	2.87		10.3%
Axonal Guidance Signaling	2.77		3.0%
Superpathway of D-myo-insositol (1,4,5)-triphosphate	2.56		13.0%
Regulation Of The Epithelial Mesenchymal Transition Pathway	2.56	0.38	4.2%

The most significantly changed pathways in common genes are related to activation of innate immunity. The unique genes in one dataset of WBES or NOES show the different biological response to these two smoke exposure scenarios. NOES has activated production of nitric oxide and reactive oxygen species and atherosclerosis signaling. WBES has activated tumor microenvironment pathway and epithelial mesenchymal transition.

Conclusions

1. NOES is more sensitive to capture adverse effect in nasal cavity. Despite the slight difference in enriched pathway of lung tissue, both exposure systems present COPD related pathological changes.
2. The lung demonstrates more pronounced differences in the baseline (Sham group). Nose is more sensitive to different exposure systems when exposed to smoke. Liver is the least affected organ. (Figure 1)
3. Activation of innate immunity-related pathways are commonly predicted in WBES and NOES CS groups at all time points. (Table 3, common genes). The lung tissue of mice exposed to smoke in NOES showed activation pathways in nitric oxide and reactive oxygen species, whereas WBES showed activation of tumor microenvironment pathways. (Table 3, unique genes)

References

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