Systems Tox Assessment to Investigate Gene Expression Changes and Disease Correlation Following 3R4F and Test Red Exposure in C57BL/6 Mice

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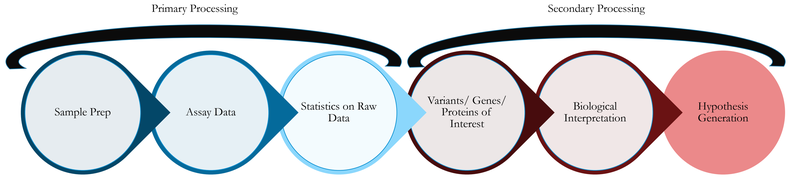
Abstract

Chronic exposure to cigarette smoke (CS) can cause inflammation and oxidative stress which leads to various diseases including chronic obstructive pulmonary disease (COPD) and cancer. As a potential reduced-risk (RR) alternative, e-vapor products deliver nicotine with significantly reduced amounts of harmful and potentially harmful constituents compared to CS. Molecular endpoints, such as transcriptomics, can aid in the mechanistic understanding of the RR potential of e-vapor aerosols in comparison to CS. Here, we analyzed transcriptomic data from lung tissue of C57BL/6 mice exposed to CS (3R4F) or e-vapor (Test Red) for up to 3, 4, or 7 months to evaluate the mechanistic correlation between changes in gene expression and known adverse outcome pathways. Data were first processed with Transcriptome Analysis Console (TAC) to determine the differentially expressed genes (DEGs) which were subjected to Qiagen's Ingenuity Pathway Analysis (IPA). The IPA database provided the biological relevance of molecules, genes, networks, and disease pathways changed by either exposure. We first compared 3R4F vs Sham, Test Red vs Sham, and 3R4F vs Test Red groups, followed by time-dependent changes over 3, 4, and 7 months. The 3R4F exposures induced COPD-related pathways including IL-6 Signaling, IL-17 Signaling, Inflammasome Pathway, and Production of Nitric Oxide and Reactive Oxygen species in Macrophages indicating oxidative stress and inflammation. The Tumor Microenvironment Pathway and Cell Cycle Regulation highlight additional pathways relating to cancer. These pathways also correlated with Disease and Function pathways such as Production of Reactive Oxygen Species, Proliferation of Immune Cells, Inflammatory Response, and Cancer indicating mechanistic changes typically seen in COPD. The Test Red exposure yielded no significant results using IPA. Comparison using IPA implicated similar networks and disease pathways across 3, 4, and 7 months between 3R4F vs sham and 3R4F vs Test Red groups. Overall, the results suggest that compared to 3R4F exposures, Test Red aerosol exposures induced substantially fewer changes in gene expression pathways associated with chronic lung diseases.

Materials and Methods



Primary Processing: The raw CEL files and metadata table for dataset E-MTAB-9597 were downloaded from Array Express. Dataset is comprised of several CEL files corresponding to transcriptomics data from lung tissue of different experimental groups (Figure 1). The CEL files were processed in Transcriptome Analysis Console (TAC) v. 4.0.2.15 (Thermo Fisher Scientific, Waltham, MA). TAC implements affy and limma R Bioconductor packages for processing of microarray datasets for the differential expression (DE) analyses. A probe set is considered differentially expressed in a comparison, if the fold change (FC) is greater than 2 or less than -2, at False Discovery Rate (FDR) p value of 0.05.

Secondary Processing: Differentially expressed genes from 3 months, 4 months, and 7 months within each groups 3R4F vs Sham, Test Red vs Sham, and 3R4F vs Test Red (Figure 1) were imported into Qiagen's IPA (version 65367011; build ing_diamond). In addition, Switching (exposed to Test Red after first 3 months on CS exposure) and cessation (exposed to air after first 3 months on CS exposure) groups were also compared against Sham. To evaluate gene expression and adverse outcome pathways and understand effects of exposure and duration most nodes (example: canonical pathway, disease, function, etc.) pertinent to exposure were selected. Core Analysis of each group at each time point was performed using these parameters for initial biological insights. Later, Comparison Analysis of canonical pathways was performed between 3 months, 4 months, and 7 months of each group using p-value of <0.05, absolute Z-score of 2, and trend+score. Same parameters as Comparison Analysis were repeated for Disease & Function and Upstream Analysis. Biological insights about inflammation, oxidative stress, cancer, and COPD were evaluated using genes and diseases under each analysis.

Materials and Methods (continued)

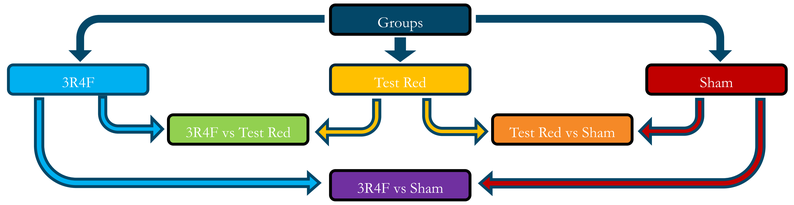


Figure 1: Test groups include 3R4F and Test Red with the control Sham. Test group pairs were generated using ratios with common denominator of either Sham or Test Red to make comparisons.

Results

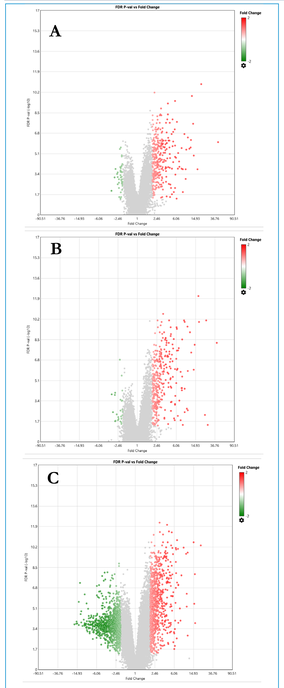


Figure 2: Volcano Plots generated upon Primary Processing demonstrate significant differentially expressed genes for
A. 3R4F vs Sham after 3 months
B. 3R4F vs Sham after 4 months
C. 3R4F vs Sham after 7 months

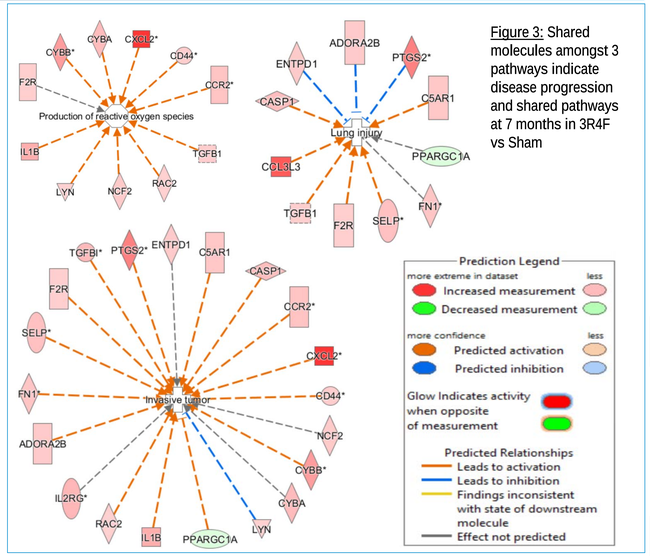


Figure 3: Shared molecules amongst 3 pathways indicate disease progression and shared pathways at 7 months in 3R4F vs Sham

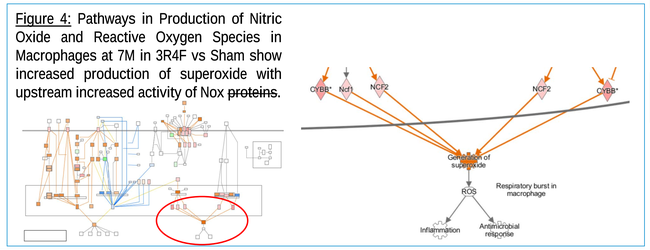


Figure 4: Pathways in Production of Nitric Oxide and Reactive Oxygen Species in Macrophages at 7M in 3R4F vs Sham show increased production of superoxide with upstream increased activity of Nox proteins.

Results (continued)

Table 1 Canonical Pathways

	3R4F vs Sham			3R4F vs Test Red			Switching vs Sham		Cessation vs Sham	
Pathways	3M	4M	7M	3M	4M	7M	4M	7M	4M	7M
Dendritic Cell Maturation	3.578	3.9	4.2	3.153	4.379	3.578	N/A	2	1	2.646
Neuroinflammation Signaling Pathway	2.524	2.982	3.43	1.604	2.982	2.183	--	--	N/A	3.162
Production of Nitric Oxide and Reactive Oxygen Species in Macrophages	2.333	2.53	2.985	2.333	3	2.496	--	--	--	--
T Cell Receptor Signaling	2.53	3.317	3.441	2.121	3.464	3.317	--	--	N/A	2.236
Antioxidant Action of Vitamin C	-2.646	-2.646	-1.89	-2.646	-2.646	-2.121	--	--	--	--
Leukocyte Extravasation Signaling	2.309	2.887	2.711	2.53	3.051	1.941	--	--	--	--
IL-6 Signaling	1.342	2.449	2.111	2	2	0.378	--	--	--	--
IL-8 Signaling	3	2.828	3.273	2.828	3	1.897	--	--	--	--
Tumor Microenvironment Pathway	3.606	3	3.273	3.317	3.317	3	--	--	--	--
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	2.828	3	2.673	2.646	3	2.333	2	1	2	2.236
Phagosome Formation	6.008	5.376	5.582	5.831	5.754	4.824	2.646	-0.302	3.317	2.646
IL-17 Signaling	3.742	3.464	3.5	3.464	3.742	3.051	--	--	2	N/A
Crosstalk between Dendritic Cells and Natural Killer Cells	2.828	2.828	1.897	2.646	3	2.449	--	--	--	--

Table 2 Disease and Function

	3R4F vs Sham			3R4F vs Test Red			Switching vs Sham		Cessation vs Sham	
Pathways	3M	4M	7M	3M	4M	7M	4M	7M	4M	7M
Lung injury	1.514	2.462	2.887	2.195	2.362	2.304	--	--	--	--
Invasive tumor	3.083	3.164	4.951	N/A	2.305	N/A	--	--	--	--
Metabolism of reactive oxygen species	3.141	3.317	4.637	--	--	--	--	--	--	--
Production of reactive oxygen species	3.25	2.977	5.008	3.461	3.404	3.617	--	--	--	--
Metastasis	3.218	3.064	4.859	2.631	2.453	2.407	--	--	--	--
Cancer	3.685	3.108	3.953	3.057	2.558	2.876	--	--	2.168	1.152
Inflammatory response	2.771	3.886	3.137	3.16	3.73	2.736	--	--	0.892	2.712

Tables 1 and 2: Canonical Pathway and Disease and Function progression following cigarette smoke and e-vapor exposure

* Values represent Z scores with positive numbers indicating pathway progression and negative indicating pathway inhibition

Conclusions

- More genes are impacted progressing from 3 months to 7 months in 3R4F group with no significant outputs for Test Red Group
- 3R4F exposure induced most canonical pathways representing inflammation, oxidative stress, and cancer-like genetic changes
- Disease and function analysis represent an increased role of the immune system when exposed to 3R4F
- Switching and Cessation groups show some indication of pathway reversal approaching initial state

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