Genomic Insights into Apoptosis and Cytokine Signaling in Long-term IQOS-exposed Mouse Lungs

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INTRODUCTION

Heat-not-burn (HNB) products like IQOS are a modern tobacco alternative designed to produce an aerosol with fewer harmful by-products than regular cigarettes ("E-Cigarettes, Heat-Not-Burn and Smokeless Tobacco Products," 2020). Marketed as a reduced-risk option, they still pose health risks due to nicotine content. While HNB-manufacturer studies suggest lesser short-term toxic effects, independent research indicates increased acute cytotoxicity, oxidative stress, and inflammation (Ghazi et al., 2024). Long-term effects remain largely unclear, but recent animal studies show potential lung damage like traditional smoking (Gu et al., 2023; Nitta et al., 2022). Novel bioinformatic methods could offer additional insights into long-term HNB exposure effects. The aim of this study was to identify new genetic drivers and pathways in long-term IQOS-exposed mice lungs using a previously published microarray dataset through integrated Differential Expression and Pathway analysis (iDEP) (Ge et al., 2018).

METHODS

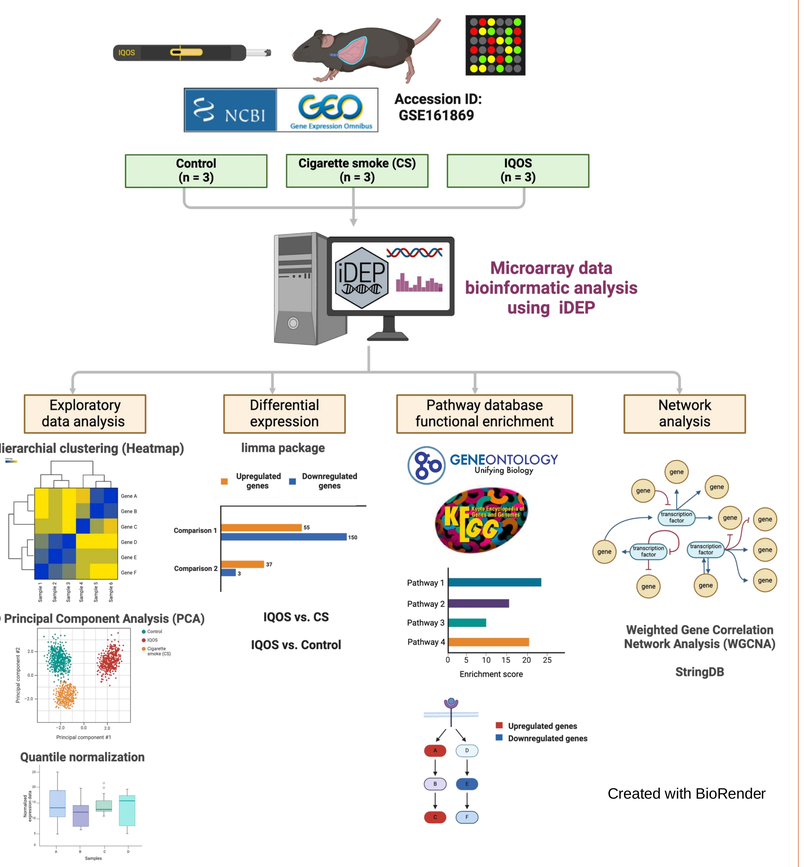

Figure 1. The study compared differential gene expression between IQOS-exposed and control mice and between IQOS-exposed and cigarette smoke (CS)-exposed mice from the mouse lung microarray data from Nitta et al. (2022) after the initial exploratory data analysis (EDA). Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway databases were used in functional enrichment analysis. Network analysis was conducted using Weighted Gene Correlation Network Analysis (WGCNA) and StringDB.

RESULTS

Exploratory data analysis revealed robust sample clustering patterns and uniform distribution of expression data across all experimental groups via hierarchical clustering, principal component analysis (PCA), and quantile normalization

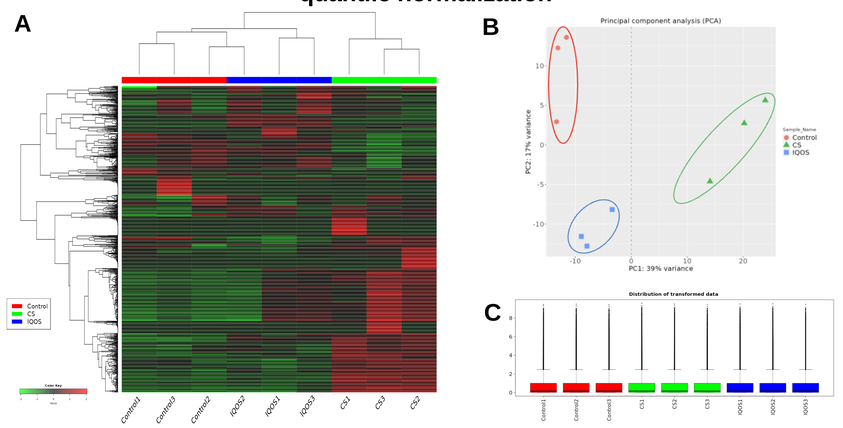

Figure 2. Hierarchical clustering **(A)** and PCA **(B)** of the microarray dataset indicated that overall expression profiles across control and IQOS groups were similar, whereas the CS group had a distinct gene expression signature. Quantile normalization **(C)** demonstrated consistent distribution of expression data across all experimental groups.

IQOS had more downregulated genes than CS, but more upregulated genes than controls

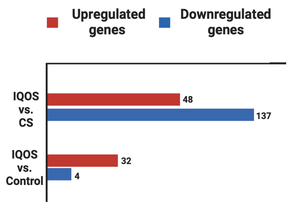

Figure 3. A total of 185 genes (48 upregulated [red] and 137 downregulated [blue]) were differentially expressed between the IQOS-exposed and CS-exposed groups. In the comparison between the IQOS-exposed and control groups, 36 genes (32 upregulated [red] and four downregulated [blue]) were differentially expressed.

GO and KEGG analysis revealed that long-term IQOS exposures reduced apoptosis and oxidative stress compared to CS, while increasing immune and cytokine responses relative to controls

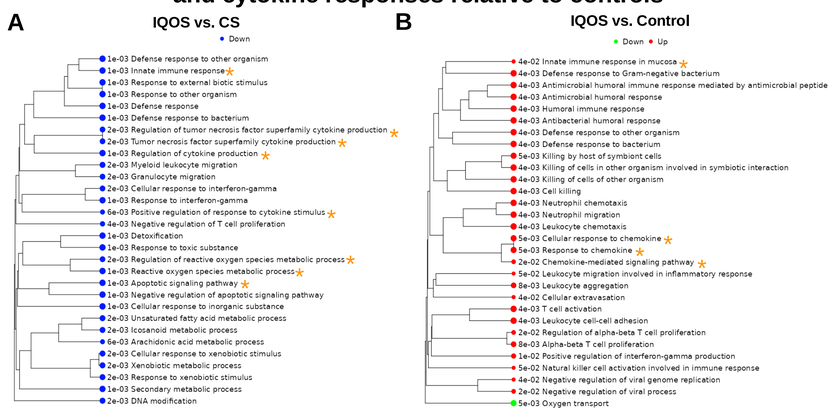

Figure 4. Pathway enrichment revealed that chronic IQOS exposure suppressed apoptotic signaling, cytokine production, oxidative stress, and innate immune responses compared to CS exposure, but enhanced chemokine responses and innate immune responses compared to controls. In both tree maps in panels **(A)** and **(B)**, gene sets closer together share more genes. Dot sizes indicate adjusted enrichment p-values, and orange asterisks indicate the relevant pathways.

Weighted gene correlation network analysis (WGCNA) and StringDB network analysis revealed novel genes involved in apoptotic and cytokine signaling pathways after IQOS exposures

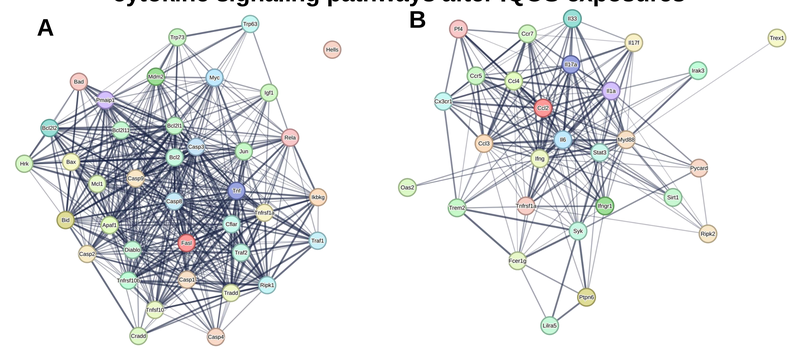

Figure 5. Thirty-eight apoptotic and 28 cytokine signaling genes from WGCNA were visualized as a medium-confidence network in StringDB. The multi-colored circles in panels **(A)** and **(B)** represent genes, with thicker lines showing stronger associations.

DISCUSSION AND CONCLUSIONS

- Apoptosis and oxidative stress were suppressed after chronic IQOS exposures compared to CS exposures, but not in comparison to controls.
- Innate immune responses and cytokine signaling were more activated after chronic IQOS exposures than controls but were suppressed compared to CS exposures.
- Novel apoptotic and cytokine signaling-related genes were identified as modulated upon long-term IQOS exposures, laying the groundwork for future studies.
- While HNB products like IQOS may pose less harm than traditional cigarette smoking in the long term, they still present risks compared to healthy controls. Stricter regulation and further investigation into their potential health effects are necessary.

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