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Genetic Impact of Tobacco Smoke on Blood and Airway Epithelium: A Transcriptional Profiling Study

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Short Title: Transcriptional Impact of Tobacco Smoke on Blood and Airway Cells
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Abstract

Tobacco smoking poses significant health risks due to its carcinogenic and toxic components. While the broad effects of smoking are well-documented, the specific genomic consequences on different body tissues remain less understood. This study seeks to address this knowledge gap by systematically analyzing the genetic alterations in blood and airway epithelium tissues of smokers, offering a clearer understanding of the molecular pathways impacted by tobacco exposure. In this study, we employed microarray-based gene expression analysis to investigate the genomic changes in smokers. Employing gene expression datasets (E-MTAB-5279 and E-GEOD-10006) from smokers and non-smokers, we conducted a detailed analysis to identify differentially expressed genes (DEGs) in blood and epithelial tissues. Our methodology included robust multi-array average processing, Limma package analysis for DEGs, and pathway enrichment analysis using the KEGG database and Gene Ontology (GO) tools. The analysis revealed 584 DEGs in the blood dataset, with 411 downregulated and 173 upregulated genes, highlighting pathways related to thermogenesis, Parkinson's disease, Alzheimer's disease, and Huntington's disease. Key genes such as ATP5C1, ATP5J, COX6C, COX7B, COX7C, NDUFA4, NDUFA5, NDUFB3, NDUFS4, UQCRB, and UQCRQ were notably enriched. In the airway epithelium dataset, 147 DEGs were identified, including 101 downregulated and 46 upregulated genes, with a significant enrichment in the ribosome pathway, particularly in genes like RPL23. In the airway epithelium dataset, we identified 147 differentially expressed genes (DEGs), consisting of 101 downregulated and 46 upregulated genes. Notably, there was a pronounced enrichment in the ribosomal pathway, exemplified by genes such as RPL23. This finding highlights a critical cellular response to tobacco smoke, emphasizing alterations in protein synthesis mechanisms within the epithelial tissues. In conclusion, our findings provide a comprehensive view of the genomic changes induced by tobacco smoke in different tissues, enhancing our understanding of smoking-related pathologies and potentially guiding future therapeutic strategies. This research not only fills a crucial gap in the understanding of tobacco's genomic impact but also lays the groundwork for improved public health interventions.

Keywords: Smoking, Gene Expression, Protein-Protein network, gene enrichment.

Introduction

Tobacco smoke is a complex mixture containing more than 5,000 compounds that are the main carcinogenic and toxicity risk factors (1). Approximately 1.3 million people are affected by tobacco smoke worldwide. Unfortunately, tobacco smoke and its contents are dangerous to the health of people who inhale it; this leads to 5 million annual deaths per year (1-6). The substance contains many carcinogens and over 100 tumor promoters (7). Once combusted, tobacco releases aromatic polycyclic hydrocarbon compounds that are laced with carcinogens (8). These dangerous products cause a variety of cancers, including oral cancer, lung cancer, and liver cancer (5). Continued abuse of the substance, while undergoing cancer treatment, is another problem because it reduces the effectiveness of the therapeutic process that facilitates healing (9, 10).

With a proper understanding of the components and causes of dreadful diseases, such as cancer, it can be possible to easily control them. Past research has shown that continued inhalation of tobacco smoke inflicts serious injuries on the epithelial cells of the oral mucosa that function to physiologically block outside stimuli (11). Additionally, tobacco smoke causes genetic disruption in that it prevents the expression of the genes in epithelial cells and tissues (12). Experimental findings have reported allelic loss in epithelial cells among most smokers (10-11). Picket at al. confirmed this claim in his empirical research, which showed that exposure to smoke from 10 different cigarettes leads to an alteration of 21 genes. Spira et al. (13) also used transcriptome profiling and found that smoking induces the formation of genes that participate in xenobiotic metabolism and stress among the epithelial cells of the airwave. In theoretical terms, it is possible to develop a transcriptome bio-marker to purposely offer protection against the effects of tobacco smoke based on identifying its carcinogenic effects (12). In this study, we obtained microarray data (E-MTAB-5279) and (E-GEOD-10006) to identify the differentially expressed genes (DEGs) in human blood and epithelial tissue in a cohort of smokers and non-smokers. We constructed a protein protein interaction (PPI) network and performed a pathway enrichment analysis with the aim of evaluating the transcriptional profiling of smokers' blood vs. smokers' epithelium cells.

Materials and Methods

We obtained gene expression datasets (E-MTAB-5279, E-GEOD-10006) from the NCBI Gene Expression Omnibus database, which can be retrieved from http://www.ncbi.nlm.nih.gov/geo/. The E-MTAB-5279 dataset contains transcriptional profiling data of blood from 30 smokers and 29 nonsmokers. The E-GEOD-10006 human airway epithelium dataset contains the expression profile of 38 smokers and 22 non-smokers. We conducted the experiments on the microarray platform in the Affymetrix Human Genome U133 Plus 2.0 Array (14-19).

DEGs and data processing

A Robust Multi-array Average (RMA) is an algorithm, which, when expressed in the R affy package, shows how the raw data were transformed into values using three processes: correction of the background, quantile normalization, and summarization of the probe (20, 21). The Limma package for analyzing gene expression data and t-tests further helped to evaluate the DEGs between smokers and non-smokers in R language. The method proposed by Benjamini and Hochberg was used to adjust the p-values in the false discovery rate system (FDR) (22). |The log2FC| >0.5 cutoff value was considered for the DEGs screening. Signal pathway enrichment and gene ontology (GO) (http://geneontology.org/) were used to analyze the DEGs (23), and David Panther (http://www.pantherdb.org/) (24) was the specialist in charge of the GO assessment and analysis as well as the analysis of the molecular functions, cellular components, and biological processes of the DEGs from the online database. Signaling pathway enrichment analysis was performed based on the method described at https://string-db.org/ of the KEGG pathways (23, 25).

Analysis of the modules and construction of the PPI network

The STRING database provided a repository location for searching the proteins that were used to identified the DEGs of the translated protein interactions (http://www.string-db.org/) (25). A visualized PPI network was then constructed using Cytoscape software (http://www.cytoscape.org/) (26). CFinder (http://www.cfinder.org/) was used to perform the cluster analysis of the PPI network. CFinder further provided a strategy for using the Clique Percolation Method algorithm to identify the network communities that are associated with k-clique (27). The complete sub-graphs have a size of k, and investigations on the numerical and analytical k are complete. For the k-cliques, values >10 are the cut-off criterion, while lower limits meet the cut-off.

Results and Discussion

Table 1: Top 5 Key Differentially Expressed Genes Identified in Blood and Airway Epithelial Tissue Datasets.

Tissue	Down-regulated genes	GLOD5	-1.0197872	0.000756157
		KIF18B	-0.869690246	0.000276436
		FAM3B	-0.862153434	0.007747086
		KIF11	-0.779838322	0.009182435
		TRIM6	-0.774527115	0.000357933
	Up-regulated genes	NKX3-1	0.754500681	0.033152755
		KANSL1-AS1	0.773544043	0.013858872
		XIST	0.802600214	0.459222892
		KANSL1-AS1	0.829954953	0.006201343
		HLA-DQA1	0.834636602	0.30136265
Blood	Down-regulated genes	HLA-DQA1	-2.014157659	0.053050403
		CALHM6	-1.259741283	3.39517E-06
		RPL22L1	-1.156703989	5.55541E-06
		MRPL47	-1.048886686	1.61243E-06
		KLRF1	-1.038081556	1.81444E-05
	Up-regulated genes	HLA-DQA1	1.307354875	0.110136451
		TRMT2B	1.32735461	3.86571E-06
		HLA-DRB4	1.345821935	0.082936296
		PAPOLA	1.399027932	1.01949E-05
		LRRN3	1.668079326	3.61436E-08

Identification of the DEGs

In the E-MTAB-5279 dataset, composed of 411 downgraded genes and 173 unregulated genes, a total of 584 DEGs were screened, as shown through heatmap (Figure:1B). In the E-GEOD-10006 dataset, we further screened 147 DEGs, which consisted of 101 downregulated and 46 upregulated genes (Figure:1B). The top 5 most significant genes are shown in Table 1.

DEGs gene ontology analysis

The GO analysis classified the DEGs into three groups: biological processes, molecular functions, and biological processes. In the E-MTAB-5279 dataset, the upregulated genes were highly involved in the MHC class II protein complex (GO:0042613) (Figure:2). Molecular function (GO:0003674) and binding (GO:0005488) molecular function were highly involved in the downregulated genes, as seen in Figure (2). For the E-GEOD-10006 dataset, the MHC protein complex (GO:0042611) and the MHC class II protein complex (GO:0042613) were enriched for the upregulated genes. The downregulated genes were mainly involved in intracellular processes (GO:0005622) (Figure:2).

Analysis of signaling pathway enrichment

Based on the KEGG databases, no significant pathway enrichments were observed in the KEGG pathways of the upregulated genes in the E-MTAB-5279 dataset. In that dataset, the top 5 enriched pathways of the downregulated genes were related to ribosome pathways, Parkinson's disease, thermogenesis, oxidative phosphorylation, and non-alcoholic fatty liver disease (NAFLD), as seen in Table 2. In the E-GEOD-10006 dataset, no significant pathway enrichments were observed in the KEGG pathways in the upregulated and downregulated genes.

Table 2: The Top 5 Pathways Enriched Among Downregulated Genes in Blood Samples.

#term ID	term description	observed gene count	background gene count	false discovery rate	matching proteins in your network (labels)
hsa03010	Ribosome	16	130	3.81E-06	MRPL1,MRPL13,MRPL3,MRPL35,MRPS14,MRPS18C,RPL22L1,RPL26L1,RPL31,RPL34,RPL36A,RPL7,RPL9,RPS27L,RPS7,RSL24D1
hsa05012	Parkinson's disease	13	142	0.00086	ATP5C1,ATP5J,CASP3,COX6C,COX7B,COX7C,NDUFA4,NDUFA5,NDUFB3,NDUFS4,PRKACB,UQCRB,UQCRQ
hsa00190	Oxidative phosphorylation	12	131	0.0013	ATP5C1,ATP5I,ATP5J,COX6C,COX7B,COX7C,NDUFA4,NDUFA5,NDUFB3,NDUFS4,UQCRB,UQCRQ
hsa04714	Thermogenesis	14	228	0.0083	ATP5C1,ATP5I,ATP5J,COX16,COX6C,COX7B,COX7C,NDUFA4,NDUFA5,NDUFB3,NDUFS4,PRKACB,UQCRB,UQCRQ
hsa04932	Non-alcoholic fatty liver disease (NAFLD)	11	149	0.0083	CASP3,COX6C,COX7B,COX7C,FASLG,NDUFA4,NDUFA5,NDUFB3,NDUFS4,UQCRB,UQCRQ

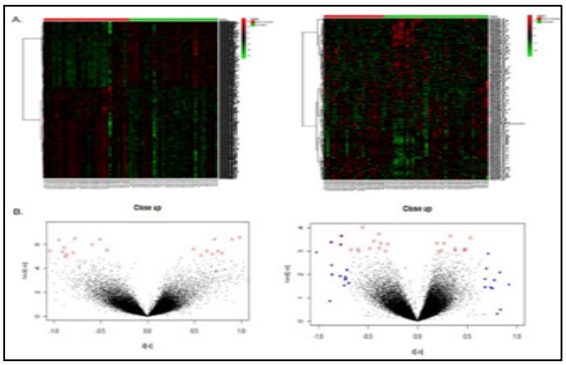

Figure: 1. A. Juxtaposes heatmaps of gene expression profiles from blood tissue (right) against those from airway epithelium of smokers and nonsmokers (left), revealing the genetic contrasts influenced by smoking. B. volcano plots further delineate these differences, plotting significant transcriptional changes in blood tissue (right) against changes in smokers' airway tissue (left), visually quantifying the impact of smoking at the molecular level. Together, these graphical representations provide a concise yet comprehensive overview of the smoking-related transcriptional alterations across two distinct tissue types.

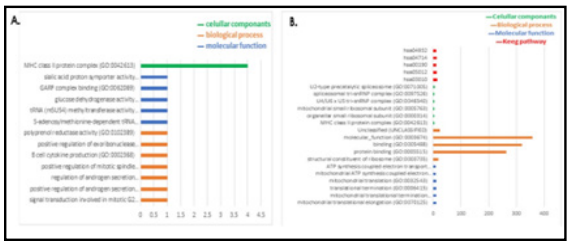

Figure: 2. A. The spectrum of DEG activities in blood, categorized into cellular components, biological processes, and molecular functions, highlighting the predominant areas of activity. B. multi-dimensional analysis of DEG, combining Gene Ontology domains with KEGG pathway associations to depict a nuanced landscape of suppressed gene functions and pathways in airway epithelium.

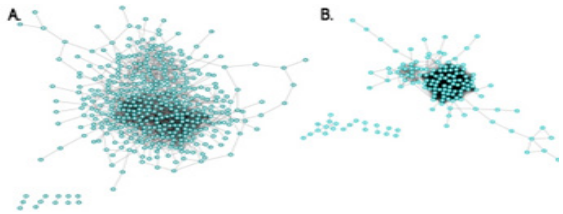

Figure: 3. A. The Protein-Protein Interaction (PPI) network in blood, depicting a dense web of interactions that suggest a highly interconnect-

ed system. B. The PPI network in tissue, with a more dispersed pattern indicating fewer or more selective interactions.

Analysis of the PPI network and modules

In the E-MTAB-5279 dataset, the PPI network complex of DEGs, contained 1714 edges and 517 nodes. In the dataset labeled E-GEOD-10006, a total of 196 nodes and 2,417 edges were identified, as illustrated in Figure 3. The application of CFinder revealed a k-cliques value exceeding 10. Regarding the E-MTAB-5279 dataset, the developed Protein-Protein Interaction (PPI) network resulted in the formation of four distinct modules. Subsequent pathway enrichment analysis revealed that module M0 was comprised of 75 edges and 13 nodes, whereas module M2 included 21 nodes and 174 edges. Module M3 was made up of 12 nodes and 65 edges, and module M4 encompassed 21 nodes and 168 edges. In the E-GEOD-10006 dataset, two modules were obtained. The pathway enrichment analysis showed that M0 consisted of 110 edges and 17 nodes and M1 consisted of 70 nodes and 2207 edges (Figures 4 and 5).

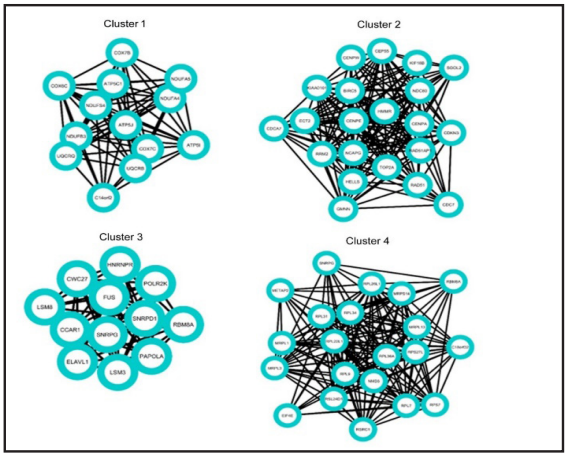

Figure: 4. The top four clusters (from Blood tissue PPI network) within a Protein-Protein Interaction network derived from blood, where each cluster represents a distinct assembly of interrelated proteins, illustrating the network's modular organization.

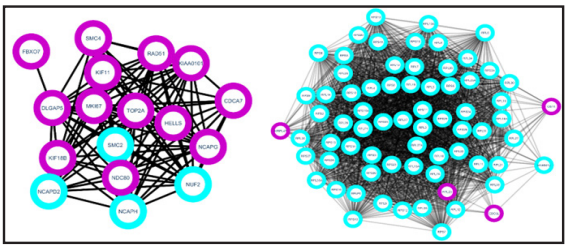

Figure: 5. Top clusters from a Protein-Protein Interaction (PPI) network in airway epithelial cells. Each node represents a protein, with the connecting lines indicating the interactions between them, thus mapping the complex biological interplay within the airway epithelium.

Hub identification
GO annotation of the cluster genes

From the significant k-cliques modules obtained from the smokers' blood (E-MTAB-5279) and the smokers' epithelium (E-GEOD-10006) datasets, we identified GO annotation modules, such as biological processes, cellular components, molecular functions, and KEGG pathways (Figure 6A). For the smokers' blood dataset, in the M0 module, the significant biological process is the mitochondrial electron transport, cytochrome c to oxygen (GO:0006123). For the smokers' epithelium dataset, in the M1 module. The significant biological process is the kinetochore organization (GO:0051383) (Figure 6B).

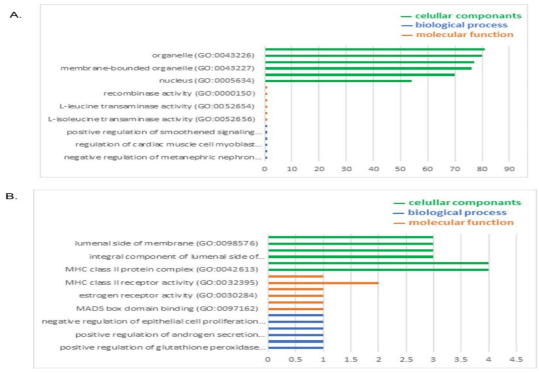

Figure: 6. GO term annotations of top clusters in smokers' blood and airway epithelium tissue DEGs.

Discussion

Cigarette smoke is linked to a variety of severe diseases because of its negative effects on human health (28). Technologies, such as molecular biomarkers, play instrumental roles in improving a diagnosis by helping to predict therapeutic behaviors. Microarray is an important diagnostic tool; it helps to probe the expression levels of a myriad of genes in the human genome. This technique is widely used to explore the biomarkers of diseases (29-31).

Additionally, one can clearly understand the effects of smoking by identifying and comparing the DEGs between smokers and non-smokers. In this study, we used computational methods to analyze gene expression data with the aim of determining transcription profiling of blood from smokers and non- smokers (E-MTAB-5279 dataset) vs. human airway epithelium of smokers and non-smokers (E-GEOD-10006 dataset). In the blood dataset, 584 differentially expressed genes (DEGs) were identified, comprising 411 genes that showed decreased expression and 173 that were more highly expressed. Notably, genes such as ATP5C1, ATP5J, COX6C, COX7B, COX7C, NDUFA4, NDUFA5, NDUFB3, NDUFS4, UQCRB, and UQCRQ predominantly featured in pathways associated with thermogenesis, as well as in the pathogenesis of Parkinson's, Huntington's, and Alzheimer's diseases. In a separate dataset examining human airway epithelium, 147 DEGs were identified, including 101 genes with reduced expression and 46 with increased expression. Of these, RPL23 was primarily involved in the ribosome pathway. Gene Ontology (GO) annotations were employed in both datasets to categorize biological processes, cellular components, molecular functions, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Specifically, in the smokers' blood dataset, the M0 module predominantly represented biological processes like mitochondrial electron transport from cytochrome c to oxygen (GO:0006123). Exposure to tobacco smoke is a high risk factor that affects mitochondrial DNA due to oxidative damage (32, 33), leading to elevated levels of reactive oxygen species (ROS) with the use of mitochondrial enhancement treatment (34). Cytochrome c oxidase has been reported to be dramatically inhibited with the increase in carbon dioxide concentration (35).

For the smokers' epithelium dataset, in the M1 module, the significant biological process is kinetochore organization (GO:0051383). Cancers are highly associated with aberrant kinetochore attachment (36, 37).

In summary, after analyzing the human airway epithelium and blood datasets, we identified the highly significant pathways that are correlated with biological processes in smokers, which are mainly associated with epithelial tissue cancer and blood toxicity.

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