Original Article

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TANAFFOS

Evaluation of E-Cigarette Effects on Gene Expression Profile of Human Bronchial Epithelial Cells: A System Biology Approach

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Background: Electronic cigarettes produce aerosols that contain flavoring agents and nicotine, a drug that can lead to dependence, which users inhale. The widespread use of e-cigarettes has prompted research projects to investigate the safety or risks of e-cigarette smoke on human health. The present study aims to discover details of the e-cigarette smoke effect on the gene expression profile of human bronchial epithelial cells (HBECs) via network analysis.

Materials and Methods: Data of GSE118431(GPL16791) from Gene Expression Omnibus (GEO) were extracted and pre-evaluated by GEO2R program. The differentially expressed genes (DEGs) were analyzed via protein-protein interaction PPI analysis, and the critical DEGs were introduced.

Results: A total of 192 significant DEGs among the 17058 targeted genes were selected for PPI network analysis. The 177 recognized genes were interacted to construct a network. Six DEGs were common between the hubs and bottlenecks of the network.

Conclusion: E-cigarettes target IL1B, PTGS2, SOD2, NFKBIA, TXNRD1, and DUSP1, the critical genes that are associated with progression of inflammation, cell proliferation and cell invasion in cancer, chromatin condensation, DNA fragmentation, and cell death.

Keywords: E-cigarette; Human; Gene; Cell; Network

INTRODUCTION

Electronic cigarettes are electrical devices that aerosolize a liquid containing solvents, flavorants, and the dependence-producing drug, nicotine, for user inhalation (1). While the e-cigarettes could be safer than traditional cigarettes, there is no evidence supporting the safety of this alternative (2). However, the flavoring compounds of these cigarettes could be a big health issue, especially for lung cells (3). There are reports about many hazardous chemical compounds which are produced via e-cigarettes. The carbonyl compounds such as acetaldehyde, acrolein, formaldehyde, and glyoxal that are found in e-cigarette aerosols are the harmful substances and health risk factors (4). Based on preclinical data, the inhaled nicotine of e-cigarettes is associated with activation of the sympathetic nervous system, a process that can stimulate cancer development (5). Recent research showed that chronic exposure to e-cigarettes has changed gene expression of human alveolar macrophages. It is reported that alveolar macrophages of vapers were widely affected and 124 genes were downregulated (6).

Study of gene expression changes in diseases is a well-known method to explore the molecular mechanism of different disorders (7). Assessment of smoking effects on human health via gene expression analysis provides useful information about hazardous compounds of cigarettes that have threatened human health. Based on these evaluations, the expression of 323 genes was significantly associated with smoking performance (8, 9).

Genomics associated with bioinformatics has appeared as a suitable method to study the molecular mechanism of diseases. In such analysis, large numbers of DEGs have been analyzed via bioinformatic tools to introduce the crucial individuals (10, 11). Network analysis as a bioinformatic approach is frequently applied to analyze the results of genomic experiments. Chen et al studied differentially expressed smoking-associated mRNAs, lncRNAs, and miRNAs, which are connected to lung cancer via network analysis (12). In the present study, gene expression profiles related to the effect of e-cigarettes on primary human airway epithelial cells, which are recorded in GEO, are extracted and analyzed via PPI network analysis to find the molecular events after smoking e-cigarettes.

MATERIALS AND METHODS

Data collection

Data of GSE118431(GPL16791) from GEO is related to the report of Park et al (13). This data set was selected to assess via network analysis. The transcriptome profiles of normal human bronchial epithelium cells (HBECs) treated in duplicate with e-cigarette smoke solution containing 2 ppm diacetyl (GSM3330066 and GSM3330067) versus the control cells (GSM3330060 and GSM3330061) were nominated for analysis.

Pre-evaluation of data

The significant DEGs which discriminate gene expression profiles of the treated cells from control were determined based on $P_{adj} < 0.05$. Mean-variance trend was applied to assess accuracy of test results. Separation of samples was explored via UMAP plot analysis.

Network analysis

The significant DEGs were imported in a PPI network via “Protein query” of STRING database by Cytoscape software v 3.7.2. The PPI network was analyzed by the “Network analyzer” application of Cytoscape software. The nodes of the network were laid out based on degree value. The top 10 nodes based on degree value were pointed out as hubs and the 10 bottlenecks were identified based on normalized betweenness centrality parameter. The common hubs and bottlenecks were introduced as hub-bottlenecks.

Statical analysis

Significant DEGs were selected based on $P_{adj} < 0.05$. The PPI network was constructed based on confidence score=0.2.

RESULTS

As it is shown in Figure 1, e-cigarette smoke has changed expression of 17058 genes including 192 DEGs. Mean-variance trend (Figure 2) has indicated that data are fitted with suitable variation and results are satisfactory and reliable. Based on UMAP plot (Figure 3), the gene expression profiles of the treated and control cells are separated. It has revealed that both treated and control samples are differentiated by the DEGs.

The 192 DEGs were included in a PPI network. Number of 177 DEGs were recognized by STRING database and the network including a main connected component and 6 isolated DEGs was constructed. As it is shown in Figure 4, a few numbers of nodes were pointed out as hubs. The hub-bottleneck nodes (IL1B, PTGS2, SOD2, NFKBIA, TXNRD1, and DUSP1) were determined and presented in Table 1.

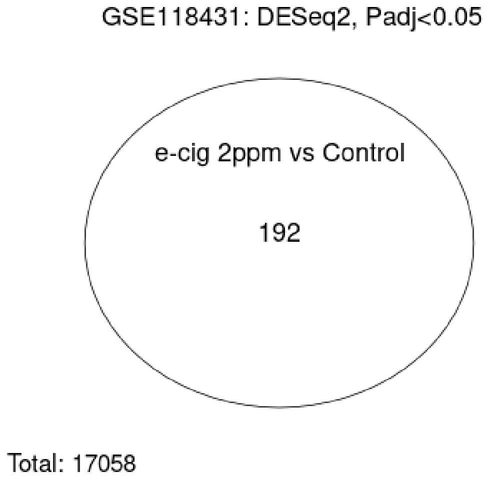

Figure 1. Presentation of 192 DEGs and 17058 genes which are dysregulated by e-cigarette smoke in HBECs

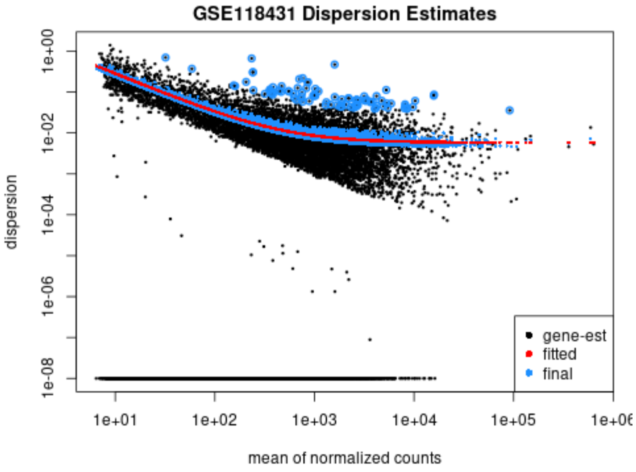

Figure 2. Mean-variance trend plot. Each point represents a gene. The red line is the mean-variance trend approximation that can be taken into account during differential gene expression analysis. The blue line is a constant variance approximation

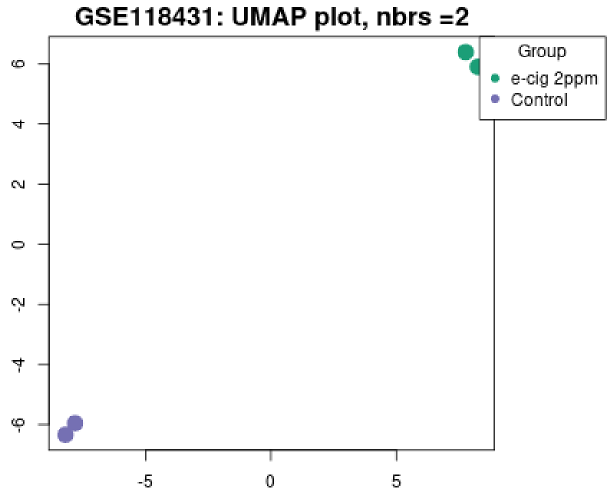

Figure 3. UMAP plot; separation of treated and control samples via analysis

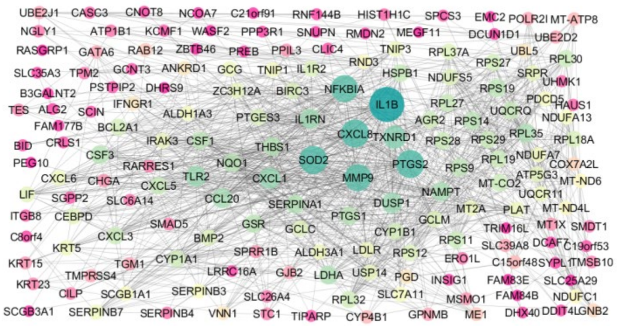

Figure 4. Main connected component of PPI network including 171 nodes. Red to green refers to the increment of degree value. Nodes are laid out based on degree value

Table1. The list of hub-bottleneck genes of the PPI network

No.	Display name	Degree	Betweenness Centrality	Log2 (fold change)
1	IL1B	66	1	0.856
2	PTGS2	51	0.3	0.716
3	SOD2	51	0.59	0.743
4	NFKBIA	49	0.44	0.455
5	TXNRD1	40	0.52	0.457
6	DUSP1	35	0.36	0.751

DISCUSSION

E-cigarette smoke has changed the expression of a large number of genes (17058 individuals) including 192 significant DEGs. It can be concluded that considerable functional changes are associated with these processes. Determining the key targeted genes was the aim of this study. Yang et al published evidence about a decrement of mouse lung function associated with increased levels of inflammation markers. These alterations are combined with pathological changes in the airways and lungs after inhalation of cigarette smoke (CS) and e-cigarette aerosol (ECA). Based on this report, fewer damages are observed in ESA-treated mice relative to the individuals with an equal dose of the CS. Results of proteomic analysis were assessed via PPI network analysis. PPI network analysis revealed that both ECA and CS significantly have targeted ribosome and complement system-related proteins in the lung tissue of treated mouse (14). Here, pre-evaluation analysis (Figures 1,2,3) showed e-cigarettes smoke solution containing 2 ppm diacetyl has effectively changed the gene expression profiles of the treated cells. The treated cells are separated from the control individuals by the differentially expressed genes.

PPI network analysis showed that the determined DEGs have different centrality properties (Figure 4). IL1B, PTGS2, SOD2, NFKBIA, TXNRD1, and DUSP1 were introduced as the central nodes (hub-bottlenecks) of the constructed network. Hub-bottleneck genes are used frequently as the critical genes to describe molecular events in the studied conditions (15, 16). As it is shown in Table 1, all critical DEGs are upregulated in the treated cells by the e-cigarette smoke solution containing 2 ppm diacetyl.

Interleukin-1B (IL1B) is a principal cytokine that is involved in starting the innate immune response in vertebrates. High correlation between acute lung inflammation related to degree of particle exposure and IL1B concentration is highlighted by researchers (17). Up-regulation of PTGS2, which is a rate-limiting enzyme for prostaglandin synthesis by IL1B, is reported by Seo et al (18). As it is presented in Table 1, PTGS2 is the second central gene.

It is reported that endoperoxidase synthase 2 (COX2/PTGS2) is strongly regulated and normally expressed at very low levels under physiological conditions. Based on this report, both expression and activation of PTGS2/COX2 are directly induced by pro-inflammatory cytokines and growth factors, the features that activate intracellular pathways that are related to inflammation (19). Superoxide dismutase 2 (SOD2) belongs to the family of iron/manganese superoxide dismutase family, the gene that encodes a mitochondrial protein. This gene is involved in cell proliferation and cell invasion in breast cancer cells (20).

NFKBIA is the fourth central gene that is upregulated under the effect of e-cigarette smoke solution containing 2 ppm diacetyl. Nuclear factor-kappa-B (NFKB) is a protein complex that controls the activity of other genes via binding to DNA (21). Investigation showed that cell viability of human mesenchymal stem cells, which were exposed to nanoparticles, is reduced. Chromatin condensation and DNA fragmentation are accompanied by cell death. Significant upregulation of NFKBIA in response to presence of nanoparticles is observed in the treated cells (22). Evaluations revealed that cigarette smoke extraction simulation led to upregulation of thioredoxin reductase-1 (TXNRD1) in human bronchial epithelial cells (23).

Dual-specificity protein phosphatase 1 (DUSP1) is involved in metabolism and energy spending. Examinations show that DUSP1 overexpression in obese subjects and physical exercise is associated with reduction of its expression (24). Lohani et al published data about normal human fetal lung fibroblasts (MRC-5) that were cultured in a niacin deficient media, and exposed to a cigarette smoke carcinogen compound (nicotine-derived nitrosamine ketone). Results showed raised expression of DUSP1 and 7 genes that are critical in promotion of cancer (25).

CONCLUSION

E-cigarettes changed gene expression profiles of normal human bronchial epithelium cells. IL1B, PTGS2, SOD2, NFKBIA, TXNRD1, and DUSP1 are the critical genes that are targeted by e-cigarette smoke solution containing 2 ppm diacetyl. The crucial genes are upregulated and associated with progression of inflammation, cell proliferation and cell invasion in cancer, chromatin condensation and DNA fragmentation, and cell death. In conclusion, e-cigarettes may be less hazardous than cigarettes, but contain harmful compounds that threaten human health.

Conflict of interest

There is no conflict of interest.

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