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Identification of gene and microRNA changes in response to smoking in human airway epithelium by bioinformatics analyses

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Abstract

Smoking is a substantial risk factor for many respiratory diseases. This study aimed to identify the gene and microRNA changes related to smoking in human airway epithelium by bioinformatics analysis.

From the Gene Expression Omnibus (GEO) database, the mRNA datasets GSE11906, GSE22047, GSE63127, and microRNA dataset GSE14634 were downloaded, and were analyzed using GEO2R. Functional enrichment analysis of the differentially expressed genes (DEGs) was enforced using DAVID. The protein–protein interaction (PPI) network and differentially expressed miRNAs (DEMs)- DEGs network were executed by Cytoscape.

In total, 107 DEGs and 10 DEMs were determined. Gene Ontology (GO) analysis revealed that DEGs principally enriched in oxidation-reduction process, extracellular space and oxidoreductase activity. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway demonstrated that DEGs were principally enriched in metabolism of xenobiotics by cytochrome P450 and chemical carcinogenesis. The PPI network revealed 15 hub genes, including *NQO1, CYP1B1, AKR1C1, CYP1A1, AKR1C3, CEACAM5, MUCL1, B3GNT6, MUC5AC, MUC12, PTGER4, CALCA, CBR1, TXNRD1,* and *CBR3*. Cluster analysis showed that these hub genes were associated with adenocarcinoma in situ, squamous cell carcinoma, cell differentiation, inflammatory response, oxidative DNA damage, oxidative stress response and tumor necrosis factor. Hsa-miR-627-5p might have the most target genes, including *ITLN1, TIMP3, PPP4R4, SLC1A2, NOVA1, RNFT2, CLDN10, TMCC3, EPHA7, SRPX2, PPP1R16B, GRM1, HS3ST3A1, SFRP2, SLC7A11*, and *KLHDC8A*.

We identified several molecular changes induced by smoking in human airway epithelium. This study may provide some candidate genes and microRNAs for assessing the risk of lung diseases caused by smoking.

Abbreviations: COPD = chronic obstructive pulmonary disease, DEGs = differentially expressed genes, DEMs = differentially expressed miRNAs, EMT = epithelial mesenchymal transition, FAK = focal adhesion kinase, FC = fold change, GEO = gene expression omnibus, GO = gene ontology, JNK = Jun NH2-terminal kinase, KEGG = Kyoto Encyclopedia of Genes and Genomes, miRNAs = microRNAs, PPI = protein–protein interaction, STRING = search tool for the retrieval of interacting genes.

Keywords: bioinformatics analysis, human airway epithelium, molecular changes, smoking

1. Introduction

There are about 1.3 billion people smoking cigarettes all over the world.[1] Smoking is one of the remarkable risk factors for respiratory diseases, including chronic obstructive pulmonary disease (COPD) and lung cancer.[2–3] Numerous studies revealed that smoking might lead to some molecular changes in the airway epithelium, such as epithelial mesenchymal transition (EMT)[2–3] and airway inflammation.[4] Genetic changes were also found in normal airway epithelium of smokers, and existed many years after stopping smoking.[5]

MicroRNAs (miRNAs) belong to noncoding RNAs and regulate the expression of genes.[6] MiRNAs participate in many cellular processes, including proliferation, differentiation and apoptosis.[6] Aberrant expression of miRNAs can lead to many diseases, including lung cancer,[7] asthma,[8] and COPD.[9] By microarray profiles, some miRNAs changes were identified in the airways of smokers and nonsmokers, such as mir-218.[10] In this study, we further analyzed the interactions between the abnormal miRNAs and the abnormal genes in smokers, and constructed a network among them.

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This study aimed to identify and analyze DEGs and DEMs in airway epithelial cells in response to smoking, which might provide some candidate genes and microRNAs for assessing the risk of lung diseases induced by smoking, and further provide new clues for experimental studies.

2. Materials and methods

2.1. Gene expression data

From the GEO database (https://www.ncbi.nlm.nih.gov/geo/), the microRNA microarray dataset GSE14634[10] and the mRNA datasets GSE11906,[11] GSE22047[12] and GSE63127[13] were downloaded. GSE14634[10] used the platform GPL8131, and the three mRNA datasets[11–13] used GPL570.

2.2. Identification of DEGs and DEMs

The GEO2R online analysis tool (https://www.ncbi.nlm. nih.gov/geo/geo2r/) was used to obtain the DEGs and DEMs between smoking and nonsmoking samples.[14] $P < .05$ and |log fold change (FC) |≥ 1 were the criterion to define the DEGs, and the top 10 |log FC| and $P < .05$ were used to define the DEMs. The 3 mRNA datasets intersected using the Venn diagrams, and the common genes were taken as DEGs. The Venn diagrams were enforced using R software.

2.3. Functional enrichment analysis

We used GO analysis and KEGG pathway analysis to obtain the biofunctions of the DEGs. The GO and KEGG analyses of DEGs were enforced using DAVID (https://david.ncifcrf.gov/).[15] $P < .05$ was treated as the threshold.

2.4. Protein–protein interaction network

DEGs were imported to the Search Tool for the Retrieval of Interacting Genes (STRING) database to enforce a PPI network,[16] and visualized by Cytoscape software.[17] The hub genes were confirmed using cytoHubba, and the top 20 hub genes were obtained by mcc, mnc, and dmnc methods. The common genes of the 3 methods were taken as the hub genes. Cluster analysis of hub genes was enforced by GenCLip 2.0.

2.5. The target genes of DEMs

The candidate target genes of DEMs were obtained by TargetScan (http://www.targetscan.org/),[18] and the common genes between the candidate target genes and the DEGs in the 3 microarray datasets were taken as the target genes. At last, miRNA- DEGs network analyses were enforced by Cytoscape.

3. Results

3.1. Identification of DEGs and enrichment analysis

We identified the DEGs of GSE11906, GSE22047 and GSE63127 datasets using GEO2R tool, and 178, 213, 249 DEGs were obtained, respectively (Fig. 1). A total of 107 common genes were screened in the 3 gene datasets, including 85 upregulated genes and 22 downregulated genes (Fig. 1). Next, the GO analysis and KEGG pathway analysis were conducted through DAVID, and the 5 top GO terms and pathways were shown in Table 1. GO analysis results showed that in the biological process, DEGs principally enriched in oxidation-reduction process. In the cellular component analysis, DEGs principally enriched in extracellular space, organelle membrane and extracellular exosome. Molecular function analysis principally enriched in oxidoreductase activity, indanol dehydrogenase activity and monooxygenase activity. KEGG pathway enrichment analysis showed that DEGs were significantly enriched in metabolism of xenobiotics by cytochrome P450, arachidonic acid metabolism, and chemical carcinogenesis.

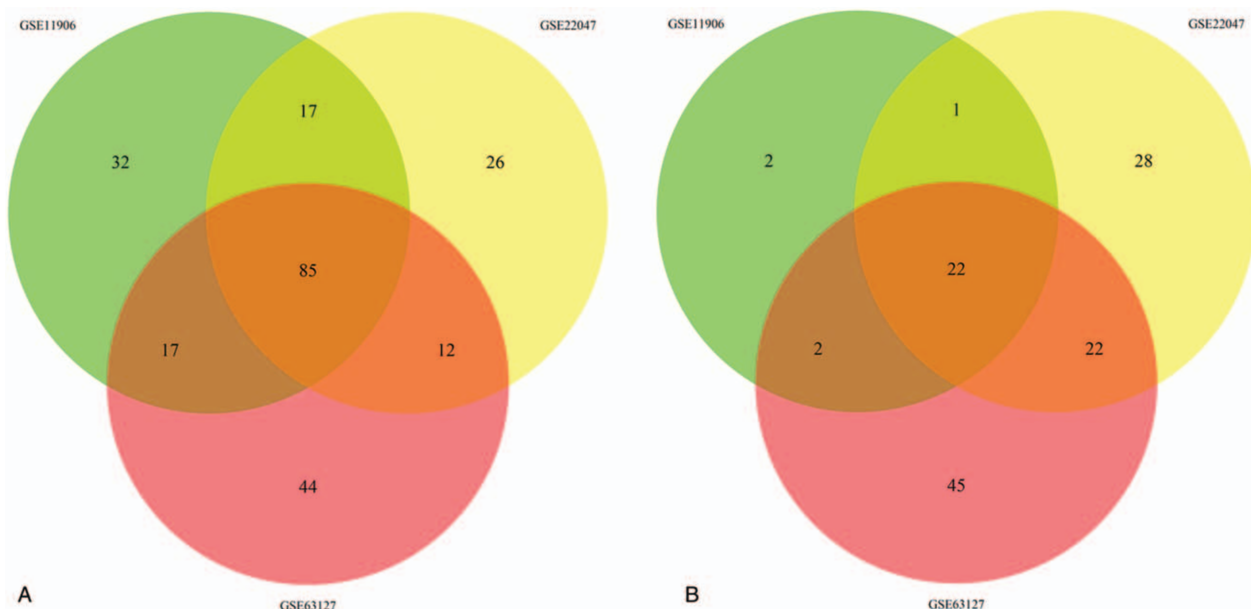

Figure 1. Venn diagrams of the DEGs in the 3 gene datasets. (A) The upregulated genes in the 3 gene datasets. (B) The downregulated genes in the 3 gene datasets. DEGs = differentially expressed genes.

Table 1

The top 5 enriched gene ontology terms and pathways of DEGs.

Category	Term	Count	*P* value	Genes
KEGG_PATHWAY	hsa00980:Metabolism of xenobiotics by cytochrome P450	7	6.45E−06	*CBR1, CYP1B1, CYP1A1, ADH7, CBR3, AKR1C1, ALDH3A1*
KEGG_PATHWAY	hsa00590:Arachidonic acid metabolism	6	3.87E−05	*AKR1C3, GPX2, CBR1, CYP4F3, CBR3, CYP4F2*
KEGG_PATHWAY	hsa01100:Metabolic pathways	18	8.28E−04	*ME1, CYP1A1, MAOB, HGD, TKT, ADH7, CBR3, GPAT3, ALDH3A1, AKR1C3, CSGALNACT1, CBR1, AKR1B10, B3GNT6, CYP4F3, CYP4F2, ATP6V0A4, GAD1*
KEGG_PATHWAY	hsa00350:Tyrosine metabolism	4	1.39E−03	*MAOB, HGD, ADH7, ALDH3A1*
KEGG_PATHWAY	hsa05204:Chemical carcinogenesis	5	1.62E−03	*CBR1, CYP1B1, CYP1A1, ADH7, ALDH3A1*
GOTERM_MF_DIRECT	GO:0016491~oxidoreductase activity	8	5.29E−05	*AKR1C3, CYP1A1, MAOB, OSGIN1, ADH7, TXNRD1, AKR1C1, ALDH3A1*
GOTERM_MF_DIRECT	GO:0047718~indanol dehydrogenase activity	3	6.97E−05	*AKR1C3, AKR1B10, AKR1C1*
GOTERM_MF_DIRECT	GO:0004497~monooxygenase activity	5	1.80E−04	*CYP1B1, CYP1A1, CYP4F11, CYP4F3, CYP4F2*
GOTERM_MF_DIRECT	GO:0020037~heme binding	6	5.41E−04	*CYP1B1, CYP1A1, CYP4F11, CYP4F3, CYP4F2, ABCB6*
GOTERM_MF_DIRECT	GO:0016655~oxidoreductase activity, acting on NAD (P)H, quinone or similar compound as acceptor	3	6.40E−04	*AKR1C3, CBR1, AKR1C1*
GOTERM_CC_DIRECT	GO:0005615~extracellular space	21	4.07E−06	*BMP4, BPIFB2, C3, CHI3L1, DPYSL3, CBR3, TCN1, TIMP3, ALDH3A1, CALCA, ELFN2, CXCL14, CCL20, SRPX2, SFRP2, APELA, MEP1A, LTF, MUC5AC, EGF, SPP1*
GOTERM_CC_DIRECT	GO:0031090~organelle membrane	5	7.98E−04	*CYP1B1, CYP1A1, CYP4F11, CYP4F3, CYP4F2*
GOTERM_CC_DIRECT	GO:0070062~extracellular exosome	26	1.13E−03	*BPIFB2, C3, ITLN1, SEC14L3, TIMP3, GPX2, AKR1C3, CBR1, LTF, CEACAM5, EGF, NQO1, AKR1C1, SPP1, MAOB, CHI3L1, HGD, TKT, ABCB6, SCEL, THSD7A, AKR1B10, MEP1A, TXNRD1, MUC5AC, ATP6V0A4*
GOTERM_CC_DIRECT	GO:0005576~extracellular region	15	1.99E−02	*CALCA, BMP4, MUCL1, CXCL14, CCL20, SFRP2, C3, APELA, LTF, ADH7, MUC5AC, EGF, TIMP3, TCN1, SPP1*
GOTERM_CC_DIRECT	GO:0016021~integral component of membrane	34	3.31E−02	*CYP1B1, CLDN10, SEC14L3, TMCC3, GPAT3, ALDH3A1, SLC1A2, APELA, RNFT2, B3GNT6, NQO1, EGF, TMEM45A, DTNA, MUC12, HS3ST3A1, PTGER4, MAOB, CYP4F11, AJAP1, GRM1, ABCB6, SLC7A11, THSD7A, ELFN2, SFRP2, AVPR1A, MEP1A, CYP4F3, CYP4F2, HTR2B, CLIP4, PHEX, ATP6V0A4*
GOTERM_BP_DIRECT	GO:0055114~oxidation-reduction process	21	4.03E−12	*ME1, CYP1B1, CYP1A1, MAOB, HGD, OSGIN1, CYP4F11, ADH7, CBR3, ALDH3A1, AKR1C3, GPX2, CBR1, PIR, AKR1B10, CYP4F3, TXNRD1, CYP4F2, NQO1, SRXN1, AKR1C1*
GOTERM_BP_DIRECT	GO:0042376~phylloquinone catabolic process	3	6.87E−05	*CYP4F11, CBR3, CYP4F2*
GOTERM_BP_DIRECT	GO:0021537~telencephalon development	4	7.00E−05	*BMP4, SLC1A2, AVPR1A, SIX3*
GOTERM_BP_DIRECT	GO:0044597~daunorubicin metabolic process	3	6.32E−04	*AKR1C3, AKR1B10, AKR1C1*
GOTERM_BP_DIRECT	GO:0044598~doxorubicin metabolic process	3	6.32E−04	*AKR1C3, AKR1B10, AKR1C1*

DEGs = differentially expressed genes.

3.2. PPI of the DEGs and hub genes

The connections among the 107 DEGs in human airway epithelium of smokers were further performed using the STRING database. Next, PPI network was visualized by the Cytoscape, and it contained 47 nodes and 78 edges (Fig. 2). The hub genes were selected by cytoHubba plugin, and the top 20 hub genes were obtained by mcc, mnc, and dmnc methods, respectively (Fig. 3). At last, 15 hub genes, including *NQO1, CYP1B1, AKR1C1, CYP1A1, AKR1C3, CEACAM5, MUCL1, B3GNT6, MUC5AC, MUC12, PTGER4, CALCA, CBR1, TXNRD1*, and *CBR3* (Table 2), were determined by the intersection of the 3 methods. Only the expression of PTGER4 decreased, while the others genes increased. Cluster analysis of hub genes showed gene-term association positively reported, including adenocarcinoma in situ, squamous cell carcinoma, cell differentiation, inflammatory response, oxidative DNA damage, oxidative stress response and tumor necrosis factor (Fig. 4).

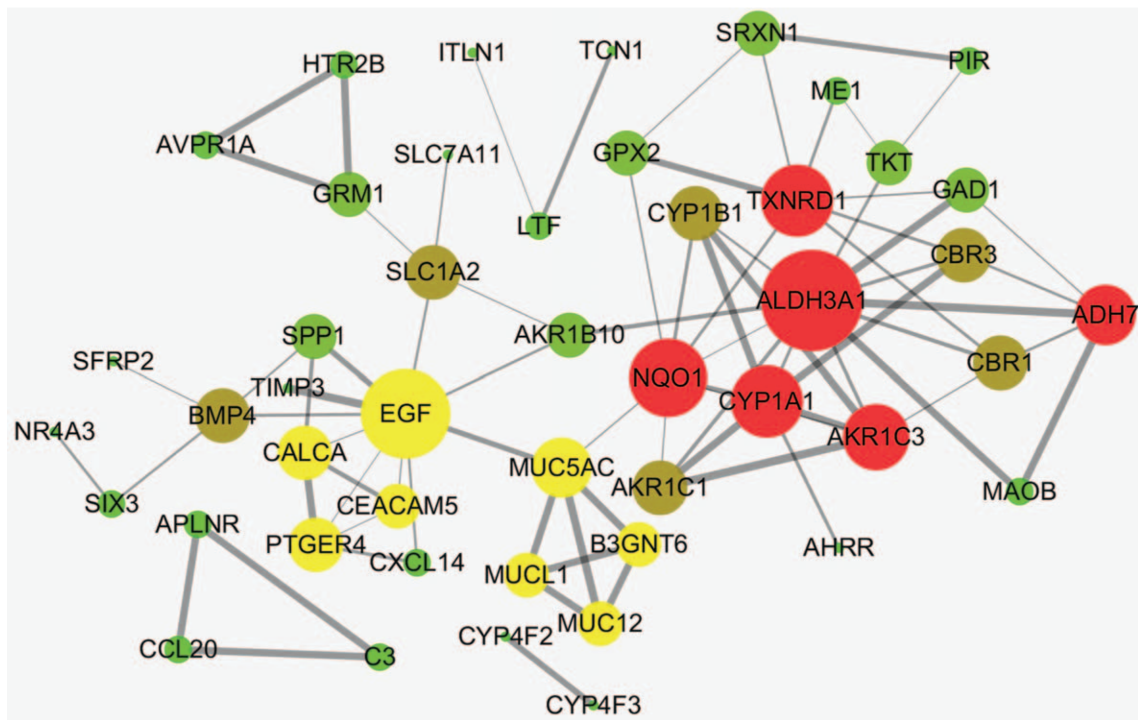

Figure 2. PPI network of DEGs. The PPI network was visualized using Cytoscape. It contained 47 nodes and 78 edges. DEGs = differentially expressed genes, PPI = protein–protein interaction.

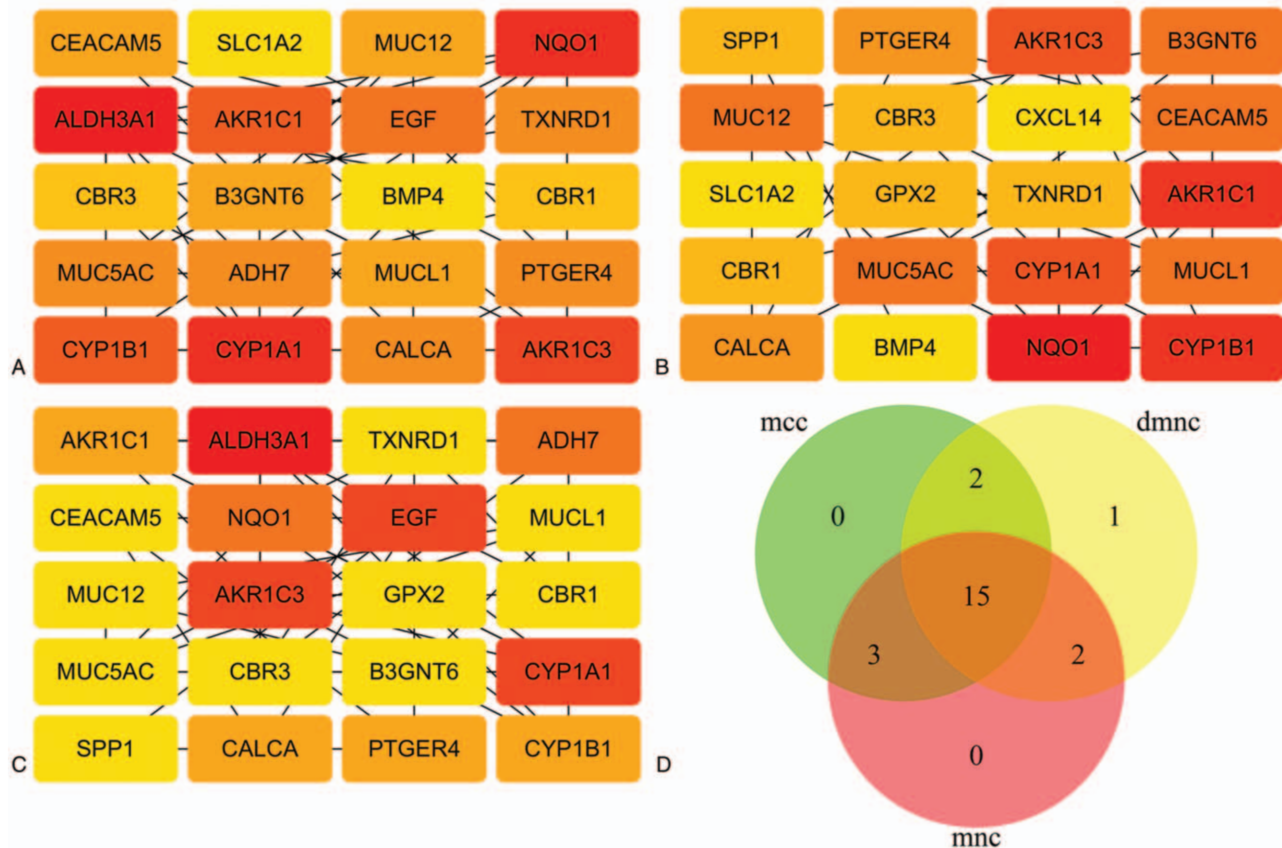

Figure 3. The hub genes of DEGs. The hub genes were obtained by cytoHubba. (A) The top 20 hub genes obtained by mcc. (B) The top 20 hub genes obtained by dmnc. (C) The top 20 hub genes obtained by mnc. (D) 15 hub genes determined by intersection of the 3 methods. DEGs = differentially expressed genes.

Table 2

The 15 hub genes of DEGs in the 3 gene datasets.

Gene symbol	GSE11906		GSE22047		GSE63127		Degree
	Log FC	*P* value	Log FC	*P* value	Log FC	*P* value	
TXNRD1	1.1842977	6.10E−15	1.10938	3.92E−30	1.176964	9.32E−47	8
PTGER4	−1.0522499	3.02E−06	−1.03131	1.68E−05	−1.23226	3.55E−04	8
NQO1	1.8612315	1.98E−26	1.7840761	6.83E−32	1.950195	4.85E−51	51
MUCL1	2.7191775	1.70E−31	2.67603168	6.46E−36	2.938821	3.95E−54	6
MUC5AC	1.5279379	2.28E−06	1.565562	2.53E−12	2.18128	9.00E−29	8
MUC12	1.299354	1.42E−08	1.415967	5.52E−08	1.677413	9.29E−17	6
CYP1B1	5.1127601	5.80E−36	5.03212953	5.91E−36	5.673674	2.85E−56	24
CYP1A1	3.9462927	1.73E−16	4.3943017	3.48E−18	3.907757	1.02E−20	51
CEACAM5	2.2760657	3.02E−16	2.42544144	1.79E−16	2.619588	2.91E−27	6
CBR3	1.1807599	1.89E−12	1.18793	5.87E−15	1.196482	4.46E−26	5
CBR1	1.5413911	9.15E−19	1.722658	9.05E−26	1.549443	6.62E−28	5
CALCA	1.435297	1.60E−09	1.237081	2.28E−05	1.791914	1.25E−13	8
B3GNT6	1.7181431	1.46E−11	1.762006	1.85E−11	1.560917	4.93E−13	6
AKR1C3	1.6136873	1.09E−21	1.587655	3.02E−31	1.673757	7.34E−46	50
AKR1C1	2.1617338	3.47E−20	2.14213189	1.80E−21	2.142024	3.08E−29	24

DEGs = differentially expressed genes.

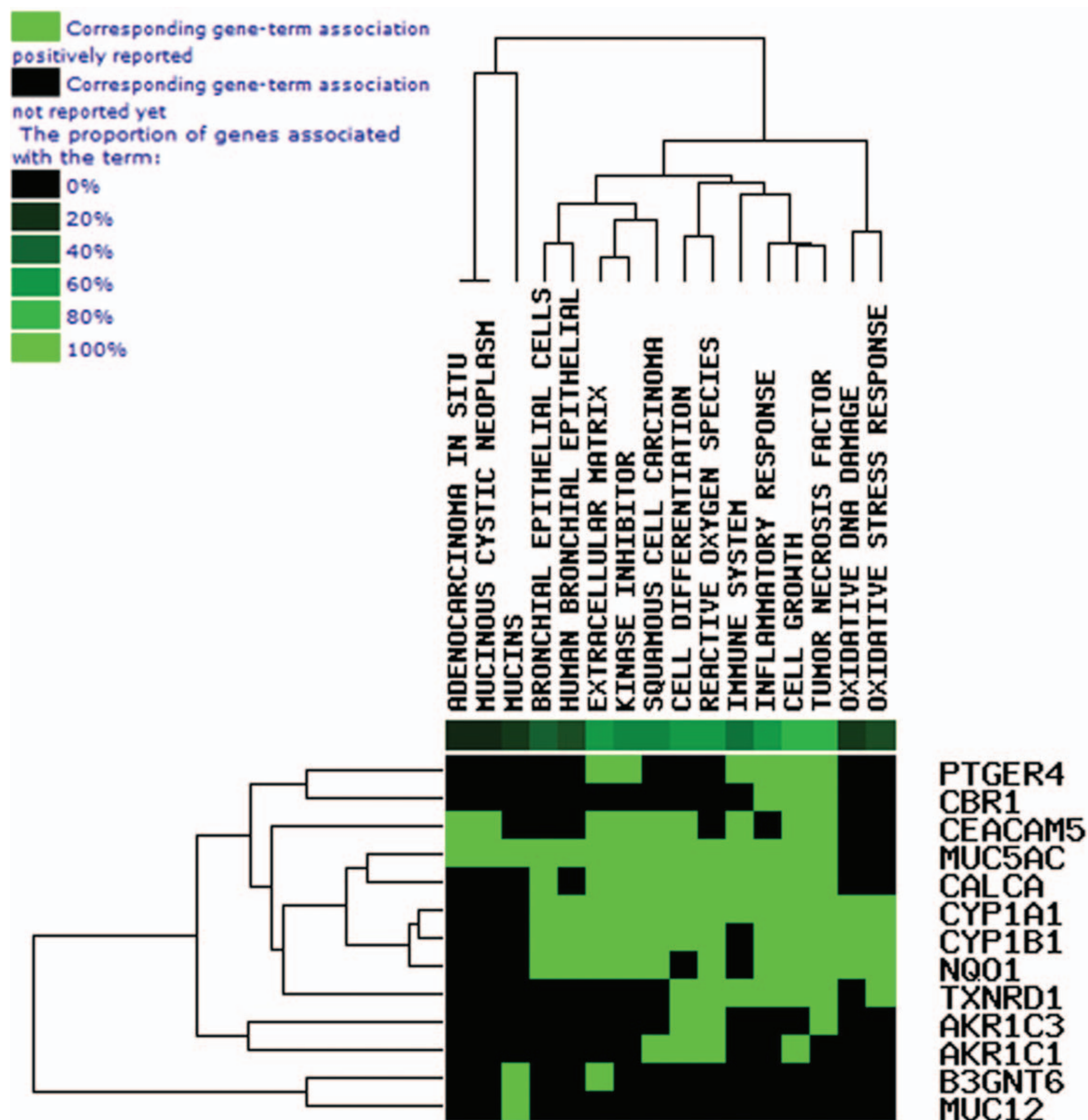

Figure 4. Cluster analysis of the hub genes. Cluster analysis of the hub genes was enforced using GenCLip 2.0.

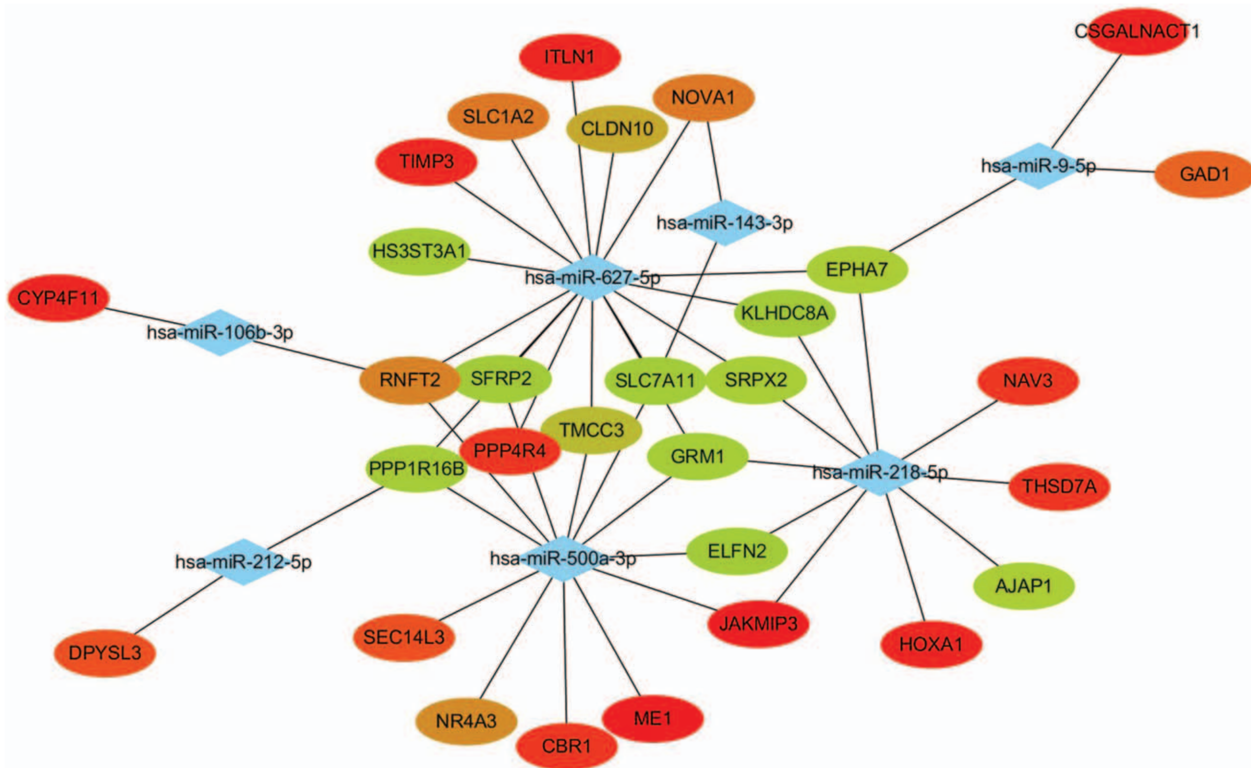

Figure 5. The network between DEMs and DEGs. The networks between DEMs and DEGs were visualized by Cytoscape. DEGs = differentially expressed genes, DEMs = differentially expressed miRNAs.

3.3. The network between DEMs and DEGs related to smoking in human airway epithelium

We also used GEO2R tool to screen DEMs in smoking and nonsmoking groups in GSE14634, and then used TargetScan database to obtain the candidate genes of DEMs. Ten DEMs were obtained, and all of them were reduced. At last, the common genes between the candidate genes and the 107 DEGs were taken as the target genes. The network between DEMs and target genes was enforced by Cytoscape (Fig. 5). We found 7 DEMs owned the common genes (Fig. 5, Table 3). Among them, hsa-miR-627-5p had the most target genes, including *ITLN1, TIMP3, PPP4R4, SLC1A2, NOVA1, RNFT2, CLDN10, TMCC3, EPHA7, SRPX2, PPP1R16B, GRM1, HS3ST3A1, SFRP2, SLC7A11,* and *KLHDC8A* (Fig. 5, Table 3).

4. Discussion

Smoking is one of the primary causes of many respiratory diseases, such as COPD and lung cancer.[2–3] Lung cancer is the

Table 3

DEMs and their target genes in 107 DEGs.

microRNA	hsa-miR-627-5p	hsa-miR-500a-3p	hsa-miR-218-5p	hsa-miR-9-5p	hsa-miR-212-5p	hsa-miR-143-3p	hsa-miR-106b-3p
Target	ITLN1	JAKMIP3	HOXA1	CSGALNACT1	PPP1R16B	NOVA1	RNFT2
	TIMP3	ME1	KLHDC8A	GAD1	DPYSL3	SLC7A11	CYP4F11
	PPP4R4	CBR1	THSD7A	EPHA7			
	SLC1A2	RNFT2	NAV3				
	NOVA1	SFRP2	EPHA7				
	RNFT2	SEC14L3	GRM1				
	CLDN10	GRM1	JAKMIP3				
	TMCC3	NR4A3	SRPX2				
	EPHA7	TMCC3	ELFN2				
	SRPX2	PPP1R16B	AJAP1				
	PPP1R16B	SLC7A11					
	GRM1	ELFN2					
	HS3ST3A1						
	SFRP2						
	SLC7A11						
	KLHDC8A						

DEGs = differentially expressed genes, DEMs = differentially expressed miRNAs

major cancer in humans, and epidemiological evidences show smoking is a substantial cause of lung cancer.[19–20] Almost 87% of lung cancer was caused by cigarette smoking.[19] Cigarette smoking is also the most primary risk factor of COPD.[21–22] Compared to nonsmokers, smokers have higher risk of respiratory symptoms and COPD mortality.[21] More and more studies revealed that smoking induced a series of genetic changes in lung, which were closely related to lung cancer and COPD.[23–24] Studies also revealed the pathogenesis of lung cancer and COPD were closely connected with the abnormal expression of miRNA and mRNA.[25–26] However, genetic changes in epithelial cells caused by smoking had not to be fully elucidated. In this study, we identified and analyzed the key genes, microRNAs and the connections between miRNAs and mRNA related to smoking in human airway epithelium by bioinformatics analysis.

In this study, 3 mRNA datasets GSE11906,[11] GSE22047[12] and GSE63127[13] were analyzed, and a total of 107 DEGs were found in human airway epithelium of smokers, including 85 upregulated genes and 22 downregulated genes. GO analysis revealed that DEGs mainly enriched in oxidation-reduction process, extracellular space and oxidoreductase activity, and KEGG pathway showed that DEGs were involved in metabolism of xenobiotics by cytochrome P450 and chemical carcinogenesis. Next, 15 hub genes, including *NQO1, CYP1B1, AKR1C1, CYP1A1, AKR1C3, CEACAM5, MUCL1, B3GNT6, MUC5AC, MUC12, PTGER4, CALCA, CBR1, TXNRD1,* and *CBR3*, were determined by cytoHubba plugin. All the hub genes increased in their expression except PTGER4. In this study, *CYP1A1* and *CYP1B1*, belonging to cytochrome P-450 family enzymes family,[27] dramatically increased in human airway epithelium of smokers. After the rats were treated with incense smoke, the levels of *CYP1A1* and *CYP1B1* dramatically increased in the lung tissues.[27] A study reported that cigarette smoke extract could increase the level of *CYP1A1* and *CYP1B1* in normal bronchial epithelial cells, and the abnomal levels of *CYP1A1* and *CYP1B1* were associated with cnacer.[28] The expression of *AKR1C1* and *AKR1C3*, belonging to aldo-keto reductase family, also increased in human airway epithelium of smokers. *AKR1C3* might be a new marker of radioresistance in lung cancer.[29] In human oral cells, investigator found cigarette smoke condensate might aggrandize the expressions of *CYP1A1, CYP1B1, AKR1C1, AKR1C3,* and *AKR1B10*.[30] NQO1, one of flavoprotein, increased in human airway epithelium of smokers. A study found, compared to normal lung tissue, NQO1 increased in lung cancer tissue.[31] The level of MUC5AC, one kind of secretory mucin, was abnormal in numerous cancers.[32] In lung cancer, the incremental level of *MUC5AC* meant a poor prognosis.[32] A study found that *MUC5AC* promoted the migration of lung cancer cells by focal adhesion kinase (FAK) signaling.[32] MUCL1 was one of breast-specific genes, and played a remarkable role in the metastasis or progression of breast cancer.[33] MUCL1 might mediate the proliferation of breast cancer cells by FAK/ Jun NH2-terminal kinase (JNK) signaling pathway.[33]*PTGER4* was the only decreased hub gene in this study. Recently, a study found PTGER4 had anti-inflammatory and anti-hyperpermeability effects in acute lung injury mice model.[34]

MicroRNAs could regulate the expression of genes, and abnormal expression of miRNAs was associated with many lung diseases, including COPD, lung cancer, asthma and sarcoidosis.[35] In this research, we obtained target genes of DEMs by the intersection between candidate target genes and DEGs, then enforced a network between DEMs and target genes. Compared to never smokers, miR-218-5p was dramatically downregulated in both healthy smokers and COPD patients.[36] In this study, we found miR-218-5p might target *HOXA1, KLHDC8A, THSD7A, NAV3, EPHA7, GRM1, JAKMIP3, SRPX2, ELFN2,* and *AJAP1*. Among them, EPHA7, one kind of receptor kinases, played a principal role in the occurrence of cancer, and was connected with lung cancer cells proliferation.[37] A study found in the lung cancer tissues and lung cancer cells the expression of miR-212 decreased, and was closely associated with poor prognostis.[38] MiR-212 was regarded as a tumor suppressor, and inhibited cell migration, cell invasion and EMT by SOX4 signaling.[38] We found hsa-miR-212-5p might target DPYSL3. DPYSL3, one kind of cell-adhesions proteins, was connected with metastatic lung cancer.[39] In this study, we found hsa-miR-627-5p had the most target genes (*ITLN1, TIMP3, PPP4R4, SLC1A2, NOVA1, RNFT2, CLDN10, TMCC3, EPHA7, SRPX2, PPP1R16B, GRM1, HS3ST3A1, SFRP2, SLC7A11,* and *KLHDC8A*). Lectins belong to innate immune defense proteins, and ITLN1 may defend against bacteria.[40] Both protein and gene expressions of ITLN1 were lower in airway epithelial cells of healthy smokers than in healthy non-smokers.[40] Reduced expression of ITLN1 also existed in smokers with lone emphysema and COPD.[40]

5. Conclusion

In summary, our study attempted to reveal the molecular changes induced by smoking in airway epithelium cells by bioinformatics analysis. In this work, we screened 107 DEGs and 10 DEMs. Fifteen hub genes (*NQO1, CYP1B1, AKR1C1, CYP1A1, AKR1C3, CEACAM5, MUCL1, B3GNT6, MUC5AC, MUC12, PTGER4, CALCA, CBR1, TXNRD1,* and *CBR3*) were determined by cytoHubba plugin. Cluster analysis revealed hub genes were associated with adenocarcinoma in situ, squamous cell carcinoma, cell differentiation, inflammatory response, oxidative DNA damage, oxidative stress response and tumor necrosis factor. At last, we performed a microRNA-target genes network, and found that hsa-miR-627-5p, hsa-miR-218-5p and hsa-miR-9-5p might target EPHA7, hsa-miR-212-5p might target DPYSL3 and hsa-miR-627-5p might target ITLN1. In the future, more experimental studies are needed to validate the molecular changes and the connection between microRNA and target genes in response to smoking in human airway epithelium cells.

Author contributions

Conceptualization: Jizhen Huang, Yuan Zhang.
Methodology: Wanli Jiang.
Supervision: Xiang Tong, Hong Fan.
Writing – original draft: Jizhen Huang, Li Zhang.

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