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

Jizhen Huang, MMa, Wanli Jiang, MDb, Xiang Tong, MDa, Li Zhang, MMa, Yuan Zhang, MMb, Hong Fan, MDa,∗

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 GSE14834 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, CEBAC5, MUC1, 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 ITL1N1, 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 non-smokers, 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.
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/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.
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, CEACAMS5, MUC12, 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).

### Table 1

| 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   | hsa00990: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, GP3, ALDH3A1, CBR1, CBR3, |
|                |                                                                      |       |           | CSGALNACT1, CBR1, CBR3, 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, SSN1, ADH7, TXNRD1, AKR1C1, ALDH3A1         |
| GOTERM_MF_DIRECT | GO:0047718~indanol dehydrogenase activity                             | 3     | 6.97E-05  | AKR1C3, AKR1B10, AKR1C1                                           |
| GOTERM_MF_DIRECT | GO:0004497~monoxygenase 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:0016555~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, BRF4, C3, CH3L1, DPLS3, CBR3, TCTN, TIMP3, ALDH3A1, CALCA, ELF2, |
|                |                                                                      |       |           | CCL2,4, CCL20, SRP2, SRRP2, APELA, MEPIA, LIF, MUC5AC, EGF, SP1   |
| GOTERM_CC_DIRECT | GO:0031090~organelle membrane                                         | 5     | 7.98E-04  | CYP1B1, CYP1A1, CYP4F11, CYP4F3, CYP4F2                             |
| GOTERM_CC_DIRECT | GO:0007082~extracellular exosome                                      | 26    | 1.13E-03  | CYP1B2, C3, ITU1, SEC14L3, TIMP3, GPX2, AKR1C3, CBR1, LIF, CEA5M5, EGF, NQO1, |
|                |                                                                      |       |           | AKR1C1, SPP1, MAOB, CH3L1, HGd, TKT, AB2B6, SCEL, THSD7A, AKR1B10, |
|                |                                                                      |       |           | MEPIA, TXNRD1, MUC5AC, ATP6V0A4                                    |
| GOTERM_CC_DIRECT | GO:0005576~extracellular region                                       | 15    | 1.99E-02  | CALCA, BMF4, MUC1, C3L2, CCL2,3, SRP2, C3, APELA, LIF, ADH7, MUC5AC, EGF, |
|                |                                                                      |       |           | TIMP3, TCTN, SP1                                                   |
| GOTERM_CC_DIRECT | GO:0016021~integral component of membrane                            | 34    | 3.31E-02  | CYP1B1, C3L2, SEC14L3, TMCC2, GPAT3, ALDH3A1, SLCA2, APELA, RMF2, |
|                |                                                                      |       |           | B3GNT6, NQO1, EGF, TME4A, DTA, MUC12, HSSST3A1, PTGER4, MAOB, CYP4F11, |
|                |                                                                      |       |           | AAAP1, GM41, ABCB6, SLCTA1, THSD7A, ELF2, SRRP2, APE1A, MEPIA, CYP4F3, |
|                |                                                                      |       |           | CYP4F2, HTR2B, QPI4, PHX, ATP6V0A4                                 |
| GOTERM_BP_DIRECT | GO:0055114~oxidation-reduction process                               | 21    | 4.03E-12  | ME1, CYP1B1, CYP1A1, MAOB, HGd, SSN1, CYP4F11, ADH7, CBR3, ALDH3A1, |
|                |                                                                      |       |           | AKR1C1, GPX2, CBR1, PR, AKR1B10, CYP4F3, TXNRD1, CYP4F2, NQO1, |
|                |                                                                      |       |           | AKR1C1, CBR3, CYP4F2                                               |
| 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  | BMF4, SLCA2, APE1A, SIK3                                          |
| GOTERM_BP_DIRECT | GO:0044597~doxorubicin 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.
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.
| Gene symbol | GSE11906 | GSE22047 | GSE63127 |
|-------------|-----------|-----------|-----------|
| TXNRD1      | 1.1842977 | 1.10938   | 1.176964  |
| PTGER4      | 1.0522499 | 1.03131   | 1.176964  |
| NQO1        | 1.8612315 | 1.7840761 | 1.759625  |
| MUC5AC      | 1.5279379 | 1.565562  | 1.950195  |
| MUC12       | 1.299334  | 1.419667  | 1.677413  |
| CYP1B1      | 1.1527061 | 2.0219303 | 5.021295  |
| CYP1A1      | 3.9462927 | 4.3943414 | 3.907575  |
| CEACAM5     | 2.2769657 | 2.4254444 | 2.619968  |
| CBRI        | 1.1907599 | 1.127893  | 1.16452   |
| CALCA       | 1.5413911 | 1.722658  | 1.729414  |
| B3GNT6      | 1.433297  | 1.23701   | 1.291914  |
| AKR1C3      | 1.6136873 | 1.762066  | 1.609917  |
| AKR1C1      | 2.1617338 | 2.1428149 | 2.142024  |

DEGs = differentially expressed genes.

Figure 4. Cluster analysis of the hub genes. Cluster analysis of the hub genes was enforced using GenCLip 2.0.
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
major cancer in humans, and epidemiological evidences show smoking is a substantial cause of lung cancer. Almost 87% of lung cancer was caused by cigarette smoking. Cigarette smoking is also the most primary risk factor of COPD. Compared to nonsmokers, smokers have higher risk of respiratory symptoms and COPD mortality. More and more studies revealed that smoking induced a series of genetic changes in lung, which were closely related to lung cancer and COPD. 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, GSE22047 and GSE63127 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 for 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, MUC1, 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, 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. A study reported that cigarette smoke extract could increase the level of CYP1A1 and CYP1B1 in normal bronchial epithelial cells, and the abnormal levels of CYP1A1 and CYP1B1 were associated with cancer. 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. In human oral cells, investigator found cigarette smoke condensate might aggravate the expressions of CYP1A1, CYP1B1, AKR1C1, AKR1C3, and AKR1B10. 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. The level of MUC5AC, one kind of secretory mucin, was abnormal in numerous cancers. In lung cancer, the incremental level of MUC5AC meant a poor prognosis. A study found that MUC5AC promoted the migration of lung cancer cells by focal adhesion kinase (FAK) signaling. MUC1 was one of breast-specific genes, and played a remarkable role in the metastasis or progression of breast cancer. MUC1 might mediate the proliferation of breast cancer cells by FAK/ Jun NH2-terminal kinase (JNK) signaling pathway. 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. 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. 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. In this study, we found miR-218-5p might target HOXAI, 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. A study found in the lung cancer tissues and lung cancer cells the expression of miR-212 decreased, and was closely associated with poor prognosis. MI5-212 was regarded as a tumor suppressor, and inhibited cell migration, cell invasion and EMT by SOX4 signaling. We found hsa-miR-212-5p might target DPYSL3. DPYSL3, one kind of cell-adhesions proteins, was connected with metastatic lung cancer. In this study, we found hsa-miR-627-5p had the most target genes (ITLN1, TIP3, PPP4R4, SLCA12, NOVA1, RNFT2, CLDN10, TMCC3, EPHA7, SRPX2, PPP1R16B, GRM1, HS3ST3A1, SFRP2, SLCA11, and KLHDC8A). Lectins belong to innate immune defense proteins, and ITLN1 may defend against bacteria. Both protein and gene expressions of ITLN1 were lower in airway epithelial cells of healthy smokers than in healthy nonsmokers. Reduced expression of ITLN1 also existed in smokers with lone emphysema and COPD.

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, MUC1, 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.

References
[1] Sridhar S, Schembri F, Zeskind J, et al. Smoking-induced gene expression changes in the bronchial airway are reflected in nasal and buccal epithelium. BMC Genomics 2008;9:259.
[2] Jiang B, Guan Y, Shen HJ, et al. Akt/PKB signaling regulates cigarette smoke-induced pulmonary epithelial-mesenchymal transition. Lung Cancer 2018;122:44–53.
[3] Milara J, Peiro T, Serrano A, et al. Epithelial to mesenchymal transition is increased in patients with COPD and induced by cigarette smoke. Thorax 2013;68:410–20.
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[4] Cui Y, Liu KW, Liang Y, et al. Inhibition of monoamine oxidase-B by selegiline reduces cigarette smoke-induced oxidative stress and inflammation in airway epithelial cells. Toxicol Lett 2017;268:44–50.

[5] Wistuba II, Lam S, Behrens C, et al. Molecular damage in the bronchial epithelium of current and former smokers. J Natl Cancer Inst 1997;89:1366–73.

[6] Sonkoly E, Pivarcsi A. Advances in microRNAs: implications for immunity and inflammatory diseases. J Cell Mol Med 2009;13:24–38.

[7] Qi J, Mu D. MicroRNAs and lung cancers: from pathogenesis to clinical implications. Front Med 2012;6:134–55.

[8] Pua HH, Ansel KM. MicroRNA regulation of allergic inflammation and asthma. Curr Opin Immunol 2015;36:101–8.

[9] Huang X, Zhu Z, Guo X, et al. The roles of microRNAs in the pathogenesis of chronic obstructive pulmonary disease. Int Immunopharmacol 2019;73:337–47.

[10] Schembri F, Sridhar S, Perdomo C, et al. MicroRNAs as modulators of smoking-induced gene expression changes in human airway epithelium. Proc Natl Acad Sci U A 2009;106:2319–24.

[11] Raman T, O’Connor TP, Hackett NR, et al. Quality control in microarray assessment of gene expression in human airway epithelium. BMC Genomics 2009;10:493.

[12] Butler MW, Fukui T, Salit J, et al. Modulation of cystatin A expression in human airway epithelium related to genotype, smoking, COPD, and lung cancer. Cancer Res 2011;71:2572–81.

[13] Tilley AE, Staudt MR, Salit J, et al. Cigarette smoking induces changes in airway epithelial expression of genes associated with monogenic lung disorders. Am J Respir Crit Care Med 2016;193:215–7.

[14] Barrett T, Wilhite SE, Ledoux P, et al. NCBI GEO: archive for functional genomics data sets-update. Nucleic Acids Res 2013;41:D991–5.

[15] Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009;4:44–57.

[16] Soklarczyk D, Franceschini A, Wyder S, et al. STRING v10: protein–protein interaction networks, integrated over the tree of life. Nucleic Acids Res 2015;43:D447–52.

[17] Su G, Morris JH, Demchak B, et al. Biological network exploration with Cytoscape 3. Curr Protoc Bioinformatics 2014;47:8.13.1–24.

[18] Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 2005;120:15–20.

[19] Hecht SS. Cigarette smoking and lung cancer: chemical mechanisms and approaches to prevention. Lancet Oncol 2002;3:461–8.

[20] Tohl CK. The changing epidemiology of lung cancer. Methods Mol Biol 2009;472:397–411.

[21] Vestbo J, Hurld SS, Agusti AG, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med 2013;187:347–65.

[22] Kim V, Rogers TJ, Criner GJ. New concepts in the pathophysiology of chronic obstructive pulmonary disease. Proc Am Thorac Soc 2006;5:478–85.

[23] Harvey BG, Heguy A, Leopold PL, et al. Modification of gene expression of the small airway epithelium in response to cigarette smoking. J Mol Med (Berl) 2007;85:39–53.

[24] Zhang DL, Qu LW, Ma L, et al. Genome-wide identification of transcription factors that are critical to non-small cell lung cancer. Cancer Lett 2018;434:132–43.

[25] Molma-Pinelo S, Pastor MD, Suarez R, et al. MicroRNA clusters: dysregulation in lung adenocarcinoma and COPD. Eur Respir J 2014;43:1740–9.

[26] Ezzi ME, Crawford M, Cho JH, et al. Gene expression networks in COPD: microRNA and mRNA regulation. Thorax 2012;67:122–31.

[27] Hussain T, Al-Attas OS, Al-Dagheri NM, et al. Induction of CYP1A1, CYP1A2, CYP1B1, increased oxidative stress and inflammation in the lung and liver tissues of rats exposed to incense smoke. Mol Cell Biochem 2014;391:127–36.

[28] Han W, Pentecost BT, Pietropaolo RL, et al. Estrogen receptor alpha increases basal and cigarette smoke extract-induced expression of CYP1A1 and CYP1B1, but not GSTP1, in normal human bronchial epithelial cells. Mol Carcinog 2003;44:202–11.

[29] Xie L, Yu J, Guo W, et al. Aldo-keto reductase 1C3 may be a new radiosensitivity marker in non-small-cell lung cancer. Cancer Gene Ther 2013;20:260–6.

[30] Nagaraj NS, Beckers S, Mensah JK, et al. Cigarette smoke condensate induces cytochromes P450 and aldo-keto reductases in oral cancer cells. Toxicol Lett 2006;165:182–94.

[31] Kolekar JM, Pritchard SC, Kerr KM, et al. Evaluation of NQO1 gene expression and variant allele in human NSCLC tumors and matched normal lung tissue. Int J Oncol 2002;21:1119–24.

[32] Lakshmanan I, Rachaganti S, Hauke R, et al. MUC5AC interactions with integrin beta4 enhances the migration of lung cancer cells through FAK signaling. Oncogene 2016;35:4112–21.

[33] Conley SJ, Bosco EE, Tice DA, et al. HER2 drives Mucin-like 1 to control proliferation in breast cancer cells. Oncogene 2016;35:4225–34.

[34] Oskolkova O, Gawluk G, Tian Y, et al. Prostaglandin E receptor-4 inhibitor mediates endothelial barrier-enhancing and anti-inflammatory effects of oxidized phospholipids. FASEB J 2017;31:4187–202.

[35] Alipoor SD, Adcock IM, Garssen J, et al. The roles of miRNAs as potential biomarkers in lung diseases. Eur J Pharmacol 2016;791:395–404.

[36] Conickx G, Mestdagh P, Avila Cobos F, et al. MicroRNA Profiling Reveals a Role for MicroRNA-218-5p in the Pathogenesis of Chronic Obstructive Pulmonary Disease. Am J Respir Crit Care Med 2017;195:43–56.

[37] Liu M, Zhou K, Cao Y. MicroRNA-944 affects cell growth by targeting EPHA7 in non-small cell lung cancer. Int J Mol Sci 2016;17:24.

[38] Tang T, Huan L, Zhang S, et al. MicroRNA-212 functions as a tumor-suppressor in human non-small cell lung cancer by targeting SOX4. Oncol Rep 2017;38:2243–50.

[39] Yang Y, Jiang Y, Xie D, et al. Inhibition of cell-adhesion protein DPYSL3 promotes metastasis of lung cancer. Respir Res 2018;19:41.

[40] Carolan BJ, Harvey BG, De BP, et al. Decreased expression of integrin 1 in the human airway epithelium of smokers compared to nonsmokers. J Immunol 2008;181:5760–7.