European Review for Medical and Pharmacological Sciences 2013; 17: 1923-1931

Integration microarray and regulation datasets for chronic obstructive pulmonary disease

J.-Y. YANG, J. JIN, Z. ZHANG, L. ZHANG, C. SHEN

Respiratory Department, Affiliated Sixth People's Hospital, Shanghai Jiaotong University, Shanghai, China

Abstract. – BACKGROUND: Pulmonary disease has become one of the major health problems.

AIM: To investigate the regulation mechanism of Chronic Obstructive Pulmonary Disease (COPD) on gene expression and pathway level.

MATERIALS AND METHODS: We mapped the differentially expressed genes to a regulation network and pathways, using transcriptome profiles and regulation data. We constructed a TF-target gene regulation network, TF-pathway regulation network, and pathway crosstalk network.

RESULTS: STAT1, NFKB1, SMAD4, and STAT3 played an important role in COPD through participating in a number of pathways. Although these related pathways all have been demonstrated associated with COPD in previous reports, the detail mechanism may be not very clear.

CONCLUSIONS: Our results may help to further understanding the mechanism of COPD. And Identified multiple pathways will also provide novel avenues in the treatment of COPD.

Key words:
Chronic obstructive pulmonary disease, Gene regulatory networks, Pathway analysis.

Introduction

Chronic Obstructive Pulmonary Disease (COPD) is a major and increasing global health problem, which is predicted to become the fourth leading cause of death and the fifth commonest cause of disability in the world by 2030[1]. COPD is characterized by chronic irreversible airflow limitation[2].

COPD is caused by noxious particles or gas, most commonly from cigarette smoke (CS), which triggers an abnormal inflammatory response in the lung. This is characterized by increased numbers of neutrophils, macrophages, T-lymphocytes, and the release of multiple inflammatory mediators (lipids, chemokines, cytokines, growth factors)[3,4]. Neutrophil recruitment to the airways and parenchyma involves adhesion to endothelial cells in the airways of COPD patients. Neutrophils can secrete serine proteases, including neutrophil elastase, cathepsin G and proteinase-3, as well as matrix metalloproteinase (MMP)-8 and MMP-9. There is a marked increase in the numbers of macrophages in airways, lung parenchyma, bronchoalveolar lavage (BAL) fluid and sputum in patients with COPD. Macrophages may be activated by CS extract to release TNF-α, IL-8, other CXC chemokines, monocyte chemotactic peptide (MCP)-1, LTB4 and reactive oxygen species (ROS). Dendritic cells (DCs) may migrate from the airways to regional lymph nodes and stimulate proliferation of CD8+ and CD4+ T-lymphocytes. T-lymphocytes in peripheral airways of COPD patients show increased expression of CXCR3, a receptor activated by IP-10, Mig and I-TAC. In a word, the increasing of the inflammatory response cell and subsequently mediators release further amplify the normal inflammatory response to CS in COPD disease[5].

Proof that genetic factors are involved in the pathogenesis of COPD comes from the observation that individuals with severe deficiency for alpha-1-antitrypsin, a major inhibitor of serine proteases, have an increased risk of developing COPD. Individuals with a severe deficiency for alpha-1-antitrypsin tend to develop more severe COPD at an earlier age[6].

Gene expression profiling of human diseased tissues may provide insights into the molecular mechanisms of human disease and may eventually lead to the identification of novel therapeutic targets[7]. A high-throughput microarray experiment was designed to analyze genetic expression patterns and identify potential genes to target for COPD. The identification of potential differen-

Corresponding Author: Ce Shen, MD; e-mail: ce.shen.sh@gmail.com

tially expressed genes may assist for improved COPD diagnosis[8].

In this study, we analyzed gene expression profiles that distinguish COPD patients from healthy control subjects. Furthermore, the relevant transcription factors, target genes and pathways in the network are used to construct regulation network and explain potential regulation mechanisms in COPD.

Materials and Methods

Affymetrix microarray data

One transcription profile of GSE16972 was obtained from a public functional genomics data repository GEO (http://www.ncbi.nlm.nih.gov/geo/).

Bronchoalveolar lavage (BAL) fluid samples were collected from 5 COPD patients and 5 healthy controls, with all of them being smokers. Besides, the control and COPD groups were homogenous regarding age and smoking habits, but only differed in lung function parameters. All subjects were between 40 and 65 years old. In COPD patients, percent of predicted one-second forced expiratory volume (FEV_1) (FEV_1%) was < 80% and FEV_1 to percent of predicted forced vital capacity (FVC) (FVC%) was < 70%; in control patients, FEV_1% was ≥ 80% and FEV_1/FVC% was ≥ 70%. COPD patients all belong to stage 2-3 according to the Global Initiative for Chronic Obstructive Lung Disease[9]. Alveolar macrophages were isolated using Percoll gradient centrifugation, isolated using CD14+ magnetic beads, and total RNA was extracted using Qiagen RNeasy Kit (Qiagen, Hilden, Germany). Total RNA was labeled with biotin and individual samples were hybridized to Affymetrix HG U133A GeneChips (Affymetrix, Cleveland, OH, USA).

Regulation data

There are approximately 2600 proteins in the human genome that contain DNA-binding domains, and most of these are presumed to function as transcription factors (TFs)[10]. The combinatorial use of a subset of the approximately 2000 human transcription factors easily accounts for the unique regulation of each gene in the human genome during development[11].

TRANSFAC database (http://www.gene-regulation.com/pub/databases.html) contains data on transcription factors, their experimentally-proven binding sites, and regulated genes[12]. Transcriptional Regulatory Element Database (TRED, http://rulai.cshl.edu/TRED/) has been built in response to increasing needs of an integrated repository for both cis- and trans- regulatory elements in mammals[13]. TRED did the curation for transcriptional regulation information, including transcription factor binding motifs and experimental evidence. The curation is currently focusing on target genes of 36 cancer-related TF families.

Combined the two regulation datasets, total 6328 regulatory relationships between 276 TFs and 3002 target genes were collected (Table I).

Pathway data

Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.genome.jp/kegg/)is a collection of online databases dealing with genomes, enzymatic pathways, and biological chemicals[14]. The PATHWAY database records networks of molecular interactions in the cells, and variants of them specific to particular organisms. Total 130 pathways, involving 2287 genes, were collected from KEGG.

Differentially expressed genes (DEGs) analysis

For the GSE16972 dataset, the limma method[15] was used to identify differentially expressed genes (DEGs). The original expression datasets from all conditions were processed into expression estimates using the RMA (Robust Multi-array Average) method with the default settings implemented in Bioconductor, and then construct the linear model. The DEGs only with the fold change value larger than 1.5 and *p*-value less than 0.05 were selected.

Co-expression analysis

For demonstrating the potential regulatory relationship, the Pearson Correlation Coefficient (PCC) was calculated for all pair-wise comparisons of gene-expression values between TFs and the DEGs. The regulatory relationships whose absolute PCC are larger than 0.6 were considered as significant.

Table I. Regulation data

Database	Regulationship	TFs	Targets
TRANSFAC	774	219	265
TRED	5722	102	2920
Total	6328	276	3002

Gene Ontology analysis

DAVID[16] is a high-throughput and integrated data-mining environment analyzing gene lists derived from high-throughput genomic experiments. Use the DAVID to identify over-represented GO categories in biological process.

Regulation and PPI network construction

Using the regulation data that have been collected from TRANSFAC database and TRED database, we matched the relationships between differentially expressed TFs and its differentially expressed target genes.

Base on the above two regulation datasets and the pathway relationships of the target genes, we build the regulation networks by Cytoscape[17]. Base on the significant relationships (PCC > 0.6 or PCC < –0.6) between TFs and its target genes, 17 putative regulatory relationships were predicted between 12 TFs and 16 target genes.

Pathway crosstalk analysis

To investigate the relationship between pathways which were regulated by the same TF in the TF to pathway regulation network, we used the PPI-network approach to find the crosstalk of pathways.

Here the crosstalk pathways are defined as those pathways which have the overlapping genes and edges with each other. The overlapping genes mean both of the two pathways included and the overlapping edges mean both of the two pathways included the PPI interaction edges.

To determine the co-expressed significance of a gene pair in disease cases, we used the PCC test to calculate the *p*-value.

Map those *p*-values to the nodes and edges in the PPI network collected from the human protein reference database (HPRD)[18] and BIOGRID[19] database. The following formula is used to define a function as the combination of statistical significance of an interaction by a scoring scheme. The detail description could be seen in Liu et al[20].

$$S(e) = f(\text{diff}(x), \text{cor}(x, y), \text{diff}(y)) = -2\sum_{1-1}^{k} \log_e(pi)$$

The diff(x) and diff(y) are differential expression assessments of gene x and gene y, respectively. cor (x,y) represents their correlation between gene x and gene y. f is a general data integration method that can handle multiple data sources differing in statistical power. Where k = 3, p1 and p2 are the *p*-values of differential expression of two nodes, p3 is the *p*-value of their co-expression.

The detailed analysis of crosstalk of relationships among pathways is then investigated, especially that with overlap of two significant pathway analysis results.

To define the interaction significance between pathways, we summarize all the scores of edges S(e) of all non-empty overlaps. Specifically, the interaction score between two pathways is estimated by their overlapping status of weighted pathways in the following formula:

$$C(pi, pj) = \sum_{e \in Oij} S(e)$$

To estimate the significance of the overlapping between different pathways, we random sample 10^5 times of the same size two pathways in the edges of pathway network and calculate their overlapping scores. The frequency larger than C is regarded as the interaction significance *p*-value. At last the pathway crosstalk with the *p*-value < 0.05 as the significant crosstalk.

Results

Regulation network

To get DEGs of COPD, we obtained publicly available microarray data sets GSE16972 from GEO. 207 DEGs with the fold change value larger than 1.5 and *p*-value less than 0.05 were selected. To get the regulatory relationships, 0.6 is as the co-expressed value (PCC) threshold. Finally, 17 regulatory relationships including 12 different expressed TFs and their 16 differently expressed target genes were selected. By integrating the regulatory relationships above, a regulation network of COPD was built between TFs and its target genes (Figure 1). In our network, signal transducers and activators of transcription (STAT)1, NFKB1 (nuclear factor K B1) and CDKN1A with higher degrees form a local network which suggesting that these genes may play an important role in COPD. STAT1 actives 3 target genes and NFKB1 actives 4 target genes. The target CDKN1A was regulated by both SMAD4 and STAT3 was observed in this network.

GO analysis of the regulation network

Several Gene Ontology (GO) categories were enriched among these genes in the regulatory network, including immune response, inflamma-

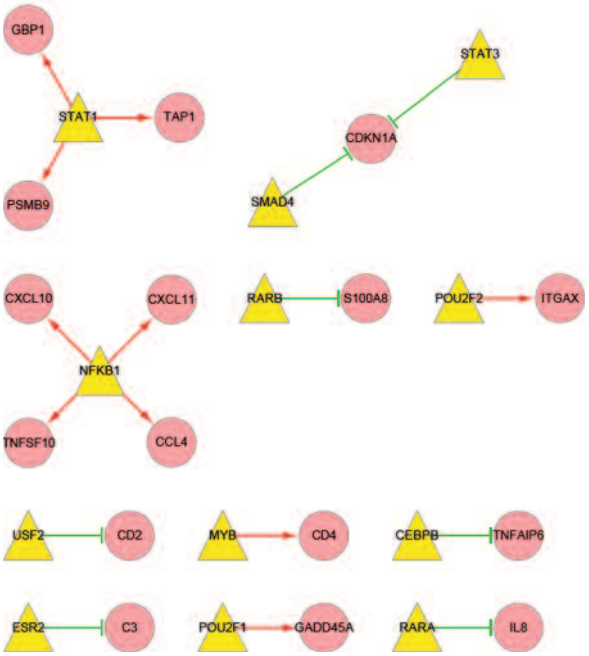

Figure 1. Regulation network of COPD.

tory response, defense response, response to wounding and so on (Table II).

Regulation network of TF to pathways

To investigate the regulatory relationships between TFs and pathways, we mapped DEGs to pathways and got a regulation network between TFs and pathways (Figure 3).

In the network, SMAD4, STAT3, RARA and NFKB1 were as hub nodes linked to lots of COPD related pathways. Some of TFs interaction regulated lots of pathways, such as SMAD4, STAT3 and RARA total regulated the bladder cancer and pathways in cancer; STAT1 and MYB both regulated the antigen processing and presentation and primary immunodeficiency.

Pathway crosstalk analysis

Further, we considered the pathway crosstalk between significant pathways detected by the overlaping score to confirm above regulation network. As we expected, 18 significant pathways crosstalk between each other was identified (Figure 3).

Of them, antigen processing and presentation pathway and primary immunodeficiency pathway have been demonstrated crosstalk with ABC transporters pathway with the p-value < 0.05 (Figure 3) which was in accordance with our analysis that these three pathways were all regulated by the STAT1 gene (Figure 2). Identically, natural killer cell mediated cytotoxicity pathway, apoptosis pathway, and cytokine-cytokine receptor interaction pathway were significant crosstalk with chemokine signaling pathway, which were regulated by the NFKB1 gene. We also found that bladder cancer, chronic myeloid leukemia pathway, which crosstalk with ErbB signaling pathway significantly, were regulated by the SMAD4 and STAT3.

In the graph, the blue point means pathways and the pink line means the p-value of crosstalk is less than 0.05.

Table II. GO enrichment analysis of biological process

Category	Term	Counts	*p*-value	FDR
BP	GO:0006955~immune response	10	8.43E-09	1.15E-05
BP	GO:0006954~inflammatory response	7	7.65E-07	0.001042
BP	GO:0006952~defense response	8	1.81E-06	0.002469
BP	GO:0009611~response to wounding	7	1.30E-05	0.017701
BP	GO:0006935~chemotaxis	4	6.65E-04	0.902068
BP	GO:0042330~taxis	4	6.65E-04	0.902068
BP	GO:0002684~positive regulation of immune system process	4	0.002092	2.810571
BP	GO:0007626~locomotory behavior	4	0.003122	4.166664
BP	GO:0007267~cell-cell signaling	5	0.003536	4.707006
BP	GO:0007050~cell cycle arrest	3	0.005651	7.423657
BP	GO:0002696~positive regulation of leukocyte activation	3	0.005975	7.833621
BP	GO:0007155~cell adhesion	5	0.006138	8.039181
BP	GO:0022610~biological adhesion	5	0.006169	8.078366
BP	GO:0050867~positive regulation of cell activation	3	0.006534	8.536473

BP: biological process

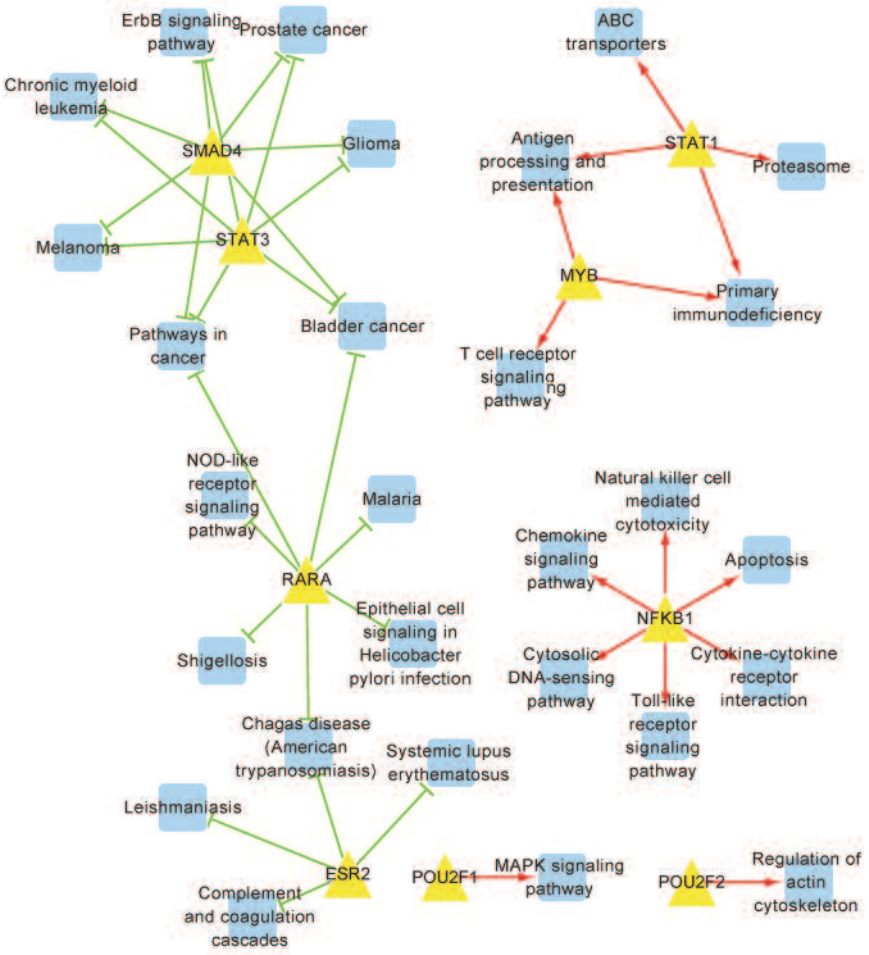

Figure 2. Regulation network of TF-PATHWAY

Discussion

From the results section, we could find that many genes have been identified high correlation with COPD. Of them, STAT1, NFKB1 and CDKN1A were indicated play an important role in COPD because of higher degrees to form a local network. As transcription factor, STAT1 could active 3 target genes, but NFKB1 active 4 target genes. The target gene, CDKN1A was regulated by both SMAD4 and STAT3. These genes all have been demonstrated associated with COPD in previous reports.

STAT1 protein can be phosphorylated by the receptor associated kinases, and then form homo- or heterodimers that translocate to the cell nucleus where they act as transcription activators. This protein mediates the expression of a variety of genes, which is thought to be important for cell viability in response to different cell stimuli and pathogens. STAT1 was significant associations to the binary COPD phenotype in two independent populations[21].

NFkB1 is a transcription regulator that is activated by various stimuli such as cytokines, oxidant-free radicals, ultraviolet irradiation, etc. Inappropriate activation of NFKB has been associated with a number of inflammatory diseases. Study showed a significant reduction in the percentage of sputum neutrophils undergoing spontaneous apoptosis in subjects with COPD compared to non-smokers. And a significant increase was observed in the expression of both the p50 (p = 0.006) and p65 (p = 0.006) subunits of NF-kB in neutrophils from COPD subjects compared to non-smokers. These results give an effective support that NFKB signaling could delay constitutive neutrophil apoptosis, which contribute to the ineffective resolution of inflammation in COPD[22].

SMAD4 gene encodes a member of the smad family of signal transduction proteins. Smad/TGFβ1 (transforming growth factor-β1) is an important signaling pathway to mediate fibrosis stimulating the secretion of extracellular ma-

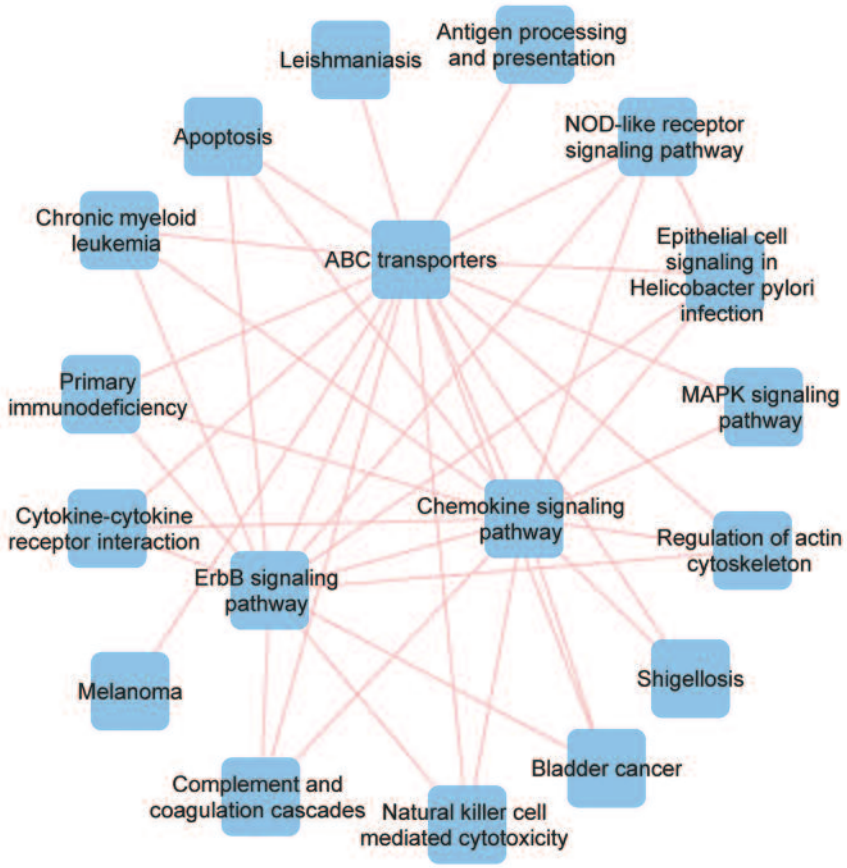

Figure 3. Pathway crosstalk analysis.

trix proteins and involve in airway remodeling in COPD. The expression of secretory leukocyte proteinase inhibitor (SLPI) in the COPD rat models significantly decreased, which may be caused by the increased expression of TGFβ1. Furthermore, effect of TGF-beta1-inhibited expression of SLPI was disengaged by SMAD4 both at the mRNA level and the protein level. Hence, SMAD4 may prove to be an important target for future development of new therapeutic strategies for COPD[23,24].

STAT3 protein is a member of the STAT protein family. This protein mediates the expression of a variety of genes in response to cell stimuli, and thus plays a key role in many cellular processes such as cell growth and apoptosis. The STAT3 gene was up-regulated in COPD caused by smoking. Furthermore, a panel of STAT3-up-regulated downstream genes was also modestly up-regulated in lung tissue with COPD from patients with a history of smoking. Therefore, STAT3 and its downstream genes can serve as biomarkers for COPD diagnosis and prognosis in humans[25].

CDKN1A (p21) gene encodes a potent cyclin-dependent kinase inhibitor which binds to and inhibits the activity of cyclin-CDK2 or -CDK4 complexes, in turn inhibiting cell proliferation so as to allow cells to repair the DNA damage. Previous study about the relationship between CS and COPD inflammation revealed that p21 was also an important modifier of lung inflammation, and genetic ablation of p21 (p21-/-) could attenuate CS-mediated lung inflammation[26].

Further, regulation network between TFs and pathways was constructed. As we expected, STAT1, NFkB1, SMAD4, and STAT3 were still as hub nodes linked to lots of COPD related pathways. For example, STAT1 regulated the antigen processing and presentation, Primary immunodeficiency and ABC transporters pathway; SMAD4 and STAT3 could regulate the bladder cancer, ErbB (also know as EGFR) signaling pathway and pathways in cancer; NFkB1 regulated natural killer cell mediated cytotoxicity pathway, apoptosis pathway, cytokine-cytokine receptor interaction pathway and chemokine signaling pathway.

These results were in agreement with our subsequent crosstalk analysis. All of these pathways have been demonstrated involved in COPD in direct or indirectly manner in previous reports.

Antigen processing and presentation have been reported related with COPD progression. The mature lymphoid collections are rarely observed in the lungs of nonsmokers, but they are present in the airways of smokers with COPD. This increase has been attributed to the large antigen load associated with bacterial colonization and more frequent infection of the lower respiratory tract. The epithelium covering the surface of the mucosal lymphoid follicles contains specialized M cells that transport antigens from the epithelial surface to the lamina propria in the mucosal immune system. Subsequently, antibody or effector T cells were induced to complete immune response[27].

Primary immunodeficiency may also contribute to persistent airway inflammation and progressive airway remodeling in COPD. Air-liquid interface epithelial cell cultures revealed that complete epithelial differentiation was required for normal pIgR expression and IgA transcytosis. However, areas of bronchial epithelial remodeling was found reduced pIgR expression, localized selective IgA deficiency, and increased CD4(+) and CD8(+) lymphocyte infiltration in patients with COPD. These results indicated that epithelial structural abnormalities lead to localized selective IgA deficiency (most common primary immunodeficiency) in COPD airways[28].

As an ATP-binding cassette (ABC) transporter, multidrug resistance-associated protein 1 (MRP1) expression was found diminished in bronchial epithelium of COPD patients (ex-smokers) and that lower expression is related to worse lung function. In addition, bronchial MRP1 expression was higher during smoking than after one year of smoking cessation, indicating functional activity of MRP1 in the lung may play an important role in the antioxidant defence against toxic compounds generated by cigarette smoke[29].

COPD was investigated associated with a significantly worse survival in cancer patients at the time of cancer diagnosis, being about 15% in elderly patients with bladder cancer. Therefore, closer involvement of pulmonologists and COPD nurses in cancer patients might be warranted[30]. A number of pathways in cancer have been suggested underlying mechanism to understand of how lung cancer is associated with COPD. Such as downregulation of NF-kB activation may improve the efficacy of first-line therapy in both COPD and lung cancer. In addition, immune dysfunction, altered adhesion signaling pathways, epithelial-to-mesenchymal transition, and oxidant/inflammation-driven extracellular matrix (ECM) degradation were all suggested associated with COPD[31].

There was some evidence that ErbB signaling pathway involved in the pathophysiology of COPD. Mucus hypersecretion from hyperplastic airway goblet cells is a hallmark of COPD. Activation of epidermal growth factor receptors (EGFR, also know as ErbB) is responsible for mucin production after inhalation of cigarette smoke in airways. In the airway epithelial cell line and rat airway model, exposure to CS upregulated the EGFR mRNA expression and induced activation of EGFR-specific tyrosine phosphorylation, resulting in upregulation of MUC5AC mRNA and protein production, effects that were inhibited completely by selective EGFR tyrosine kinase inhibitor[32].

Natural killer mediated cell cytotoxic activity was found to be significantly decreased in patients with COPD compared with levels in healthy volunteers. This defect could be partially rescued by glycophosphopeptical[33,34].

Recently, an increasing number of data suggest apoptosis of structural cells in the lung might possibly be an important upstream event in the pathogenesis of COPD. There is an increase in apoptotic alveolar epithelial and endothelial cells in the lungs of COPD patients. Since this is not counterbalanced by an increase in proliferation of these structural cells, the net result is destruction of lung tissue and the development of emphysema[35,36].

As mentioned in introduction, recruitment of neutrophils and release of inflammatory mediators, such as CXCR1 and CXCR2 in the lung parenchyma of COPD patients appears to be a critical event in disease development and progression. Therefore, cytokine-cytokine receptor interaction pathway and chemokine signaling pathway were no doubt associated with COPD[37]. CXCR3/CXCL10 axis also has been proposed involved in the T cell recruitment that occurs in peripheral airways of smokers with COPD[38].

Conclusions

We used the regulation network approach to identify the most robust and consistent set of

genes for discrete and quantitative COPD phenotypes. This strategy used microarray data extraction methods and regulation datasets. The genes STAT1, NFKB1, STAT3 and CDKN1A had been proved high related to COPD with previous studies. Although SMAD4 had not been proved to be responded to the COPD directly, our results suggest that it involve in. Importantly, many pathways have been linked to those genes, which may help to understanding the mechanism of COPD. Identified multiple pathways will also provide novel avenues in the treatment of COPD.

Conflict of Interest

None.

Reference

1) Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med 2006; 3: e442.
2) Di Stefano A, Caramori G, Ricciardolo FL, Capelli A, Adcock IM, Donner CF. Cellular and molecular mechanisms in chronic obstructive pulmonary disease: an overview. Clin Exp Allergy 2004; 34: 1156-1167.
3) Barnes PJ. Mechanisms in COPD: differences from asthma. Chest 2000; 117(2 Suppl): 10S-14S.
4) Chung K. Cytokines in chronic obstructive pulmonary disease. Eur Respir J 2001; 18(34 suppl): 50s-59s.
5) MacNee W. Pathogenesis of chronic obstructive pulmonary disease. Proc Am Thorac Soc 2005; 2: 258-266; discussion 90-91.
6) Pauwels RA, Rabe KF. Burden and clinical features of chronic obstructive pulmonary disease (COPD). Lancet 2004; 364(9434): 613-620.
7) Chen ZH, Kim HP, Ryter SW, Choi AM. Identifying targets for COPD treatment through gene expression analyses. Int J Chron Obstruct Pulmon Dis 2008; 3: 359-370.
8) Bhattacharya S, Srisuma S, Demeo DL, Shapiro SD, Bueno R, Silverman EK, Reilly JJ, Mariani TJ. Molecular biomarkers for quantitative and discrete COPD phenotypes. Am J Respir Cell Mol Biol 2009; 40: 359-367.
9) Poliska S, Csanky E, Szanto A, Szatmari I, Mesko B, Szeles L, Dezso B, Scholtz B, Podani J, Kilty I, Takacs L, Nagy L. Chronic Obstructive Pulmonary Disease-Specific gene expression signatures of alveolar macrophages as well as peripheral blood monocytes overlap and correlate with lung function. Respiration 2011; 81: 499-510.
10) Wachi S, Yoneda K, Wu R. Interactome-transcriptome analysis reveals the high centrality of genes differentially expressed in lung cancer tissues. Bioinformatics 2005; 21: 4205-4208.
11) Brivanlou AH, Darnell JE, Jr. Signal transduction and the control of gene expression. Science 2002; 295(5556): 813-818.
12) Wingender E. The TRANSFAC project as an example of framework technology that supports the analysis of genomic regulation. Brief Bioinform 2008; 9: 326.
13) Jiang C, Xuan Z, Zhao F, Zhang MQ. TRED: a transcriptional regulatory element database, new entries and other development. Nucleic Acids Res 2007; 35(Database issue): D137-140.
14) Kanehisa M. The KEGG database. Novartis Found Symp 2002; 247: 91-101; discussion -3, 19-28, 244-252.
15) Smyth GK. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. Stat Appl Genet Mol Biol 2004; 3: Article3.
16) 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.
17) Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003; 13: 2498-2504.
18) Keshava Prasad T, Goel R, Kandasamy K, Keerthikumar S, Kumar S, Mathivanan S, Telikicherla D, Raju R, Shafreen B, Venugopal A, Balakrishnan L, Marimuthu A, Banerjee S, Somanathan DS, Sebastian A, Rani S, Ray S, Harrys Kishore CJ, Kanth S, Ahmed M, Kashyap MK, Mohmood R, Ramachandra YL, Krishna V, Rahiman BA, Mohan S, Ranganathan P, Ramabadran S, Chaerkady R, Pandey A. Human protein reference database–2009 update. Nucleic Acids Res 2009; 37(Database issue): D767-D772.
19) Stark C, Breitkreutz BJ, Chatr-aryamontri A, Boucher L, Oughtred R, Livstone MS, Nixon J, Van Auken K, Wang X, Shi X, Reguly T, Rust JM, Winter A, Dolinski K, Tyers M. The BioGRID Interaction Database: 2011 update. Nucleic Acids Res 2011; 39(Database issue): D698-D704.
20) Liu ZP, Wang Y, Zhang XS, Chen L. Identifying dysfunctional crosstalk of pathways in various regions of Alzheimer's disease brains. BMC Syst Biol 2010; 4(Suppl 2): S11.
21) Bakke PS, Zhu G, Gulsvik A, Kong X, Agusti AG, Calverley PM, Donner CF, Levy RD, Make BJ, Pare PD, Rennard SI, Vestbo J, Wouters EF, Anderson W, Lomas DA, Silverman EK, Pillai SG. Candidate genes for COPD in two large data sets. Eur Respir J 2011; 37: 255-263.
22) Brown V, Elborn JS, Bradley J, Ennis M. Dysregulated apoptosis and NFkappaB expression in COPD subjects. Respir Res 2009; 10: 24.
23) Luo BL, Niu RC, Feng JT, Hu CP, Xie XY, Ma LJ. Downregulation of secretory leukocyte proteinase inhibitor in chronic obstructive lung disease: the role of TGF-beta/Smads signaling pathways. Arch Med Res 2008; 39: 388-396.

24) Niu RC, Luo BL, Feng JT, Wang LJ, Hu CP. Expression of secretory leukocyte proteinase inhibitor in the bronchi and lung tissues of chronic obstructive pulmonary disease rat models and the regulatory mechanism by transforming growth factor beta(1). Zhonghua Jie He He Hu Xi Za Zhi 2007; 30: 851-856.

25) Qu P, Roberts J, Li Y, Albrecht M, Cummings OW, Eble JN, Du H, Yan C. Stat3 downstream genes serve as biomarkers in human lung carcinomas and chronic obstructive pulmonary disease. Lung Cancer 2009; 63: 341-347.

26) Yao H, Yang SR, Edirisinghe I, Rajendrasozhan S, Caito S, Adenuga D, O'Reilly MA, Rahman I. Disruption of p21 attenuates lung inflammation induced by cigarette smoke, LPS, and fMLP in mice. Am J Respir Cell Mol Biol 2008; 39: 7-18.

27) Hogg JC, Timens W. The pathology of chronic obstructive pulmonary disease. Annu Rev Pathol 2009; 4: 435-459.

28) Polosukhin VV, Cates JM, Lawson WE, Zaynagetdinov R, Milstone AP, Massion PP, Ocak S, Lee JW, Bowler RP, Kononov AV, Randell SH, Blackwell TS. Bronchial secretory IgA deficiency correlates with airway inflammation and progression of COPD. Am J Respir Crit Care Med 2011; 184: 317-327.

29) Van Der Deen M, De Vries E, Timens W, Scheper RJ, Timmer-Bosscha H, Postma DS. ATP-binding cassette (ABC) transporters in normal and pathological lung. Respir Res 2005; 6: 59.

30) van de Schans SA, Janssen-Heijnen ML, Biesma B, Smeenk FW, van de Poll-Franse LV, Seynaeve C, Coebergh JW. COPD in cancer patients: higher prevalence in the elderly, a different treatment strategy in case of primary tumours above the diaphragm, and a worse overall survival in the elderly patient. Eur J Cancer 2007; 43: 2194-2202.

31) Yao H, Rahman I. Current concepts on the role of inflammation in COPD and lung cancer. Curr Opin Pharmacol 2009; 9: 375-383.

32) Takeyama K, Jung B, Shim JJ, Burgel PR, Dao-Pick T, Ueki IF, Protin U, Kroschel P, Nadel JA. Activation of epidermal growth factor receptors is responsible for mucin synthesis induced by cigarette smoke. Am J Physiol-Lung Cell Mol Physiol 2001; 280: L165.

33) Prieto A, Reyes E, Bernstein Ed, Martinez B, Monserrat J, Izquierdo Jl, Callol L, De Lucas P, Alvarez-Sala R, Alvarez-Sala Jl. Defective natural killer and phagocytic activities in chronic obstructive pulmonary disease are restored by glycophosphopeptical (inmunoferon). Am J Resp Crit Care Med 2001; 163: 1578.

34) Fairclough L, Urbanowicz R, Corne J, Lamb J. Killer cells in chronic obstructive pulmonary disease. Clin Sci 2008; 114: 533-541.

35) Demedts IK, Demoor T, Bracke KR, Joos GF, Brusselle GG. Role of apoptosis in the pathogenesis of COPD and pulmonary emphysema. Respir Res 2006; 7: 53.

36) Park JW, Ryter SW, Choi AMK. Functional significance of apoptosis in chronic obstructive pulmonary disease. COPD: J Chron Obstruct Pulmon Dis 2007; 4: 347-353.

37) Panina P, Mariani M, DAmbrosio D. Chemokine receptors in chronic obstructive pulmonary disease (COPD). Curr Drug Targets 2006; 7: 669-674.

38) Saetta M, Mariani M, Panina-Bordignon P, Turato G, Buonsanti C, Baraldo S, Bellettato CM, Papi A, Corbetta L, Zuin R. Increased expression of the chemokine receptor CXCR3 and its ligand CXCL10 in peripheral airways of smokers with chronic obstructive pulmonary disease. Am J Resp Crit Care Med 2002; 165: 1404-1409.