

Landscape of Genetic and Modular Networks Between Non-Small Cell Lung Cancer and Chronic Obstructive Pulmonary Disease

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Research

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Abstract

The relationship between non-small cell lung cancer (NSCLC) and chronic obstructive pulmonary disease (COPD) has been demonstrated in many studies. However, the underlying connection is still unknown. The present study explored the landscape between them. Based on literature research and modularized analysis, NSCLC and COPD network were constructed. We employed functional annotation to measure the relationships among the annotation terms of overlapping modules. The two diseases share 154 overlapping genes, 2374 common biological processes and 601 pathways. MMP9, BCL2, BAX, TP53, PIK3CA were common hub genes of the top three modules between NSCLC and COPD. The most common significant biological process was inflammatory response. Via validation, MMP9 and BCL2 were highly expressed in the NSCLC patients with COPD than healthy donors, while BAX and TP53 were lower expressed. Our results provide novel molecular connection between NSCLC and COPD, which may facilitate the diagnosis and treatment of multiple diseases.

Introduction

Non-small cell lung carcinoma (NSCLC) is the most common malignancy, accounts for approximately 85% of lung cancers ^[1]. Chronic obstructive pulmonary disease (COPD) also has a high prevalence and mortality rates ^[2]. The association between COPD and the NSCLC, including gene pathogenicity, endothelial cell remodeling and inflammatory mechanisms, has been observed in multiple studies, ^[3,4].

Comparing to healthy people, COPD patients are relatively at a high risk of developing NSCLC ^[5]. Advanced NSCLC patients are more likely to be accompanied by COPD, and have more respiratory symptoms than those without COPD ^[6]. Chronic activation of airway immune system by infections increased the incidence of NSCLC. Meanwhile, airway chronic inflammation occurs throughout the course of COPD, which may induce bronchial epithelial carcinogenesis ^[7]. Smoking is recognized as an important risk factor for NSCLC and COPD, either active or passive. It also aggravates respiratory symptoms such as dyspnea, coughing and wheezing. Smoking cessation has a positive effect on the prevention and control of NSCLC, while the respiratory symptoms of COPD can also be alleviated to some extent.

Although the two diseases are closely related, the molecular mechanism between them remains unclear. Network construction helps to understand the biological interaction of cancer ^[8]. The regulation network of single molecule was explored in previous report, and provided guideline for future NSCLC therapies ^[9]. In this study, we applied gene network to reveal the systemic biological functions, and bridged the connection between the two diseases.

Materials And Methods

Obtaining the genes and network construction

The terms “non small cell lung cancer” or “chronic obstructive pulmonary disease” were inputted into to the search box of the Online Mendelian Inheritance in Man (OMIM) database, (<http://www.ncbi.nlm.nih.gov/omim>), a knowledge database of human genes and genetic disorders (13). Disease-associated genes were then submitted to Agilent Literature Search software, version 3.2.2 (<http://www.agilent.com/labs/research/litsearch.html>), which automatically querying multiple text-based searching and extracting associations among genes of interest. The overview network of gene associations was constructed.

Network analysis

Cytoscape software version 2.71 (<http://www.cytoscape.org>) was used for visualization of disease-associated networks and analysis of the network properties. Network parameters including the network coefficient, diameter, centralization and radius were calculated.

Identification of modules

MCODE (version 1.32) is a program that is used for network module division (<http://baderlab.org/Software/MCODE>). Subsequent to the disease network data being obtained, each disease network was imported and MCODE was used to divide it into several modules using the following parameters: Connectivity threshold, 2; corethreshold K, 2; node score threshold, 0.2.

Functional enrichment analysis

Hypergeometric distribution tests were performed to analyze the function of the modules that contained the most genes in each network (COPD-associated and NSCLC-associated gene networks) using the Database for Annotation, Visualization and Integrated Discovery (<http://david.abcc.ncifcrf.gov>). The following parameters were used: Count,2; EASE, 0.01; and species and background, Homo sapiens. Using the gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes annotation, the biological processes and pathway corresponding to the modules were identified, and the P-values were ranked.

RNA extraction and quantitative polymerase chain reaction

Serum samples from five NSCLC patients with COPD and five healthy donors were obtained from The Third Affiliated Hospital of Soochow University (Changzhou, China). Total RNA was isolated using the Trizol kit (Life Technologies, US). The DNA levels of MMP9, BCL2, TP53, PIK3CA and BAX were quantified by qRT-PCR which performed on the StepOnePlus™ Real-Time PCR Systems (ABI, US). The primers for amplification of these genes were obtained from Life Technologies (Supplementary Table 4). The cycle condition was set as follows: predenaturation at 95 °C for 1 min, denaturation at 95 °C for 5s, annealing at 60 °C for 30s, extension at 70 °C for 30 s, 40 cycles in total.

Data analysis

Stepone plus V2.0 software was used to calculate the PCR amplification efficiency. The average $2^{-\Delta\Delta CT}$ of each reaction was used for comparison of the genes expression by t test. Data was analyzed using SPSS 23.0 software (SPSS Inc., USA).

Results

NSCLC, COPD-associated genes

After searching 'COPD' and 'NSCLC' in the OMIM database (on May 24, 2019), we got 1615 NSCLC-associated and 417 COPD-associated genes (supplement Table I). The two diseases share 125 overlapping genes, which accounted for 7.74% (125/1615) of the identified NSCLC-associated genes, 29.98% (125/417) of COPD-associated genes.

Diseases network topological analysis

NSCLC network (Fig. 1A) and COPD network (Fig. 1B) spread outward from center. As shown in (Table 1), topological parameters of the two disease networks are compared. NSCLC network include 1615 associated genes and 4,405 edges (interactions), which was more complex than COPD 417 genes and 907 edges). The increase in the node degree (the number of node-edges in the network) in the NSCLC (Fig. 1C) and COPD (Fig. 1D) networks followed a power-law distribution.

Functional enrichment analysis of NSCLC

DAVID functional annotation was used to enrich the genes of the two diseases. After gene ontology, 6327 GO terms and 1702 pathways were obtained, and the number of GO term connections was 317051 (Fig. 2). The most significant functions were regulation of apoptotic process, cellular protein metabolic process, and positive regulation of protein metabolic process. Cellular protein modification process, regulation of cellular macromolecule biosynthetic process, and RNA metabolic process had the largest number of genes, which were 569, 561 and 546 respectively. The most significant pathways were IRS-mediated signaling, SOS-mediated signaling, and downstream signaling events of B Cell Receptor (BCR) (Table 2).

Functional enrichment analysis of COPD

The COPD network consists of 1515 GO terms and 716 pathways, the number of GO term connections was 107254 (Fig. 3). The most significant functions was inflammatory response, regulation of apoptotic process, and cellular response to chemical stimulus. Cellular protein modification process, regulation of cellular macromolecule biosynthetic process, and RNA metabolic process had the largest number of genes. The most significant pathways was cytokines and inflammatory response (Table 3).

Modules of NSCLC and its functional enrichment analysis

A total of 124 modules analyzed by MCODE were identified from the NSCLC network. 315 biological processes were involved in the top 3 modules of NSCLC-associated network (Fig. 4), including positive regulation of transcription from RNA polymerase II promoter, negative regulation of apoptotic process and inflammatory response. There are 12 hub genes in the top 3 modules (genes with the most connections above 10 in the network).

Modules of COPD and its functional enrichment analysis

Then, 35 modules were identified from the COPD network (Fig. 5). The top 3 modules of COPD-associated network have 121 biological processes, including negative regulation of apoptotic process, cell proliferation and inflammatory response. They shared 81 pathways, including the AMPK signaling pathway, pathways in cancer and non-small cell lung cancer pathway.

Validation of hub genes

We examined the expression profiles of overlapping hub genes in the top three modules of NSCLC and COPD. Finally, MMP9 and BCL2 were highly expressed in the blood of NSCLC patients with COPD compared to healthy donors, while BAX and TP53 were lowly expressed (Fig. 6).

Discussion

Numerous molecular characteristics were explored by network construction, and the common biological backgrounds bridged the connection between NSCLC and COPD. The overlapping genes, similar biological processes and pathways provide an efficient method to study the genetic correlation of them. The top three modules of NSCLC and COPD shared 5 overlapping hub genes, MMP9, BCL2, TP53, PIK3CA and BAX.

MMP9 is a member of Matrix metalloproteinases (MMPs), which degrade the basement membrane and promote the invasion of lung cancer^[10]. The analysis of DEGs in lung cancer tissues and construction of regulatory networks showed that MMP9 played an important role in the development of lung cancer^[11]. MMP9 also participated in the progression of COPD, it change the normal alveolar structure and is involved in the airway remodeling. Positive correlation between MMP9 and COPD assessment test score in COPD patients was previously demonstrated^[12].

TP53 mutation occurs in many human malignancies and is involved in cell apoptosis, senescence and DNA repair^[13]. The expression of mutant TP53 is associated with platinum-based chemo-resistant^[14]. Emphysema is one of the pathological type of COPD, TP53 take part in the development of emphysema. Inflammatory factors may be lead to the accumulation of TP53 protein and increase the Bax/Bcl-x (L) ratio in emphysema patients lung tissue^[15]. Recently, a study used bioinformatic analyses indentified that TP53 was target gene of miRNAs which was found significantly downregulated in COPD patients^[16].

MMP9 and TP53 are hub genes in the same module of NSCLC and widely studied separately before. We found that MMP9 was highly expressed and TP53 was lowly expressed in the NSCLC patients with COPD. Functional enrichment demonstrate that cellular and protein metabolic processes are common biological processes they mainly involved in NSCLC. Apoptotic process is related to both NSCLC and COPD. These may supply a new perspective on the connection between MMP9 and TP53 in NSCLC and COPD.

BCL2 gene family is an important regulator of apoptosis. BCL2 can inhibit cells procedural death and BAX that is involved in programmed cell death, which are both closely linked to NSCLC. The overexpression of BCL2 and the low expression level of BAX can decrease lung cancer cell apoptosis [17].

Other non-overlapping hub gene such as KRAS, EGFR and ALK also play important roles in the two diseases. The PFS (progression free survival) of KRAS/TP53 or PIK3CA/TP53 mutation patients is shorter than mono-mutation of KRAS and TP53. KRAS, PIK3CA and TP53 mutations are associated with distant metastases and poor prognosis. Patients with NSCLC should receive routine genetic test, the mutations of ALK, KRAS, PIK3CA and TP53 mutations determine the clinical decision and prognosis [18].

The top three modules of NSCLC and COPD shared 82 common biological process and 78 pathways. The most significant biological process was inflammatory response. Inflammatory response is effective prognostic indicator in NSCLC patients [19] and also worsen the situation of COPD [20].

According to our findings, inflammatory and apoptotic process were exert a enormous function on both diseases. Activation of the inflammatory process or apoptotic process are assumed to cause tumor progression promotion [21]. Anti-inflammation or increase apoptosis have anticancer effect in cancer induced mice [22]. COPD is an inflammatory lung condition that features incompletely reversible airflow obstruction [22]. Thus, therapies targeting the apoptotic and anti-inflammatory signaling pathways regulated by hub genes may represent promising treatments for NSCLC or COPD.

With the explosive growth of the study amount, the potential molecular function and pathways are developed as targeted agents for NSCLC. EGFR tyrosine kinase inhibitors (TKIs) and ALK inhibitors are used as first-line treatment for NSCLC patients in the NCCN Guidelines. Nevertheless, there are many limitation for the use of TKIs and ALK for NSCLC patients. More targets and more biological functions need to be widely investigated in the treatment not only for NSCLC but COPD.

Conclusions

In this study, we provide a detailed overview of NSCLC-genes network and COPD-genes network. We illustrate the possible underlying biological processes between NSCLC and COPD. Network analysis helps us to gain new insights on pathological mechanisms and treatment of the two diseases.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The data used to support the findings of this study are available from the corresponding author upon request.

Competing interests

The authors declare that there are no conflicts of interest.

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Authors' contributions

Ye Yuan and Hongbing Shi contributed equally to this work. Ye Yuan and Hongbing Shi performed the analyses and wrote the manuscript text. Xiaodong Li and Ye Wu conceived and designed the study. All authors reviewed and proved the manuscript.

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Tables

Table1. Topological analysis of the NSCLC and COPD-associated networks

Parameter	NSCLC	COPD
Clustering coefficient	0.590	0.644
Network diameter	13	13
Network radius	1	1
Network centralization	0.077	0.077
Number of nodes	1615	417
Number of edges	4405	906
Overlapping genes	125	125
Modules	124	35
Maximum size	107	121
Minimum size	3	3
Avg. number of neighbors	5.493	4.430

Table2. Top 5 pathways and GO processes of NSCLC

Top 5 pathways according to <i>P</i> value			Top 5 GO processes according to <i>P</i> value		
GO:0000058	IRS-mediated signalling	180.0E-108	GO:0042981	Regulation of apoptotic process	350.0E-126
GO:0000061	SOS-mediated signalling	180.0E-108	GO:0032268	Regulation of cellular protein metabolic process	7.6E-111
GO:0000072	Downstream signaling events of B cell Receptor (BCR)	180.0E-108	GO:0051247	Positive regulation of protein metabolic process	170.0E-105
GO:0000073	Activation of NF-kappaB in B cells	180.0E-108	GO:0031399	Regulation of protein modification process	6.1E-99
GO:0000089	Regulation of hypoxia-inducible factor (HIF) by oxygen	180.0E-108	GO:0032270	Positive regulation of cellular protein metabolic process	8.7E-96

Table3. Top 5 pathways and GO processes of COPD

Top 5 pathways according to <i>P</i> value			Top5 GO processes according to <i>P</i> value		
GO:0000681	Cytokines and inflammatory response	18.0E-27	GO:0006954	Inflammatory response	720.3E-66
GO:0000684	IL10 negatively regulates extracellular inflammatory mediators	18.0E-27	GO:0042981	Regulation of apoptotic process	650.0E-66
GO:0000170	Expression of IL4, IL13-upregulated extracellular proteins	18.0E-27	GO:0070887	Cellular response to chemical stimulus	630.0E-66
GO:0000934	L10 negatively regulates extracellular inflammatory mediators	18.0E-27	GO:0010033	Response to organic substance	240.0E-66
GO:0005223	Expression of IL4, IL13-upregulated extracellular proteins	18.0E-27	GO:1901700	Response to oxygen-containing compound	1.5E-54

Figures

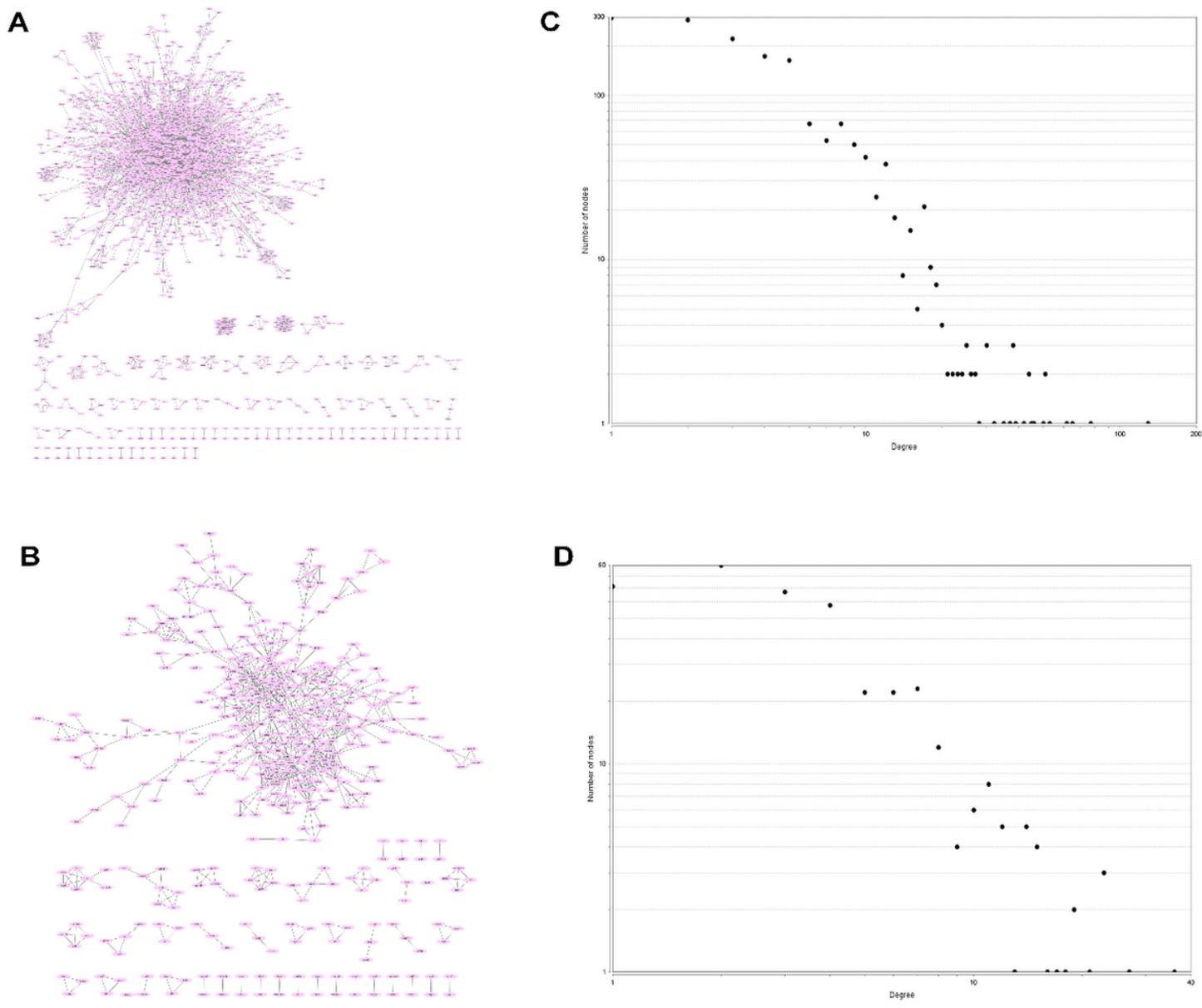


Figure 1

Characteristics of disease-associated gene interaction networks. (A) NSCLC network and (B) COPD network. Node degree and power distribution of the (C) NSCLC and (D) COPD networks.

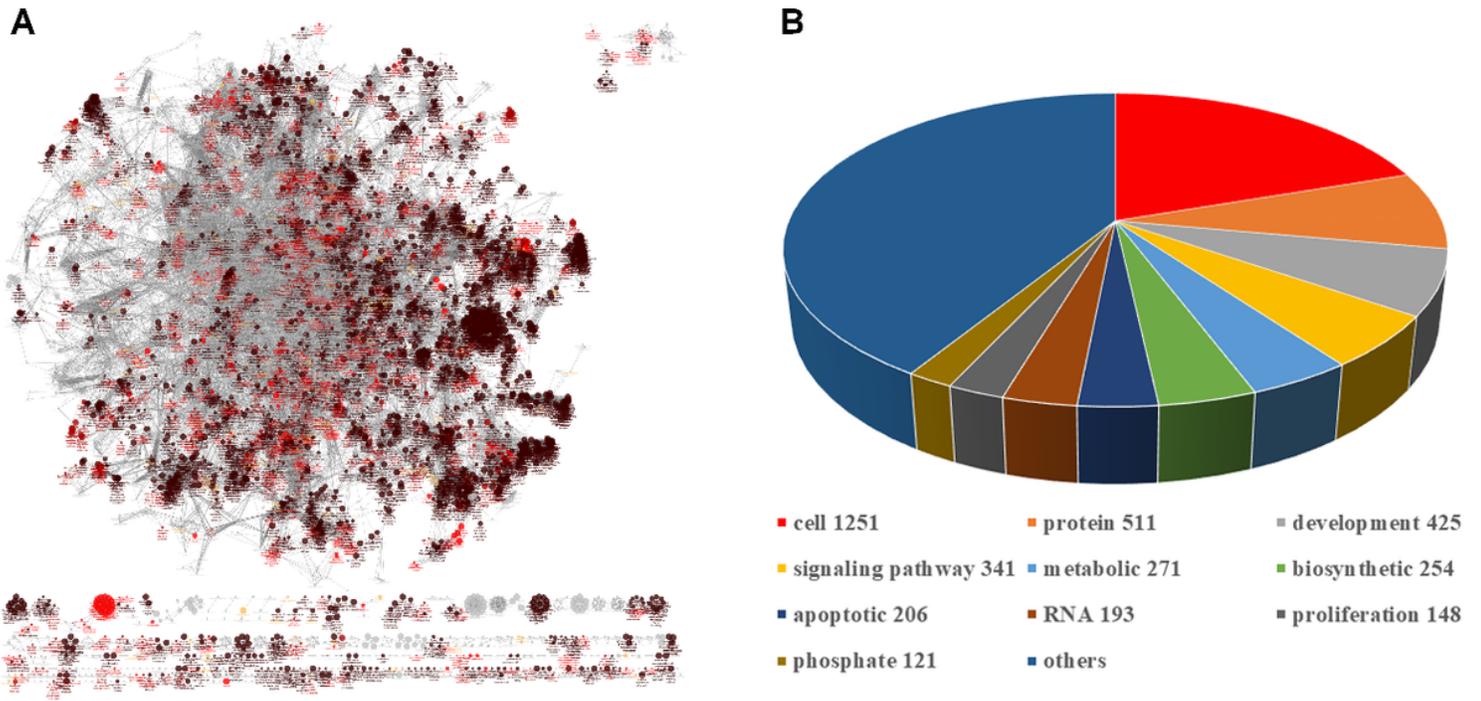


Figure 2

(A) Biological processes of NSCLC network. (B) The biological process of NSCLC.

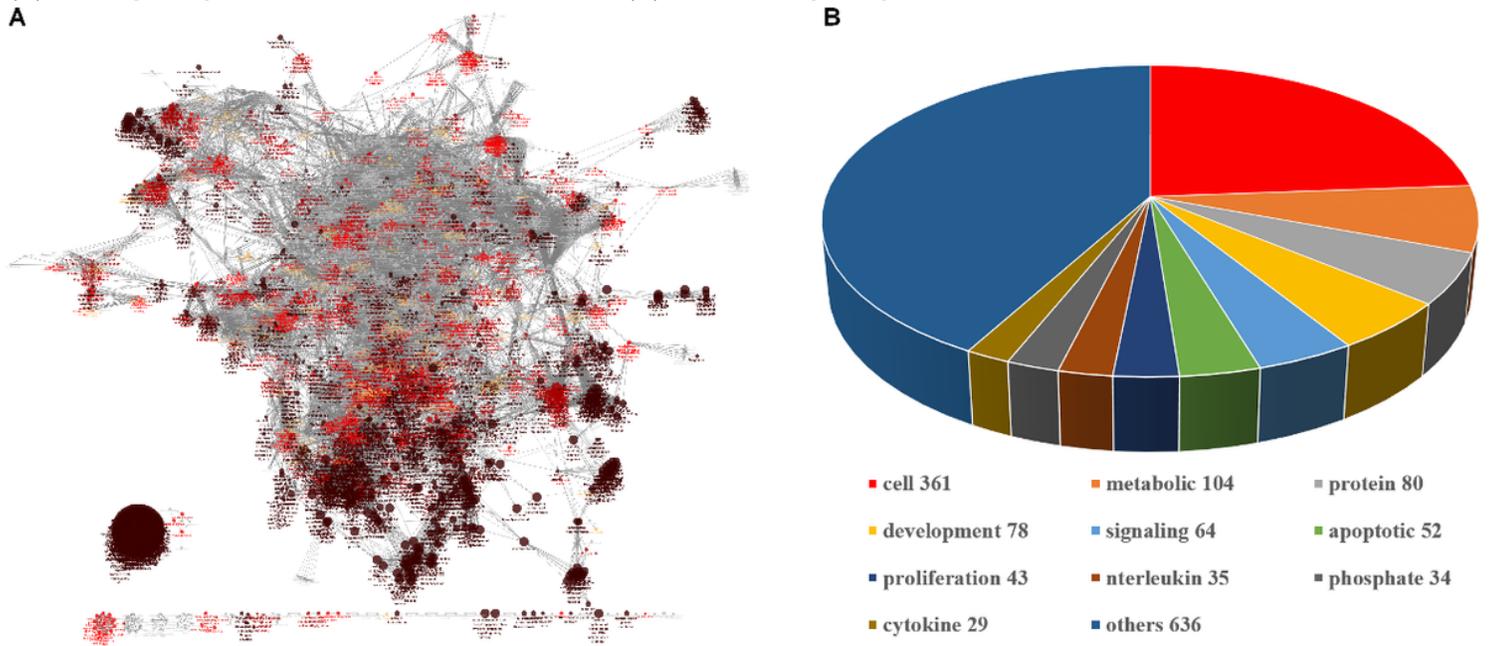


Figure 3

(A) Biological processes of COPD network. (B) The biological process of COPD.

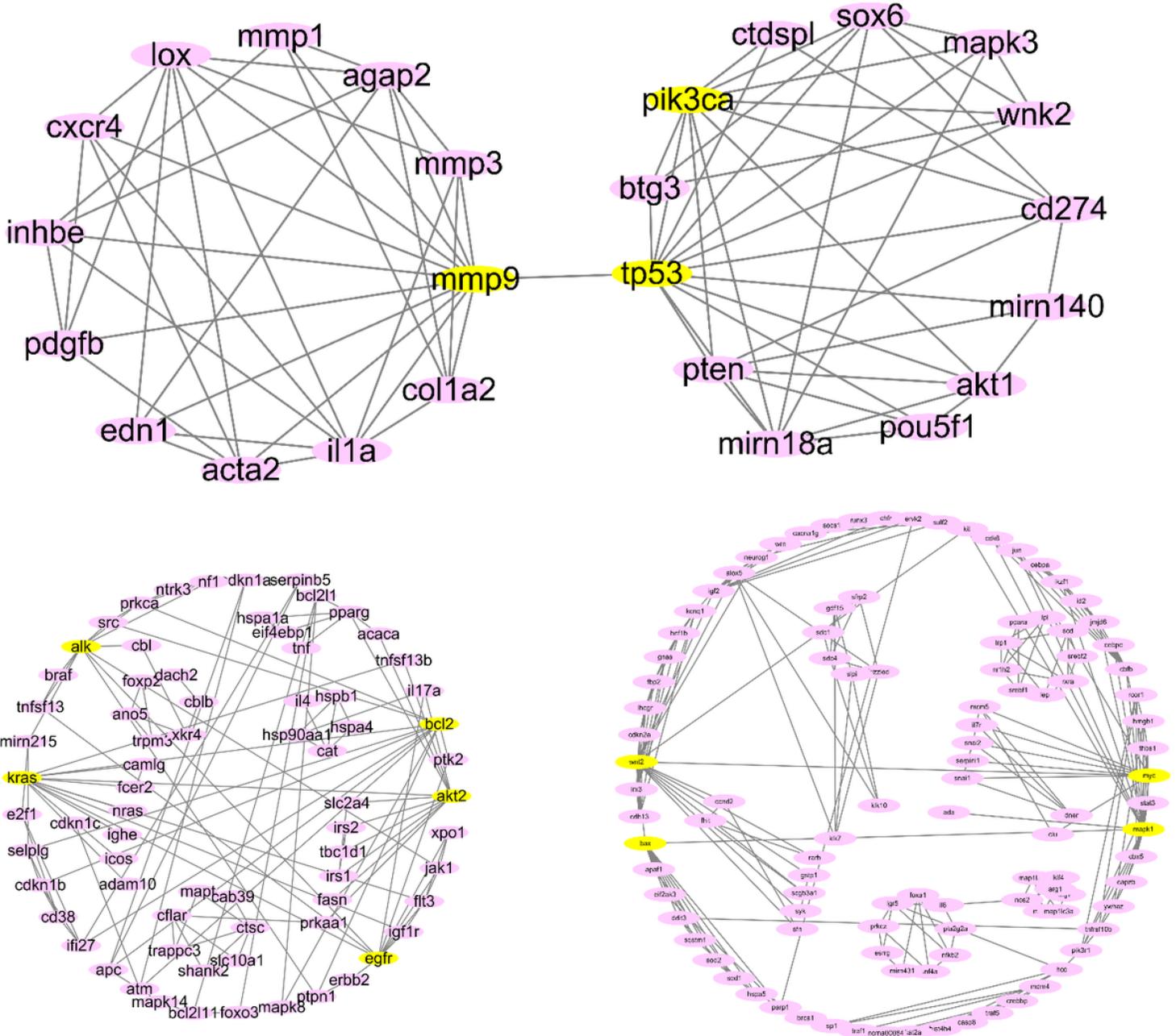


Figure 4

Top 3 functional modules and the genes of NSCLC.

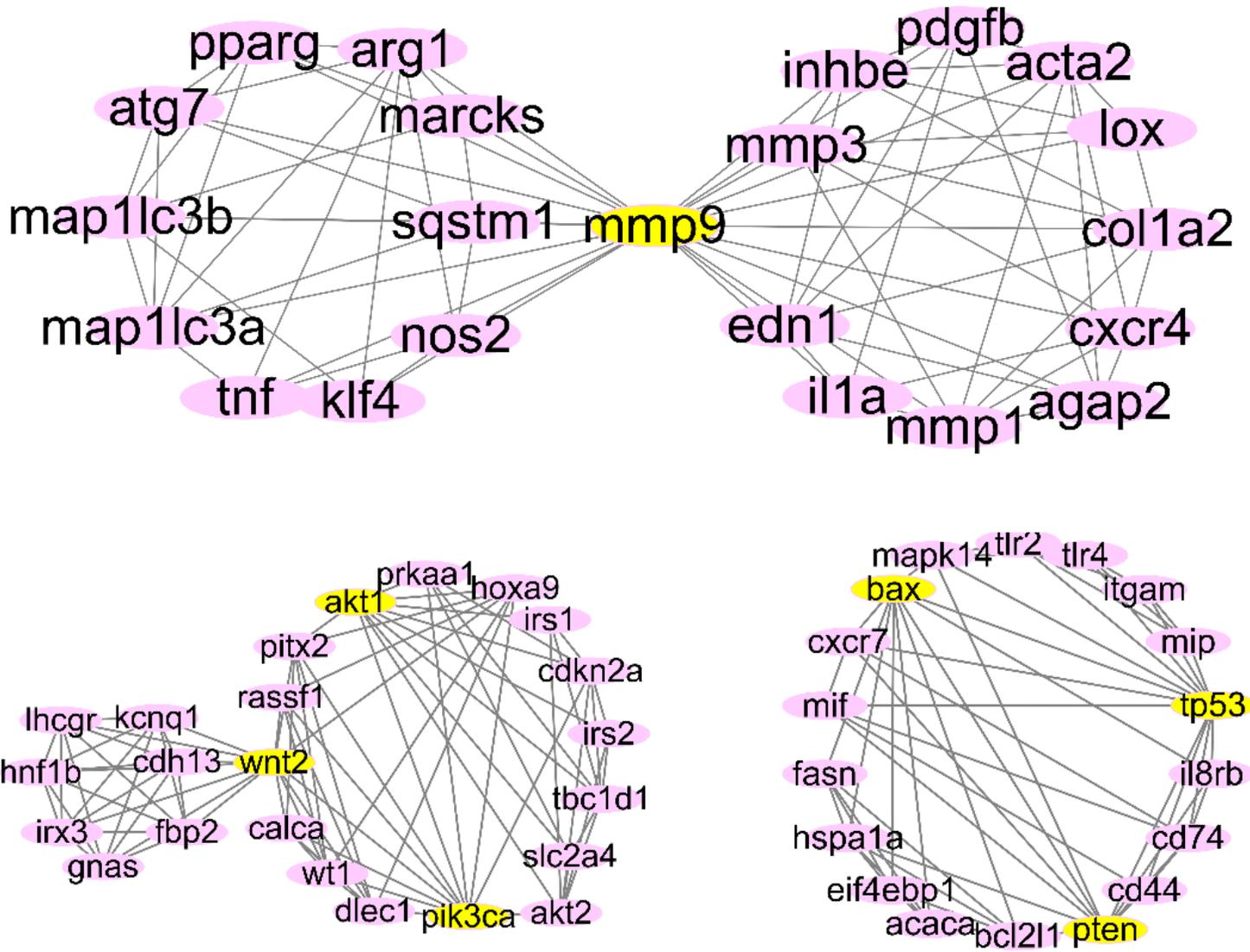


Figure 5

Top 3 functional modules and the genes of COPD.

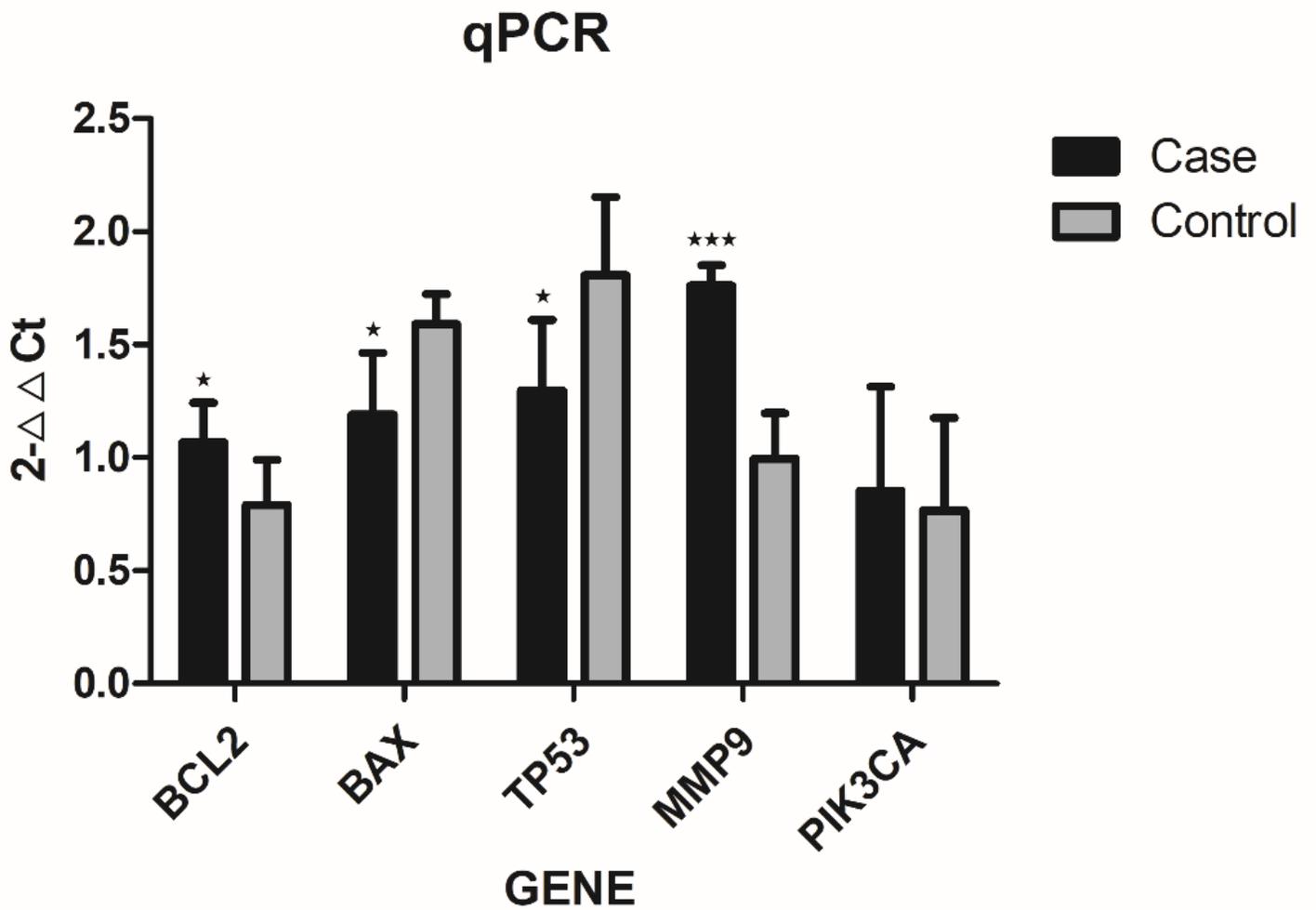


Figure 6

The expression of hub genes. MMP9 and BCL2 were highly expressed in NSCLC patients with COPD compare to healthy people, BAX and TP53 were lowly expressed. *P < 0.05; **P < 0.01; ***P < 0.001.

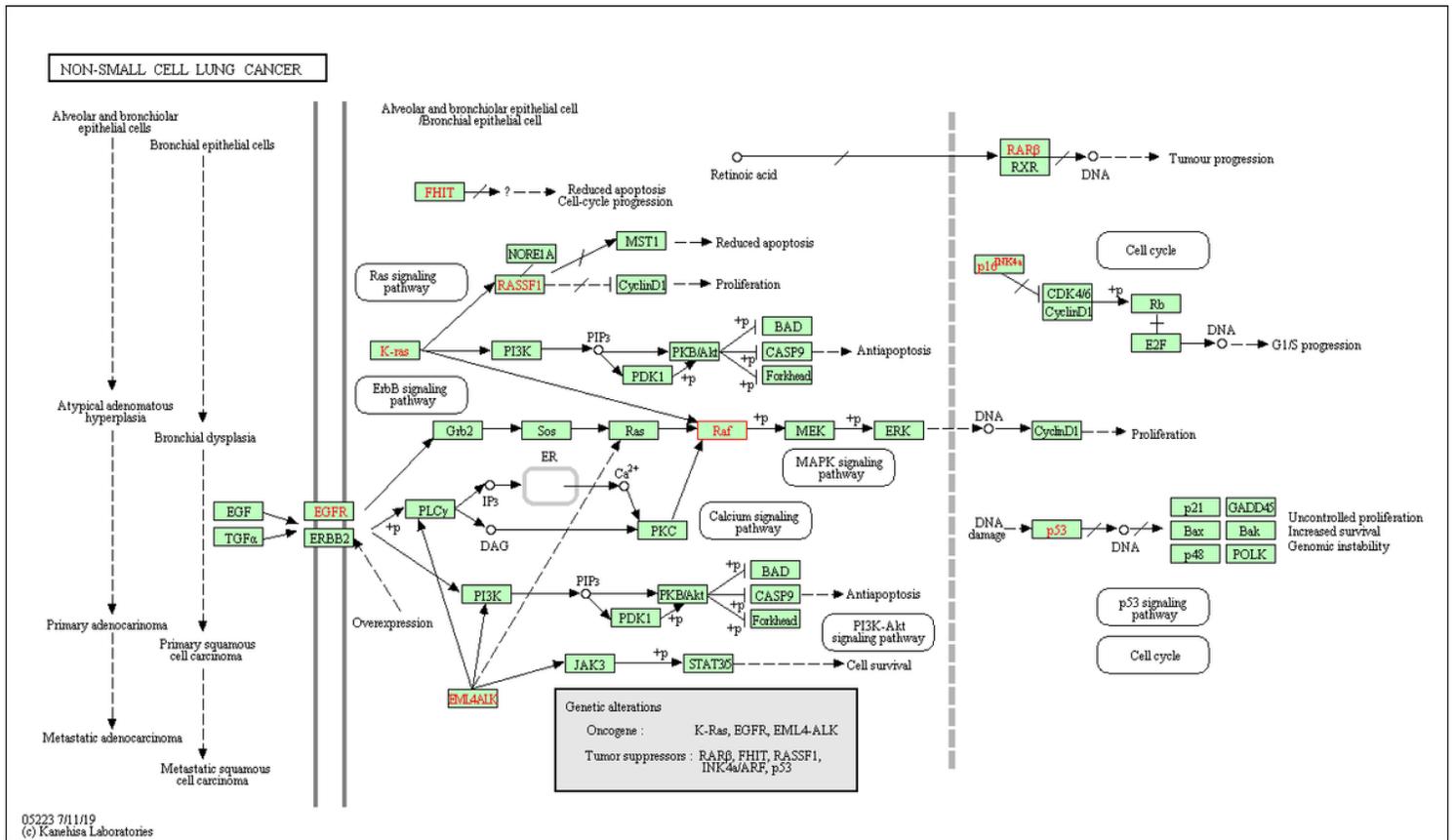


Figure 7

The pathway network of NSCLC. The hub genes such as TP53 and EGFR were all involved in this pathway.