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Original Article

Identification of key genes associated with idiopathic pulmonary fibrosis using bioinformatics analysis

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Abstract: Idiopathic pulmonary fibrosis (IPF) is a progressive and fatal interstitial lung disease. The goal of this study is to elucidate the molecular mechanism of IPF. GSE24206 was downloaded from Gene Expression Omnibus, which included 17 IPF and 6 control samples. The t-test was applied to identify differentially expressed genes (DEGs) between IPF and control samples. Pathway and functional enrichment analyses were used to investigate the functions involving these DEGs. According to the information of TRANSFAC, Tumor Associated Gene (TAG) and Tumor Suppressor Gene (TSGene) databases, the screened DEGs were further annotated. To comprehensively understand the interactions between proteins encoded by the DEGs, protein-protein interactions (PPIs) were predicted by STRING and PPI network was visualized by Cytoscape software. Additionally, module analysis for PPI network was performed using BioNet tool. Total 192 up-regulated and 28 down-regulated genes were identified. Both down-regulated *PDGFRA* and up-regulated *CCND1* were TAGs. Pathway enrichment analysis indicated that *PDGFRA* were involved in all of the 8 pathways for the 28 down-regulated genes. Besides, *LTBP3* and*THY1* separately were involved in extracellular matrix organization and cell adhesion. After PPI network analysis, we discovered that the degree of COL1A2, TGFB1, COL1A1, COL3A1, ASPN, CD4, SDC1, CXCL12, COL5A1, and *COMP* were significantly higher. In conclusions, our results showed that the pathology of IPF involved multiple dysregulated genes, and our study would pave ways for further study of IPF.

Keywords: Idiopathic pulmonary fibrosis, differentially expressed genes, enrichment analysis, protein-protein interaction network, module analysis

Introduction

Idiopathic pulmonary fibrosis (IPF) is a progressive and fatal interstitial lung disease, which is characterized by temporally heterogeneous lung architectural distortion, dense collagen and extracellular matrix (ECM) deposition in interstitium, alveolar collapse, and the presence of fibroblastic foci [1]. Nowadays, IPF affects about five million people worldwide, and its incidence is about 20 to 60 per 100,000 persons [2]. Besides, IPF occurs usually in middle-aged and older adults, and men are more susceptible to IPF [3]. Notably, the incidence of lung cancer seems to be increased in IPF patients compared with general population [4, 5]. Lung cancer may occur before, after, or at the time when IPF is diagnosed [6].

Though IPF cannot be cured, oxygen therapy, lung transplantation, and drugs have been used to help IPF patients. Nowadays, many drugs (like macitentan, sildenafil, warfarin, and bosentan) have been developed, but these drugs show little benefit [7]. Recently, in order to get novel therapeutic targets, the pathogenesis of IPF has been studied. Previous studies showed that deficiencies of surfactant protein C (SP-C, encoded by *SFTPC*) [8] and surfactant protein A2 (SP-A2, encoded by *SFTPA2*) [9] are associated with IPF. Seibold et al. identify a common variant in the putative promoter of mucin 5B (*MUC5B*), which presents in 38% of patients with IPF [10]. Disease-causing heterozygous mutations in two components of telomerase complex, telomerase reverse transcriptase (TERT) and telomerase RNA component (TERC), are also involved in IPF [11, 12]. Besides, many biological pathways which are linked to IPF have been identified. Epithelial-mesenchymal transition in alveolar epithelial cells (AECs) is hypothesized as a source of myo-

Table 1. The statistics of DEGs between IPF and control samples

	Transcript Counts	Gene Counts
Down	109	28
Up	521	192
Total	630	220

DEGs: differentially expressed genes; IPF: idiopathic pulmonary fibrosis.

Table 2. The enriched KEGG pathways for the DEGs

	Description	Gene counts	*P*-value
Up	Complement and coagulation cascades	6	0.000101308
	ECM-receptor interaction	6	0.000321922
	Staphylococcus aureus infection	5	0.00032338
	Protein digestion and absorption	5	0.001909578
	Amoebiasis	5	0.006112176
Down	Constitutive PI3K/AKT Signaling in Cancer	2	0.006803308
	PI3K events in ERBB4 signaling	2	0.008763174
	PIP3 activates AKT signaling	2	0.008763174
	PI-3K cascade	2	0.008763174
	PI3K events in ERBB2 signaling	2	0.008763174
	PI3K/AKT Signaling in Cancer	2	0.008763174
	GAB1 signalosome	2	0.009288597
	PI3K/AKT activation	2	0.009288597

KEGG: Kyoto Encyclopedia of Gene and Genomes; DEGs: differentially expressed genes.

fibroblasts which serves as the primary collagen-producing cell [13]. ECM deposition, which is regulated by matrix metalloproteinases (MMPs) and their inhibitors, can be triggered by chronic inflammation and lead to the formation of a permanent fibrotic scar [14]. Despite extensive research, the pathogenesis of IPF still remains unclear.

In 2011, Meltzer et al. screened differentially expressed genes (DEGs) between upper and lower lobe samples using paired t-tests, and identified DEGs between IPF explants and IPF biopsies using unpaired Student's t-tests [15]. Using the data deposited by Meltzer et al. [15], the DEGs between IPF and normal samples were screened, and their underlying functions were predicted by functional and pathway enrichment analyses. Besides, gene functional annotation analysis was performed. Additionally, protein-protein interaction network (PPI) network and module were constructed to investigate the interactions between these DEGs.

Materials and methods

Collection and preprocessing of mRNA expression profile data

The mRNA expression profile of GSE24206 deposited by Meltzer et al. [15] was downloaded from Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) database using the platform of Affymetrix Human Genome U133 Plus 2.0 Array. The dataset included 17 samples (GSM595421, GSM595422, GSM595423, GSM595424, GSM595425, GSM595426, GSM595427, GSM595428, GSM595429, GSM595432, GSM595434, GSM595435, GSM595437, GSM595439, GSM595441, GSM595443, GSM595445) from 11 IPF patients (6 patients contributed twain samples from upper and lower lobes, and 5 patients provided singleton samples) and 6 samples (GSM595407, GSM595411, GSM595414, GSM595416, GSM595417, GSM595419) from healthy donors (Healthy donors provided lung samples obtained from routine lung volume reduction of lung during lung transplantation). Combining with the probe annotation file of Affy [16] chip provided by Brain Array Lab, the original data were preprocessed using AFFY package in Bioconductor [17]. After Robust Multi-array Average (RMA) [18] background correction, quantile normalization and probe summarization, gene expression matrix of the samples were obtained.

DEGs screening

For the preprocessed data, t-test [19] was performed to identify DEGs between IPF and control samples. We defined FDR < 0.05 and |log-2fold change (FC)| ≥ 1 as the thresholds.

Functional and pathway enrichment analysis

To study the DEGs at a functional level, Gene Ontology (GO) functional [20] enrichment analy-

Table 3. The enriched GO functions for the DEGs

	Term	Description	Gene counts	*P*-value
Up	GO: 0030198	Extracellular matrix organization	19	8.33E-10
	GO: 0043062	Extracellular structure organization	19	8.79E-10
	GO: 0007155	Cell adhesion	31	2.25E-08
	GO: 0005576	Extracellular region	71	0
	GO: 0044421	Extracellular region part	47	0
	GO: 0005615	Extracellular space	36	1.59E-13
	GO: 0005201	Extracellular matrix structural constituent	10	4.75E-09
	GO: 0008201	Heparin binding	10	5.48E-07
	GO: 0048407	Platelet-derived growth factor binding	4	2.60E-06
Down	GO: 0034754	Cellular hormone metabolic process	3	0.000451975
	GO: 0060325	Face morphogenesis	2	0.001132088
	GO: 0060323	Head morphogenesis	2	0.001442882
	GO: 0048008	Platelet-derived growth factor receptor signaling pathway	2	0.001699747
	GO: 0060324	Face development	2	0.001789859
	GO: 0010171	Body morphogenesis	2	0.00217258
	GO: 0004745	Retinol dehydrogenase activity	2	0.000243186
	GO: 0005001	Transmembrane receptor protein tyrosine Phosphatase activity	2	0.000353342
	GO: 0019198	Transmembrane receptor protein phosphatase activity	2	0.000353342

GO: Gene Ontology; DEGs: differentially expressed genes.

Table 4. The functional statistics of DEGs between IPF and control samples

	TF counts	TF genes	TAG counts	TAG (Oncogenes)	TGA (Tumor Suppressor Gene)	TAG (other)
Down	1	NFIL3	5	PDGFRA	PTPRG, HOPX	TACC2, RGS2
Up	3	SOX4, NR1H3, MEOX1	18	CD24, CCND1	THY1, STEAP3, SCGB3A1, SCARA3, PDLIM4, NBL1, NAPEPLD, IGFBP4, HTRA1, ENC1	XAF1, TGFB1, SSPN, MUC5B, LRRC17, FHL2

DEGs: differentially expressed genes; IPF: Idiopathic pulmonary fibrosis.

sis and Kyoto Encyclopedia of Gene and Genomes (KEGG) [21] pathway enrichment analysis were performed. The *p*-value < 0.01 was set as the cut-off criterion.

Functional annotation analysis

According to the information of transcription factors provided by TRANSFAC [22] database, the DEGs were further screened and annotated to obtain genes with transcriptional regulation function. Besides, all known oncogenes and tumor suppressor genes were extracted based on Tumor Associated Genes (TAG) database [23] and Tumor Suppressor Gene (TSGene) database [24].

PPI network and module construction

Containing known and predicted protein-protein interactions, STRING database [25] has been widely used to construct PPI network. Here, STRING database was used to search interactions of the proteins encoded by DEGs. The Cytoscape software [26] was used to visualize the PPI network. Subsequently, BioNettool [27] was employed for performing module analysis for PPI network, and FDR < 0.0001 was set as the criterion.

Results

DEGs screening

Using t-test, a total of 220 DEGs were screened in IPF samples compared with normal samples, including 192 up-regulated genes (corresponding to 521 transcripts) were, and 28 down-regulated genes (corresponding to 109 transcripts) (**Table 1**).

Functional and pathway enrichment analysis

Pathway enrichment analysis indicated that the 192 up-regulated genes were enriched in 5

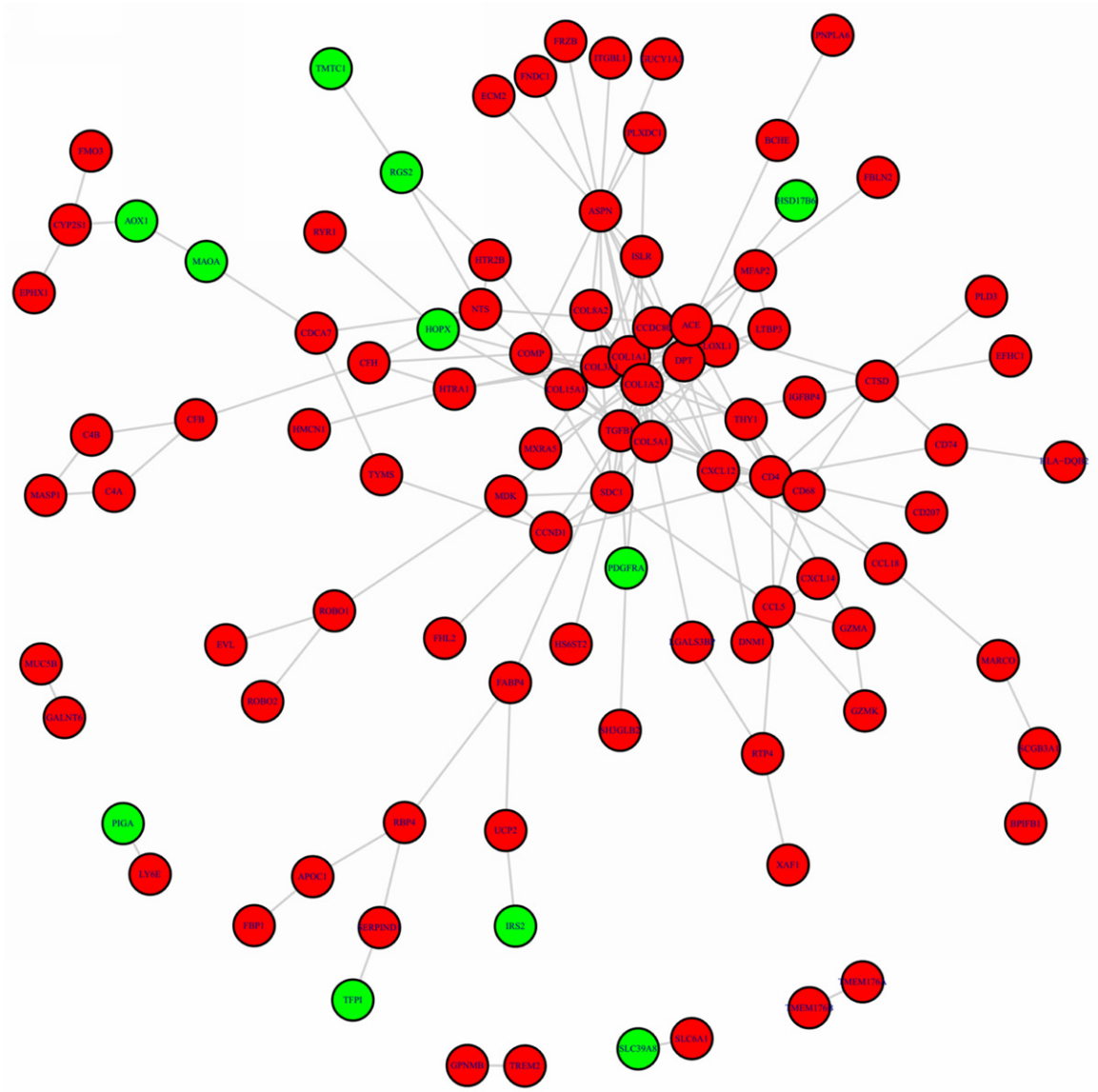

Figure 1. PPI network of DEGs between IPF and control samples. Red and green nodes represent up-regulated and down-regulated genes, respectively. PPI: protein-protein interaction; DEGs: differentially expressed genes; IPF: idiopathic pulmonary fibrosis.

pathways, such as complement and coagulation cascades (P = 0.000101308), ECM-receptor interaction (P = 0.000321922) and staphylococcus aureus infection (P = 0.000-32338). Meanwhile, the 28 down-regulated genes were enriched in 8 pathways, including constitutive PI3K/AKT signaling in cancer (P = 0.006803308) and PI3K events in ERBB4 signaling (P = 0.008763174). Notably, insulin receptor substrate 2 (*IRS2*) and platelet-derived growth factor alpha receptor (*PDGFRA*) were enriched in all of the 8 pathways for down-regulated genes (**Table 2**). Moreover, the 192 up-regulated genes were enriched in some GO functions, including extracellular matrix organization (P = 8.33E-10, which involved latent TGF-β binding protein-3, *LTBP3*) and cell adhesion (P = 2.25E-08, which involved thymus cell antigen 1, *THY1*). And the 28 down-regulated genes were also enriched in several GO functions, including cellular hormone metabolic process (P = 0.000451975) and face morphogenesis (P = 0.001132088) (**Table 3**).

Functional annotation analysis

Among the up-regulated genes, 3 genes were transcription factors, and 18 genes (e.g. cyclin

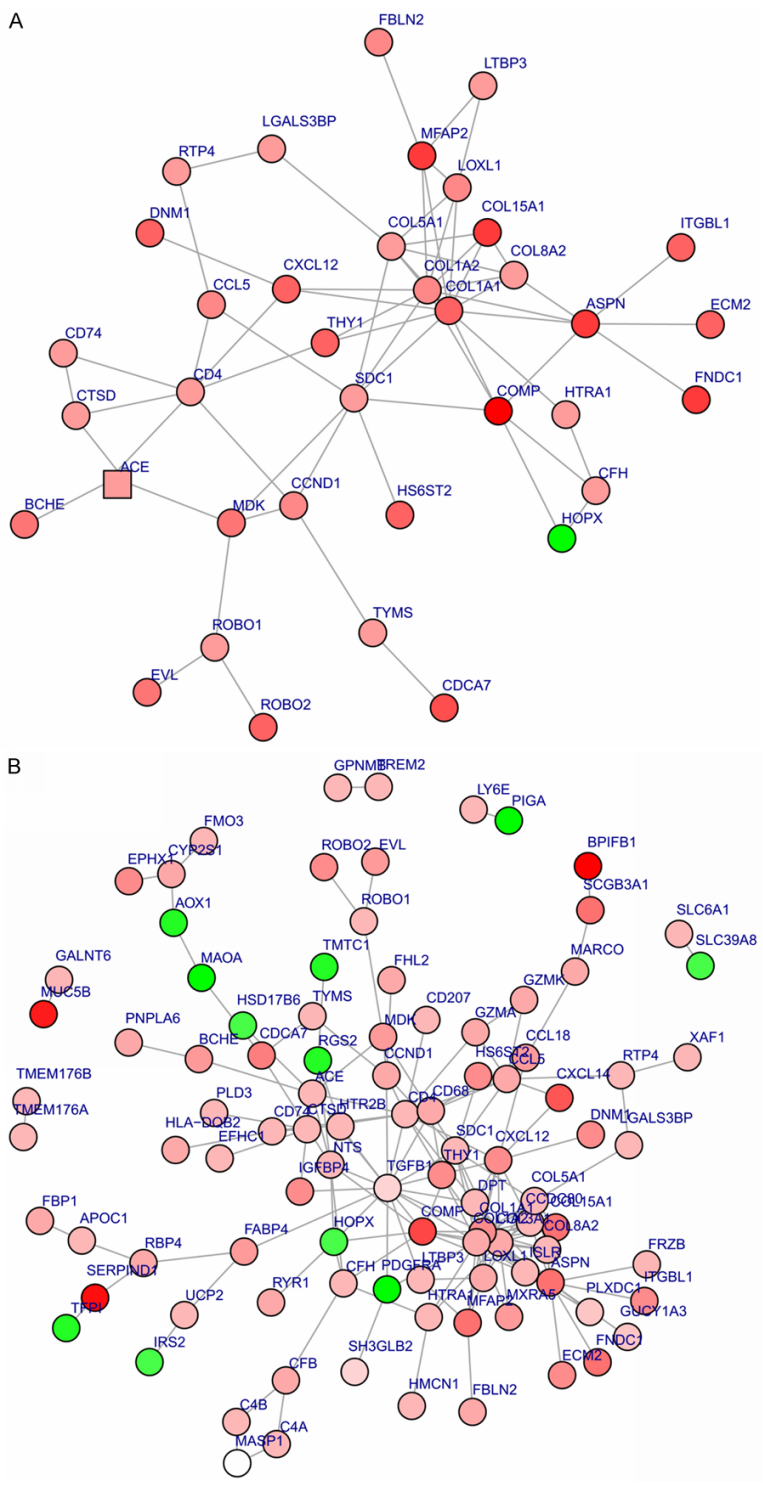

Figure 2. Module analysis of PPI network. The depth of color is proportional to |log2 FC| of DEGs. Red and green nodes represent up-regulated and down-regulated genes, respectively. Square nodes represent genes with low importance, and circular nodes represent genes with high importance. PPI: protein-protein interaction; DEGs: differentially expressed genes; FC: fold change.

D1, CCND1) were TAGs (including 2 oncogenes, 10 tumor suppressor genes and 6 uncertain genes). Among the down-regulated genes, nuclear factor interleukin-3 (NFIL3) was transcription factor, PDGFRA was oncogene, receptor protein tyrosine phosphatases gamma (PTPRG) and homeodomain-only protein X (HOPX) was tumor suppressor genes. However, as TAGs, transforming acidic coiled-coil 2 (TACC2) and regulator of G protein signaling 2 (RGS2) were uncertain genes (**Table 4**).

PPI network and module analysis

Based on STRING database, PPI network was constructed (**Figure 1**), and the top 10 genes with degree ≥ 9 were α 2 type I collagen gene (COL1A2, degree = 17), transforming growth factor-β1 (TGFB1, degree = 17), α 1 type I collagen gene (COL1A1, degree = 16), α 1 type III collagen gene (COL3A1, degree = 15), asporin (ASPN, degree = 14), CD4 (degree = 12), syndecan-1 (SDC1, degree = 10), stromal cell-derived factor 1 (CXCL12, degree = 10), α 1 type V collagen gene (COL5A1, degree = 9) and cartilage oligomeric matrix protein (COMP, degree = 9). The module involving 37 nodes was obtained from the PPI network, in which COL1A1 (degree = 11) had the highest degree (**Figure 2**). After KEGG pathway enrichment analysis, genes in this module were mainly involved in ECM-receptor interaction (P = 7.39E-06), protein digestion and absorption (P = 0.000137815) and axon guidance (P = 0.008992409) (**Table 5**). Moreover, through GO functional enrichment analysis, the genes in this module were mainly enriched in extracellular matrix organization (P = 3.12E-

Table 5. KEGG pathway and GO functional enrichment analysis of DEGs in the identified module

Enriched terms	Gene Counts	*P*-Value
KEGG pathway		
ECM-receptor interaction	5	7.39E-06
Protein digestion and absorption	4	0.000137815
Focal adhesion	5	0.000445781
Amoebiasis	3	0.005217842
Axon guidance	3	0.008992409
GO function		
Extracellular matrix organization	13	3.12E-13
Extracellular structure organization	13	3.25E-13
Multicellular organismal catabolic process	7	9.13E-10
Cell adhesion	15	3.80E-09
Biological adhesion	15	3.91E-09

KEGG: Kyoto Encyclopedia of Gene and Genomes; GO: Gene Ontology; DEGs: differentially expressed genes.

13), extracellular structure organization (P = 3.25E-13) and biological adhesion (P = 3.91E-09) (**Table 5**).

Discussion

IPF is a devastating form of interstitial lung disease [28]. However, there is no effective treatment. To understand the potential mechanism of IPF, bioinformatics might be an effective method. In this study, a total of 220 DEGs were identified in IPF samples in comparison to control samples.

Peroxisome proliferator-activated receptor γ (*PPARγ*) agonists can suppress TGF-β-induced myofibroblast differentiation and production of collagen protein, hence, *PPARγ* agonists have potential antifibrotic effects and may be used in therapy of fibrotic lung diseases [29]. The synthesis of type I collagen reacts both positively and negatively to stimulation generated by tissue injury and repair, and is accumulated in IPF patients [30, 31]. Via transcriptional activating *COL1A2*, connective tissue growth factor (*CTGF*) contributes to lung fibrosis and may serve as a promising target for treatment of fibrotic diseases [32]. ASPN can bind with collagen and calcium, and then induce collagen mineralization which is essential for ECM deposition [33]. Previous study shows that down-regulated CD28 in circulating CD4 T-cells are related to manifestations and progression of IPF [34]. Increased syndecan-1 (which is encoded by *SDC1*) have been detected in lung homogenates and lavage fluid of lungs in patients with IPF, and syndecan-1 ectodomain induces neutrophil chemotaxis, inhibits wound healing in alveolar epithelial, and promotes fibrogenesis [35]. In the bleomycin model, up-regulated *CXCL12* is the major chemokine responsible for recruiting bone-marrow derived fibrocytes to lung [36]. *COMP* was overexpressed in serum of IPF patients and it may be a novel biomarker for disease activity and TGF-β1 activity [37, 38]. In the PPI network, COL1A2, TGFB1, COL1A1, COL3A1, ASPN, CD4, SDC1, CXCL12, COL5A1, and COMP had higher degrees. Module analysis showed that COL1A1 had the highest degree in the identified module. These indicated that these genes might be key genes in IPF.

Functional enrichment indicated that *LTBP3* was involved in extracellular matrix organization. The ECM protein LTBP3 have a dual function, which is required both for the secretion of small latent TGF-beta complex and binding latent TGF-beta to ECM microfibrils [39, 40]. As stated before, growth factors TGF-beta stimulates ECM production of fibroblast, myofibroblast differentiation, and resistance to apoptosis [41, 42]. *THY1*, which involved in cell adhesion, has been proposed as a "fibrosis suppressor" gene [43]. *THY1*is present in normal lung fibroblasts [44], but absent in the fibroblasts of IPF patients because of methylation [43]. Thus, *LTBP3* and *THY1* might play an important role in IPF progression.

Additionally, gene functional annotation analysis showed that 5 down-regulated genes (e.g. *PDGFRA*) and 18 up-regulated genes (e.g. C*CND1*) are TAGs. Schwartz *et al.* hypothesis that *CCND1* plays an instrumental role in the pro-fibrogenic process, which was further validated by in situ growth factor overproduction and exaggerated extracellular matrix deposition [45]. Intedanib is a triple kinase inhibitor that blocks *PDGFR*, vascular endothelial growth factor receptor (*VEGFR*) and fibroblast growth factor receptor (*FGFR*) for the therapy of IPF

and several types of cancer [46]. Pathway enrichment analysis indicated that *PDGFRA* were involved in all of the 8 pathways for the 28 down-regulated genes. These suggested that *PDGFRA* and *CCND1* might be implicated in IPF.

Conclusions

In conclusion, to illustrate the pathological mechanism of IPF, the gene expression profile containing 23 samples was downloaded and analyzed. Total 220 DEGs were identified in IPF samples. Besides, several genes (*COL1A2*, *TGFB1*, *COL1A1*, *COL3A1*, *ASPN*, *CD4*, *SDC1*, *CXCL12*, *COL5A1*, *COMP*, *LTBP3*, *THY1*, *CCND1* and *PDGFRA*) might play important roles in IPF. However, further experimental validation is still needed to prove this speculation.

Disclosure of conflict of interest

None.

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