Identification of autophagy-related genes in idiopathic pulmonary fibrosis using bioinformatics methods

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To the Editor: Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, and fibrotic lung disease. The clinical processes of IPF include dyspnea, decreased lung function, and cough. The median survival time of patients with IPF is approximately 3 years. The 5-year survival rate is less than 30%. [1] Although some progress has been made in anti-fibrotic therapy, IPF is still an incurable disease. Therefore, it is necessary to explore the pathological process of IPF from the perspective of cell biology, and these explorations can provide a basis for the treatment of IPF.

Autophagy is a cellular process that transfers organelles, proteins, or intracellular pathogens to lysosomes for degradation. [2] Autophagy has been shown to play an important role in several diseases by regulating genes or signaling pathways in cells. However, the biological significance of autophagy and the underlying regulatory mechanisms in IPF remains elusive.

In this study, we explored potential autophagy-related differential genes in IPF using bioinformatics methods. Dataset GSE24206 was downloaded from Gene Expression Omnibus (GEO). We then analyzed the differentially expressed autophagy-related genes between patients with IPF and healthy controls. We also used bioinformatics tools to analyze the related functions and pathways of the differentially expressed autophagy-related genes and their interactions. The purpose of this study was to identify the autophagy-related genes of IPF and their associations. This will provide potential insights for exploring the development and treatment of IPF.

The Human Autophagy Database (HADb, http://www.autophagy.lu/) is an online database that contains genes involved directly or indirectly in autophagy. In this study, 222 autophagy-related gene databases were obtained from the HADb database. The GEO database (https://www.ncbi.nlm.nih.gov/gds) is a public database containing different microarray and sequencing data. One gene expression dataset, GSE24206, was collected from the GEO dataset. GSE24206 was in GPL570 ([HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array), which included 17 patients with IPF and six normal samples.

The expression and platform data were downloaded from the GSE24206 dataset. Principal component analysis (PCA) was performed to test the differences between IPF and normal samples. The R package “limma” was used to select differentially expressed autophagy-related genes from the microarray data. The differentially expressed genes were screened by the adjusted P value < 0.05 and absolute fold change > 1.5. The volcano plot, heatmap, and box plot of the differentially expressed autophagy-related genes were generated with “ggplot2,” “pheatmap,” and “ggpubr” packages of R software (R Foundation for Statistical Computing, Vienna, Austria). The correlation of differentially expressed autophagy-related genes was analyzed using the Spearman correlation in the “corrplot” package of R software. The protein-protein interaction (PPI) of 20 autophagy-related genes was performed using the STRING database (http://string-db.org/cgi/input.pl) and visualized using Cytoscape (version 3.8.1, https://cytoscape.org/). Only the minimum required interaction score > 0.4 was mapped. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted to analyze 20 autophagy-related genes using the Metascape database (https://metascape.org/gp/index.html#!/main/step1). P value < 0.05 was considered statistically significant.

To identify the differences between the IPF and the control samples, we performed a PCA. The results showed that there were differences between the two groups [Figure 1A]. We then analyzed the expression of 222 autophagy-related genes in 17 IPF and six normal samples. Using the threshold of adjusted P value < 0.05 and absolute fold change > 1.5, 20 differentially expressed autophagy-related genes of IPF were identified in GSE24206, including 11 up-regulated...
genes (tumor protein p63 [TP63], integrin subunit beta 4 [ITGB4], cathepsin D [CTSD], tumor necrosis factor superfamily member 10 [TNFSF10], distinct subgroup of the ras family member 3 [DIRAS3], tumor protein p53 [TP53], acid alpha glucosidase [GAA], eukaryotic translation initiation factor 2 alpha kinase 2 [EIF2AK2], erb-b2 receptor tyrosine kinase 2 [ERBB2], tuberous sclerosis complex subunit 2 [TSC2], and cyclin dependent kinase inhibitor 2A [CDKN2A]) and nine down-regulated genes (endoplasmic reticulum to nucleus signaling 1 [ERN1], forkhead box O1 [FOXO1], interleukin 24 [IL24], DNA damage inducible transcript 3 [DDIT3], BCL2 interacting protein 3 [BNIP3], DLC1 Rho GTPase activating protein [DLC1], C-X-C motif chemokine receptor 4 [CXCR4], MYC proto-oncogene, basic helix-loop-helix transcription factor [MYC], and nicotinamide phosphoribosyltransferase [NAMPT]). Figure 1B and 1C of Supplementary Table 1, http://links.lww.com/CM9/A661. Furthermore, the box plot shows the expression of 20 differentially expressed autophagy-related genes between patients with IPF and the
healthy samples [Supplementary Figure 1, http://links.lww.com/CM9/A661].

To determine the interactions among 20 autophagy-related genes differentially expressed in patients with IPF and the normal samples, we performed a PPI analysis and found that their encoded proteins interacted with each other [Supplementary Figure 2A, http://links.lww.com/CM9/A661]. Additionally, the connection numbers of these encoded proteins are shown in Supplementary Figure 2B, http://links.lww.com/CM9/A661. Furthermore, to explore the expression correlation of these autophagy-related genes, we performed a correlation analysis. The results revealed that there were significantly positive or negative correlations between autophagy-related genes [Supplementary Figure 3, http://links.lww.com/CM9/A661].

To explore the potential functions and pathways of the 20 differentially expressed autophagy-related genes, GO and KEGG enrichment analysis was conducted. For the GO enrichment analysis, autophagy-related genes were enriched in positive regulation of the apoptotic process, autophagy, and regulation of cellular response to stress [Supplementary Figure 4A, http://links.lww.com/CM9/A661 and Supplementary Table 2, http://links.lww.com/CM9/A661]. For the KEGG enrichment analysis, autophagy-related genes were enriched in apoptosis, autophagy, and the FOXO signaling pathway [Supplementary Figure 4B, http://links.lww.com/CM9/A661 and Supplementary Table 3, http://links.lww.com/CM9/A661].

Bioinformatic analysis of autophagy-related genes in non-tumor diseases has rarely been reported. We were the first to analyze the autophagy-related genes of IPF. The results indicated that there were 20 autophagy-related genes in IPF. In addition, the PPI analysis demonstrated that their encoded proteins interacted with each other. Many studies have reported that these autophagy-related genes affect the development of different diseases by regulating autophagy. For instance, one study reported that FOXO1 regulates autophagy and affects the development of hepatocellular carcinoma through adenosine monophosphate-activated protein kinase-FOXO1-unc-51 like autophagy activating kinase 1 signaling axis. These results suggest that autophagy-related genes play an important role in the development of diseases.

We also explored the potential functions and pathways of the 20 autophagy-related genes in IPF. Several studies have reported that the 20 autophagy-related genes can affect the pathological process of IPF. For example, CXCR4-targeted PET imaging has been used to identify disease activity and predict the outcome of patients with IPF treated with pirfenidone. Our results showed that the GO and KEGG enrichment terms were mainly related to autophagy. Evidence suggests that autophagy is involved in the progression of IPF. Kim et al found that IL-37 inhibits transforming growth factor beta 1 signaling and enhances autophagy in IPF fibroblasts. In addition to IPF, autophagy affects the pathological progression of various respiratory diseases. Cao et al found that progestin and adipoQ receptor family member 3 suppresses the growth of non-small cell lung cancer cells via modulation of epidermal growth factor receptor-mediated autophagy. However, the mechanism of autophagy in different respiratory diseases including IPF remains unclear. Therefore, further investigations are required to better explain the detailed mechanisms of autophagy.

There are still some limitations in this study. First, we did not verify the expression of these autophagy-related genes in patients with IPF and the healthy controls. We need to verify the expression of these genes and explore their detailed mechanisms in the development of IPF in the future. Second, due to the lack of patient survival information in the GSE24206 dataset, we did not analyze the impact of these genes on the prognosis of patients with IPF.

In conclusion, we identified 20 differentially expressed autophagy-related genes (11 up-regulated genes and nine down-regulated genes) that may be involved in the development of IPF. However, our bioinformatics results should be confirmed by future biological experiments. These findings may provide several potential therapeutic targets and help us to better understand the development of IPF.

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Conflicts of interest
None.

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