Network pharmacology-based mechanistic investigation of Jinshui Huanxian formula acing on idiopathic pulmonary fibrosis

Tiantian Liu
Henan University of Traditional Chinese Medicine
Pengli Xu
Henan University of Traditional Chinese Medicine
Shuishui Qi
Henan University of Traditional Chinese Medicine
Shaorui Ke
Henan University of Traditional Chinese Medicine
Qin Hu
Henan University of Traditional Chinese Medicine
Peng Zhao
Henan University of Traditional Chinese Medicine
Jiansheng Li (li_js8@163.com)
Henan University of Traditional Chinese Medicine

Research

Keywords: Jinshui Huanxian formula, Idiopathic pulmonary fibrosis, Network pharmacology, Pharmacological mechanism

Posted Date: May 7th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-26079/v1

Abstract

Background

Idiopathic pulmonary fibrosis (IPF) is a chronic respiratory disease with high incidence rate, morbidity and mortality. Jinshui Huanxian formula (JHF) is an empirical formula for the pathogenesis of lung-kidney qi deficiency and phlegm-blood stasis in pulmonary fibrosis. The purpose of this study is to explore the pharmacological mechanism of JHF action in IPF therapy by network interaction analysis.

Methods

The main active components and corresponding target genes of JHF were predicted using various databases. Two sets of IPF disease genes were obtained from the DisGeNET database and GEO database. Two sets of drug targets for IPF treatment were collected and the overlapping genes between disease genes and drug targets were analyzed. The target genes of JHF were intersected with the differentially expressed genes of IPF to obtain the predicted targets of JHF acting on IPF. The functions and pathways of predicted targets acting on IPF were analyzed by using DAVID and KEGG pathway database. Finally, the resulting drug target mechanisms were validated in a rat model of pulmonary fibrosis.

Results

494 active compounds and 1304 corresponding targets were screened. Intersection analysis showed that 4 genes were common genes of JHF targets, IPF disease genes and anti-IPF drugs in KEGG database, and these genes were targeted by several compounds of JHF respectively. 72 JHF targets were closely related with IPF, and were thus considered therapeutically relevant. The targets were screened and participated in the regulation of IPF through 18 pathways. The molecular functions of targets included regulation of oxidoreductase activity, kinase regulator activity, phosphotransferase activity and transmembrane receptor protein kinase activity. In vivo experiments showed that JHF could alleviate the degree of pulmonary fibrosis, including the decrease of collagen deposition and epithelial-mesenchymal transition.

Conclusions

This study explored the mechanisms of JHF from a systematic point of view, trying to identify the specific target pathways acing on IPF. Pharmacological network with in vivo validation explained the potential roles and mechanisms of JHF in IPF therapy.

Background

Idiopathic pulmonary fibrosis (IPF) is a chronic respiratory disease, which is characterized by progressive fibrosis of lung parenchyma, resulting in loss of function and respiratory failure. It is the most common pulmonary interstitial disease with an estimated incidence of 2.8–9.3 out of 100,000 per year in Europe and North America[1]. The mortality rate of IPF is very high, and the median survival time reported is about 3 years[2]. As a chronic and refractory disease, the incidence, disability and mortality of IPF are increasing year by year, which seriously affects the health of patients and increases the social and economic burden. Glucocorticoids and immunosuppressants (such as cyclophosphamide, cyclosporine A, azathioprine, etc.) as traditional methods for treatment of pulmonary fibrosis, although they can alleviate inflammation, they have poor prognosis in preventing the development and improvement of pulmonary fibrosis[3]. Compared with single target drugs, multi-target drugs may be more effective due to synergistic effect or negative regulation of resistance[4, 5].

Traditional Chinese medicine (TCM) is one of the main components of medical practice, it is a natural chemical library. Chinese herbal compound has the characteristics of multi-component, multi-target and complex mechanism of action. It can enhance the body function and reduce the drug toxicity through the synergistic mechanism of main active ingredients. Jinshui Huanxian formula (JHF) is an empirical formula for the pathogenesis of lung-kidney qi deficiency and phlegm-blood stasis in pulmonary fibrosis. It takes Ginseng and Radix Rehmanniae as the monarch, nourishes yin and dissipates phlegm, promotes blood circulation and regulating qi, and mainly treats the syndrome of lung-spleen deficiency and phlegm-blood stasis in the later stage. In clinical practice, it has a significant effect in improving the clinical symptoms, slowing down the development of the disease and improving the quality of life[6].

Network pharmacology integrates system level network analysis and pharmacology to gain insight into the complex mechanisms of herbal formulas used to treat complex diseases[7, 8]. Network pharmacology analysis has been applied to Chinese herbal medicine to study the role of multi-target compounds in the biological network and explore a variety of molecular mechanisms[7, 9]. In this context, the purpose of this study is to establish a comprehensive research method based on network pharmacology to explore the pharmacological mechanisms of JHF acting on idiopathic pulmonary fibrosis.

In order to explore the pharmacological mechanisms of JHF associated with idiopathic pulmonary fibrosis, we used JHF based network pharmacology to study the relationship among traditional Chinese medicine, compounds, target genes and IPF differentially expressed genes. First, through extensive data mining, we collected information on two groups of IPF-related disease genes, two groups of anti-IPF drugs and therapeutic targets. Second, we gathered information on bioactive compounds in JHF and retrieved candidate target genes from public databases. Through the analysis of the collected data sets, the possible important targets of JHF were identified, and the related pathways involved in the possible targets of JHF were recognized. Then, based on IPF differentially expressed genes, network analysis was conducted to obtain the information of therapeutic targets in JHF that might target IPF differentially expressed genes, the biological functions of targets and the important pathways involved. The predictive targets and functions of JHF were verified by in vivo experiments.

Methods

The whole workflow was illustrated in Fig. 1

Identification Of Active JHF Ingredients

TCMSP (http://tcmspw.com/tcmsp.php) is a unique Chinese herbal medicine system pharmacology platform, contains information about absorption, distribution, metabolism and excretion (ADME) characteristics of compounds[10]. Oral bioavailability (OB) and drug-likeness (DL) are the two most important indicators to evaluate the characteristics of ADME through bioinformatics. OB represents the percentage of unchanged oral dose of drugs reaching systemic circulation, indicating the convergence of ADME process. High oral bioavailability is often the key index to determine the properties of bioactive molecules[11]. DL is a qualitative concept used in drug design to evaluate how “drug-like” a compound is, such as solubility and chemical stability, which helps optimize pharmacokinetics and drug properties[12]. TCMID (http://119.3.41.228:8000/tcmid/search/) is a comprehensive database including formulae, herbs, herbal ingredients, information for drugs and diseases. This database helps researchers in the fields of traditional medicine to discover potential new drugs and the mechanisms of drug interactions.

Compounds were retained only if OB ≥ 30 and DL ≥ 0.18 to satisfy criteria suggested by TCMSP database. All 494 ingredients were collected using Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform TCMSP and TCMID.

Compound Targets For JHF

For each compound, putative targets were predicted from TCMSP database and web server STITCH (http://stitch.embl.de/, ver. 5.0) [13]with the ‘*Homo sapiens*’ species setting. The STITCH database use compounds that were structurally similar to the chemical components of JHF to identify targets (Additional file 1: Table S1). The threshold of confidence score was set as 0.8, which is a high confidence score, filter genes associated with chemicals.

Idiopathic Pulmonary Fibrosis Associated Genes

Information on IPF-associated genes was collected from the database GEO (https://www.ncbi.nlm.nih.gov/geo/)[14] and DisGeNET (http://www.disgenet.org/, ver. 6.0)[15]. Searching DisGeNET with disease name “Idiopathic pulmonary fibrosis”, 378 IPF-associated genes were obtained (Additional file 2: Table S2). The GEO database was searched and genomic expression profile in lung tissue of IPF patients was found (dataset GSE2052). The corresponding research analyzed human lung tissues from 13 IPF lung explants and 11 normal histology lung tissue samples. A total of 257 differentially expressed genes with known gene symbols were confirmed, in which 122 were up-regulated (logFC ≥ 1) and 135 were down-regulated (logFC≤-1) in IPF patients (Additional file 3: Table S3).

Anti-IPF Drugs And Their Targets

The known anti-IPF drugs and their targets were collected from KEGG[16](https://www.genome.jp/kegg/pathway.html) and DrugBank database[17](https://www.drugbank.ca/, ver. 5.1.4). From the KEGG pathway database, we found 4 drugs and their 14 target proteins by searching IPF (Additional file 4: Table S4). In DrugBank database, only drugs approved by the Food and Drug Administration (FDA) were chosen for the treatment of idiopathic pulmonary fibrosis and whose targets were human genes/proteins. In total, we extracted 2 drugs and 26 target proteins from the DrugBank database (Additional file 5: Table S5).

Construction Of The Target Networks

The constructed compound-target and target-pathway networks were visualised using Cytoscape ver. 3.6.0 (http://www.cytoscape.org/). Nodes represent chemicals and targets; edges indicate interactions between chemicals and targets, and between targets and pathways.

In order to screen out the targets of JHF regulating the differentially expressed genes of IPF, firstly, IPF-associated differentially expressed genes were mapped to High-quality INTeractomes ver. 4 (HINT; http://hint.yulab.org). The HINT database is a curated compilation of high-quality protein-protein interactions from eight interactome resources (BioGRID, MINT, iRefWeb, DIP, IntAct, HPRD, MIPS and the PDB)[18]. The target gene network which contained the selected targets and the neighbor genes. Secondly, predicted targets of JHF were mapped to IPF-associated differentially expressed gene network, small network was extracted from the selected targets, and the target information of regulating IPF-associated genes was obtained.

Functional Annotation Clustering Analysis

To clarify the functions and pathways that were involved in predicted JHF targets, the functional annotation clustering tool in Database for Annotation, Visualization and Integrated Discovery (DAVID) ver. 6.8 (https://david.ncifcrf.gov/home.jsp) was used to calculate both the Gene Ontology (GO) enrichment and the KEGG pathways.

Reagents And Animals

Jinshui Huanxian formula was provided by the Pharmaceutical Department of Henan University of Chinese Medicine. Extraction procedure used for obtaining and standardizing JHF and JHF administration in vivo were performed as previously described[6]. Bleomycin hydrochloride was purchased from the Nippon Kayaku Co. Ltd. (lot 650427). Pirfenidone (PFD) was obtained from the Beijing Kangdini Pharmaceutical Co. Ltd. (lot 150603) (Beijing, China).

The animal experiments were conducted with the approval of the Experimental Animal Care and Ethics Committee of Henan University of Chinese Medicine and were in accordance with the internationally accepted principles for laboratory animal use and care. Sprague-Dawley rats (200 ± 20 g) were obtained from the Experimental Animal Center of Henan Province (Zhengzhou, China). The rats were housed under standard conditions of temperature (26 ± 2℃), humidity (50 ± 10%) and light intensity (12 h light/dark cycle), and fed with free access to standard laboratory food and water. All animal protocols in the study were performed in accordance with international ethical guidelines and the National Institutes of Health Guide concerning the Care and Use of Laboratory Animals. The experiments were approved by the Experimental Animal Care and Ethics Committee of the First Affiliated Hospital, Henan University of Traditional Chinese Medicine.

Pulmonary Fibrosis Rat Model And Drug Administration

Pulmonary fibrosis (PF) rat model was established as described in the previous study[19]. Briefly, rats were randomly divided into four groups. The rat model of pulmonary fibrosis was prepared by tracheal infusion of bleomycin at 5 mg/kg. Then PF rats were intragastrically administrated with normal saline, JHF (10.8 g/kg), and PFD Capsules (50 mg/kg) every day for 4 weeks. Finally, all rats were sacrificed, and lung tissues were collected.

Histological Analysis

The formaldehyde-fixed lung tissues were paraffin-embedded and cut into 4 μm sections. The sections were stained with hematoxylin-eosin solution (Solarbio, Beijing, China), and Masson's Trichrome stain kit (Solarbio, Beijing, China) to determine the collagen distribution. Digital images were captured by using light microscopic.

Western Blot

Lung tissues were homogenized and lysed with RIPA containing PMSF (Solarbio, Beijing, China) for 30 min, centrifuged at 12,000 g for 10 min at 4 °C. Lysates were mixed with SDS loading buffer and boiled at 100 °C for 5 min. Proteins were separated by SDS-PAGE gel and transferred to PVDF membranes (Millipore, Bedford, MA, United States). The membranes were sealed with 5% nonfat-milk and incubated with the following primary antibodies: α-SMA (1:1000, Proteintech, China), E-cadherin (1:1000, Proteintech, China), N-cadherin (1:1000, Proteintech, China) and GAPDH (1:5000, Proteintech, China) antibodies at 4 °C overnight. The membranes were incubated with horseradish peroxidase-linked anti-rabbit or anti-mouse antibody (1:3000, Proteintech, China) for 2 h, and visualized on a Bio-Rad Chemi Doc™ MP System (Bio-Rad, United States) using Super ECL Plus reagent (Solarbio, China).

Statistical Analysis

All data were expressed as means ± standard errors of the means (S.E.M.). Statistical evaluation of the data was performed with one-way ANOVA followed by a post hoc Tukey's test, and considered significant at *P*-values < 0.05.

Results

Compounds and predicted targets in JHF

JHF contains 12 herb materials that include Renshen (Ginseng Radix et Rhizoma ,GRR), Maidong (radix ophiopogonis, RO), Dihuang (Radix Rehmanniae, RR), Gualou (Fructus et Semen Trichosanthis, FST), Zhebeimu (Bulbus Fritillariae Thunbergii, BFT), Mudanpi (Cortex Moutan Radicis, CMR), Yinyanghuo (Herba Epimedii Brevicornus, HEB), Baiguo (Semen Ginkgo, SG), Baitouweng (Radix Pulsatillae,RP), Yiyiren (Semen Coicis, SC), Chenpi (Pericarpium Citri Reticulatae, PCR), Gouqizi(inLycii Fructus, LF). We collected compounds from the TCMSP and TCMID databases by oral bioavailability and drug-likeness, including 162 compounds in GRR, 22 compounds in RO, 10 compounds in RR, 41 compounds in FST, 27 compounds in BFT, 28 compounds in CMR, 50 compounds in HEB, 53 compounds in SG, 25 compounds in RP, 11 compounds in SC, 40 compounds in PCR and 79 compounds in LF. Of the 548 compounds, 54 were duplicated and therefore removed, resulting in 494 compounds.

We used the STITCH database to predict the targets of the selected compounds. The compounds were predicted to interact with 1304 distinct protein targets with high confidence level (Additional file 1: Table S1). Figure 2 showed the JHF drug-target network, describing its multi-component and multi-target therapy. Notably, there were different numbers of mutual putative targets among the compounds in JHF, proposing that these herbs might have several interactions in the course of treatment.

Identification Of JHF's Important Targets By Intersection Analysis

We collected two sets of disease genes and two sets of drug targets associated with IPF as reference. We first looked at the overlap between these gene sets. As shown in Fig. 3A, among the IPF patients participating in the GSE2052 dataset experiment, 25 of the 378 disease genes were differentially expressed, accounting for 6.6% of all disease genes. On the other hand, there were 4 drug target genes for anti-IPF drugs in KEGG and DrugBank databases, accounting for 30.8% of the target genes in KEGG database.

In Fig. 3B we showed the overlaps of JHF' target genes with IPF disease genes and target genes for anti-IPF drugs in KEGG database. Among the 1305 target genes of JHF, 99 were IPF disease genes, accounting for 7.59% of all disease genes. Among them, 4 were anti-IPF drug target genes. The four common genes in the three data sets were TNF (Tumor necrosis factor), CCL2 (C-C motif chemokine 2), IL6 (Interleukin-6), and IL10 (Interleukin-10), indicating their important role in the treatment of IPF. From

the predictions, TNF was targeted by 13 compounds of JHF, i.e., kaempferol, quercetin, ruscogenin, luteolin, epicatechin, palmitic acid, methyl palmitate, adenosine, adenosine triphosphate, choline, ginsenoside rg1, hexadecanoic acid, spermine; CCL2 was targeted by 6 compounds of JHF (i.e., naringenin, quercetin, rutin, palmitic acid, adenosine triphosphate, hexadecanoic acid); IL6 was targeted by 8 compounds of JHF (i.e., quercetin, luteolin, palmitic acid, adenosine, adenosine triphosphate, dibutyl phthalate, hexadecanoic acid, spermine); IL10 was targeted by 3 compounds of JHF (i.e., quercetin, luteolin, adenosine) (See Additional file 1: Table S1). The four target genes were all target genes of PFD, and PFD have been approved for the treatment of IPF[20]. Therefore, in the following study, we used pirfenidone as a positive control drug.

Identification Of The Pathways And Diseases Regulated By JHF

We used Cytoscape software to construct the drug-target-pathway network of KEGG's anti-IPF drugs (Fig. 4A). At present, pirfenidone, a drug commonly used in treatment of pulmonary fibrosis, is mainly involved in TNF signaling pathway, TGF-β signaling pathway, cytokine-cytokine receptor interaction and cellular senescence, etc. We used ClueGO, a Cytoscape plugin[21], to analyze the biological processes involved in KEGG's anti-IPF drug targets. The biological processes mainly included transmembrane receptor protein kinase activity, regulation of phosphatidylinositol 3-kinase activity, vascular endothelial cell proliferation and regulation of vascular endothelial growth factor production (Fig. 4B).

As shown in Fig. 4C, a target gene participated in multiple pathways. To elucidate the biological pathways that JHF might regulate, we analyzed the important pathways involved by JHF targets through DAVID analysis, and constructed a target-pathway network of putative JHF targets. Considering that disease is an advanced biological process caused by the dysfunction of basic biological processes, we only focused on the relevant signaling pathways involved in biological processes. The targets were significantly enriched in 16 pathways ($p < 0.01$) (Fig. 4D). To uncover the therapeutic potential of the putative targets, disease ontology enrichment was conducted. Through "high" strict classification and enrichment score, a total of 59 clusters of diseases were related to JHF targets, including inflammation, bronchiolitis, coronary artery disease, etc (Additional file 6: Table S6).

Target, pathway and gene ontology analysis of IPF differential expression genes targeted in JHF

In order to further improve the reliability of the analysis, we mapped the predicted targets of JHF to the network of IPF disease genes, and obtained the target information that JHF could directly regulate IPF differential expression genes (Fig. 5A). By analyzing these targets with DAVID-KEGG, it was found that 72 targets (such as Transforming growth factor β1 (TGF-β1), SMAD3) were screened and participated in 18 pathways, including ErbB signaling pathway, Thyroid hormone signaling pathway, TGF-β signaling pathway and so on (Additional file 7: Table S7) (Fig. 5B). Through the analysis of ClueGO plug-in in the software of Cytoscape, the molecular functions of these targets mainly included regulation of

oxidoreductase activity, kinase regulator activity, phosphotransferase activity and transmembrane receptor protein kinase activity (Fig. 5C). The biological processes mainly included tube development (Fig. 5D).

Experimental Validation

To confirm our predictions and the therapeutic effects of JHF, we used a well-characterized animal model of pulmonary fibrosis. JHF or PFD was administrated to PF rats. Previous studies have shown that compared with the model group, JHF and pirfenidone could significantly inhibit the decreases of FVC (Forced Vital Capacity) and the increases of lung coefficient[19]. Histological examination showed structural changes in the alveoli of the model group, including collapsed alveolar spaces, thickening of the alveolar walls, presence of inflammatory cells and excessive collagen fiber deposition. However, JHF and PFD could alleviate the alveolar damage described above due to bleomycin (Fig. 6A). The epithelial-mesenchymal transition (EMT) plays an important role in the pathogenesis of pulmonary fibrosis, which is an important pathological process of pulmonary fibrosis[22]. TGF-β is an important inducer of EMT and the strongest inducer of extracellular matrix deposition. Therefore, we detected the role of EMT involved in TGF-β signaling pathway in the development of pulmonary fibrosis. The occurrence of pulmonary fibrosis caused the increase of Vimentin and N-cadherin expression, and the decrease of E-cadherin expression. However, JHF could reverse the expression of these proteins (Fig. 6B). The expression of TGF-β and SMAD3 were increased in PF model group compared to normal control, while in the JHF group, the protein expression returned to the normal level (Fig. 6B).

Discussion

It is well known that treatment options for pulmonary fibrosis include antioxidants, cytokine inhibitors, anti-fibrotic drugs and lung transplantation or else [23]. However, most of these studies have focused on one or two aspects of the lung injury repair process. Although pirfenidone has been proved to have a better therapeutic effect on PF in clinical practice, it has only received a conditional use recommendation, and the efficacy and safety of long-term use are not clear [24, 25]. Herbal medicine has become one of the most important sources of chemical substances or lead compounds in drug discovery[26]. Network pharmacology has been used to study the complex components, unknown targets and pharmacological mechanisms of traditional Chinese medicine prescriptions[27]. In this work, we systematically studied the anti-IPF mechanism of JHF from the perspective of target, pathway, network and efficacy levels, respectively.

In the three datasets of IPF disease genes, JHF targets and anti-IPF drug targets, the four overlapping genes were TNF, CCL2, IL6 and IL10. Inappropriate production of TNF is involved in the pathogenesis of many human diseases, including pulmonary fibrosis[28, 29]. And TNF has been shown to affect multiple responses far beyond its proinflammatory properties[30, 31]. CCL2 is associated with activation of macrophages and may have a serious impact on the overall survival of patients with IPF[32]. IL6 and

IL10 play an important role in the recruitment, activation, survival and differentiation of fibroblasts into myofibroblasts in IPF[33, 34]. However, in JHF, there are multiple compounds targeting these targets, and the same compound has different other targets, indicating the characteristics of multi-component, multi-target and synergistic action mode of traditional Chinese medicine.

Through the construction and analysis of the assumed target pathway network of JHF regulating IPF differentially expressed genes, we found that 5 pathways with the most enrichment of JHF targets and the most significant difference were as follows: thyroid hormone signaling pathway, chemokine signaling pathway, ErbB signaling pathway, neurotrophin signaling pathway and Hippo signaling pathway.

It has been proved that thyroid hormone (TH) signaling plays an important role in pulmonary fibrosis, including its effect on mitochondrial function. It was found that the increased transformation of prohormone thyroxine (T4) to active 3,5,3-triiodothyronine (T3) improved the metabolism of alveolar epithelial cells (AECs) in IPF, and thyroid hormone played an important role in the biogenesis and bioenergy of mitochondria, which regulated the apoptosis of AECs[35]. The activity and expression level of iodothyronine deiodinase 2 (DIO2), an enzyme activating TH, were higher in lungs of IPF patients than in the normal control individuals, and related to the severity of disease. TH inhibited pulmonary fibrosis by depending on PPARGC1A and PINK1 pathways, and the anti-fibrosis properties of TH were related to the protection of alveolar epithelial cells and the restoration of mitochondrial function[36].

Multiple chemokines and cytokines inducing the migration of fibroblasts and phenotype change to myofibroblasts, lead to the occurrence of IPF. Chemokines and cytokines are expressed and released in alveolar macrophages (AMs), which are mediators of the inflammatory response and fibrotic process. In the middle stage of pulmonary fibrosis, immune cells secrete chemokines and proinflammatory factors, accelerate the migration and secretion of macrophages, and thus accelerate the immune response[37]. Single cell sequencing showed that chemokine signaling pathway was upregulated in IPF[38]. Based on the RNA sequencing of bronchoalveolar lavage (BAL) cells in IPF, the differentially expressed gene symbol list Gene Ontology identified that chemokine-mediated signaling pathway and chemokine activity were the most significant enrichment of biological processes and molecular functions. Similarly, in KEGG pathway analysis, cytokine-cytokine receptor interaction, chemokine signaling pathway and TNF signaling pathway were considered to be the most significant overexpression[39].

ErbB signaling was enriched in plasma proteome of IPF patients[40]. These were tyrosine kinase receptor families, including Her1 (epidermal growth factor receptor (EGFR)), Her2, Her3, and Her4. Several of these receptors have been reported to play an important role in epithelial remodeling and epithelial hyperplasia, and have been found to play an important role in the fibrosis model[41–43]. EGFR (c-erbB1) played a key role in the maintenance and repair of epithelial tissue. The interaction between c-erbB receptors and their ligands might help to determine their role in the maintenance and repair of bronchial epithelium[44].

Neurotrophin signaling is realized by the binding of NT ligands and their homologous high-affinity receptors, so-called neurotrophic tyrosine kinase receptors. NT ligands and their homologous receptors were detected in mice and adult human lung tissues[45–47]. More and more evidences show that the

expression of NTs and of their homologous receptors in NT signaling pathways of lung diseases were changed. In chronic obstructive pulmonary disease, the concentrations of NT3 were inhibited[48]. It has been shown that the expression of NT4/5 and its homologous receptor TrkB increased in the transplanted human lungs with idiopathic pulmonary fibrosis and in lungs of mice with bleomycin-induced pulmonary fibrosis. The dysregulated TrkB/NT4/5 axis might lead to some pathological changes related to pulmonary fibrosis, including hyperplasia of alveolar type II cells and proliferation of fibroblasts[49].

Hippo signaling pathway plays a key role in many key pathological processes such as organ growth control, cell proliferation, apoptosis, tissue regeneration and tumor suppression[50, 51]. Yes-associated protein (YAP) is a key downstream effector protein of Hippo. In patients with IPF, the activity of YAP increased, and the interaction of YAP and mTOR/p-S6 signaling pathways induced cell proliferation and migration, and inhibited epithelial cell differentiation[52].It has been shown that melatonin attenuated TGF-β1-induced fibrogenesis in pulmonary fibroblasts by activating the Hippo pathway and then promoting nuclear translocation and increasing the inactivation and degeneration of YAP1 in the cytoplasm[53].

In addition, TGF-β is an important inducer of EMT and the strongest inducer of extracellular matrix deposition. TGF-β could stimulate fibroblasts to synthesize extracellular matrix components and induce matrix metalloproteinase expression[54], including Smads-dependent and non-Smads-dependent pathways. Smad-complex targeted many genes to activate or inhibit the expression of EMT-related transcription factors by interacting with DNA sequence-specific transcription factors, co-activators, or co-inhibitors[55, 56]. Vascular endothelial growth factor (VEGF) is a potent promoter of angiogenesis, and abnormal angiogenesis is a central characteristic for the development and progression of IPF. VEGF signaling was enriched in the upregulated plasma proteome of patients with IPF, indicating its role in the pathogenesis of IPF[40]. In VEGF signaling pathway, VEGF combined with vascular endothelial growth factor receptor 2 (VEGFR2) regulated cell migration, survival and permeability by activating phosphatidylinositol 3-kinase (PI3K) in PI3K-Akt signaling pathway [57, 58]. Some studies have shown that TGF-β1 could stimulate the expression of VEGF-A in human fetal pulmonary fibroblasts through Smad3 signaling pathway [59]. VEGF and VEGF signaling pathway will be the powerful and effective therapeutic targets for IPF.

In some “hub” signal molecules, there are multiple pathway routes overlapping, forming complex functional modules[60]. For this complex crosstalk among pathways and environment dependent specific functions, JHF can treat IPF through multiple targets and multiple channels.

Conclusions

Overall, this study analyzed the pharmacological mechanism of JHF in the treatment of IPF to provide guidance for clinical practice. The studies were used to infer the action mode of TCM prescription from the level of the target, pathway, network and biomedical efficacy, and obtain the information of therapeutic targets in JHF that might target IPF differentially expressed genes, the biological functions of

targets and the important pathways involved. In addition, this work lays a foundation for the treatment of complex diseases, such as IPF.

Abbreviations

IPF: idiopathic pulmonary fibrosis; JHF: Jinshui Huanxian formula; TCM: traditional Chinese medicine; TCMSP : Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform; TCMID: Traditional Chinese Medicines Integrated Database; FDA: food and drug administration; HINT: High-quality INTeractomes; DAVID: Database for Annotation, Visualization and Integrated Discovery; PFD: pirfenidone; PF: pulmonary fibrosis; TNF: tumor necrosis factor; CCL2: C-C motif chemokine 2; IL6: Interleukin-6; IL10: Interleukin-10; TGF-β: transforming growth factor β; EMT: epithelial-mesenchymal transition; TH: thyroid hormone; AECs: alveolar epithelial cells; AMs: alveolar macrophages; BAL: bronchoalveolar lavage; YAP: Yes-associated protein; EGFR: epidermal growth factor receptor; VEGF: vascular endothelial growth factor; VEGFR2: vascular endothelial growth factor receptor 2; PI3K: phosphatidylinositol 3-kinase.

Declarations

Authors' contributions

LTT designed methodology, performed experiments, analyzed data, performed literature review, wrote the original draft. XPL collected the data, performed experiments, performed literature review. QSS conceptualized the study, reviewed and edited the manuscript. KSR and HQ researched literature. ZP revised the manuscript. LJS conceptualized the study, administered the project.

Funding

This work was supported by National Natural Science Foundation of China (81673942).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All animal protocols in the study were performed in accordance with international ethical guidelines and the National Institutes of Health Guide concerning the Care and Use of Laboratory Animals. The experiments were approved by the Experimental Animal Care and Ethics Committee of the First Affiliated Hospital, Henan University of Traditional Chinese Medicine.

Acknowledgements

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflicts of interest.

References

1. Hutchinson J, Fogarty A, Hubbard R, McKeever T. Global incidence and mortality of idiopathic pulmonary fibrosis: a systematic review. Eur Respir J. 2015;46(3):795–806. doi:10.1183/09031936.00185114.
2. Ley B, Collard HR, King TE. Clinical Course and Prediction of Survival in Idiopathic Pulmonary Fibrosis. Am J Resp Crit Care. 2011;183(4):431–40. doi:10.1164/rccm.201006-0894CI.
3. Segawa S, Goto D, Yoshiga Y, Sugihara M, Hayashi T, Chino Y, et al. Inhibition of transforming growth factor-beta signalling attenuates interleukin (IL)-18 plus IL-2-induced interstitial lung disease in mice. Clin Exp Immunol. 2010;160(3):394–402. doi:10.1111/j.1365-2249.2010.04094.x.
4. Chan SY, Loscalzo J. The emerging paradigm of network medicine in the study of human disease. Circ Res. 2012;111(3):359–74. doi:10.1161/CIRCRESAHA.111.258541.
5. Jia J, Zhu F, Ma X, Cao Z, Cao ZW, Li Y, et al. Mechanisms of drug combinations: interaction and network perspectives. Nat Rev Drug Discov. 2009;8(2):111–28. doi:10.1038/nrd2683.
6. Bai Y, Li J, Zhao P, Li Y, Li M, Feng S, et al. A Chinese herbal formula ameliorates pulmonary fibrosis by inhibiting oxidative stress via upregulating Nrf2. Front Pharmacol. 2018;9:628.
7. Zhao J, Lv C, Wu Q, Zeng H, Guo X, Yang J, et al. Computational systems pharmacology reveals an antiplatelet and neuroprotective mechanism of Deng-Zhan-Xi-Xin injection in the treatment of ischemic stroke. Pharmacol Res. 2019;147:104365. doi:10.1016/j.phrs.2019.104365.
8. Guo W, Huang J, Wang N, Tan HY, Cheung F, Chen F, et al. Integrating Network Pharmacology and Pharmacological Evaluation for Deciphering the Action Mechanism of Herbal Formula Zuojin Pill in Suppressing Hepatocellular Carcinoma. Front Pharmacol. 2019;10:1185. doi:10.3389/fphar.2019.01185.
9. Zhang XW, Liu W, Jiang HL, Mao B. Dissection of Pharmacological Mechanism of Chinese Herbal Medicine Yihuo Huatan Formula on Chronic Obstructive Pulmonary Disease: A Systems Pharmacology-Based Study. Sci Rep. 2019;9(1):13431. doi:10.1038/s41598-019-50064-9.
10. Ru J, Li P, Wang J, Zhou W, Li B, Huang C, et al. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. J Cheminform. 2014;6:13. doi:10.1186/1758-2946-6-13.
11. Xu X, Zhang W, Huang C, Li Y, Yu H, Wang Y, et al. A novel chemometric method for the prediction of human oral bioavailability. Int J Mol Sci. 2012;13(6):6964–82. doi:10.3390/ijms13066964.

12. Tao W, Xu X, Wang X, Li B, Wang Y, Li Y, et al. Network pharmacology-based prediction of the active ingredients and potential targets of Chinese herbal Radix Curcumae formula for application to cardiovascular disease. J Ethnopharmacol. 2013;145(1):1–10. doi:10.1016/j.jep.2012.09.051.
13. Kuhn M, Szklarczyk D, Franceschini A, von Mering C, Jensen LJ, Bork P. STITCH 3: zooming in on protein-chemical interactions. Nucleic Acids Res. 2012;40(Database issue):D876-80. doi:10.1093/nar/gkr1011.
14. Edgar R, Domrachev M, Lash AE. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res. 2002;30(1):207–10. doi:10.1093/nar/30.1.207.
15. Pinero J, Bravo A, Queralt-Rosinach N, Gutierrez-Sacristan A, Deu-Pons J, Centeno E, et al. DisGeNET: a comprehensive platform integrating information on human disease-associated genes and variants. Nucleic Acids Res. 2017;45(D1):D833-D9. doi:10.1093/nar/gkw943.
16. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 2000;28(1):27–30. doi:10.1093/nar/28.1.27.
17. Wishart DS, Feunang YD, Guo AC, Lo EJ, Marcu A, Grant JR, et al. DrugBank 5.0: a major update to the DrugBank database for 2018. Nucleic Acids Res. 2018;46(D1):D1074-D82. doi:10.1093/nar/gkx1037.
18. Das J, Yu H. HINT. High-quality protein interactomes and their applications in understanding human disease. BMC Syst Biol. 2012;6:92. doi:10.1186/1752-0509-6-92.
19. Bai Y, Li J, Zhao P, Li Y, Li M, Feng S, et al. A Chinese Herbal Formula Ameliorates Pulmonary Fibrosis by Inhibiting Oxidative Stress via Upregulating Nrf2. Front Pharmacol. 2018;9:628. doi:10.3389/fphar.2018.00628.
20. Raghu G, Rochwerg B, Zhang Y, Garcia CA, Azuma A, Behr J, et al. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline: Treatment of Idiopathic Pulmonary Fibrosis. An Update of the 2011 Clinical Practice Guideline. Am J Respir Crit Care Med. 2015;192(2):e3–19. doi:10.1164/rccm.201506-1063ST.
21. Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, et al. ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. Bioinformatics. 2009;25(8):1091–3. doi:10.1093/bioinformatics/btp101.
22. Kim KK, Kugler MC, Wolters PJ, Robillard L, Galvez MG, Brumwell AN, et al. Alveolar epithelial cell mesenchymal transition develops in vivo during pulmonary fibrosis and is regulated by the extracellular matrix. P Natl Acad Sci USA. 2006;103(35):13180–5. doi:10.1073/pnas.0605669103.
23. Prasad J, Holland AE, Glaspole I, Westall G. Idiopathic pulmonary fibrosis: an Australian perspective. Intern Med J. 2016;46(6):663–8. doi:10.1111/imj.13078.
24. Papiris SA, Kagouridis K, Kolilekas L, Triantafillidou C, Tsangaris I, Manali ED. Pirfenidone treatment in idiopathic pulmonary fibrosis: too much of a great expectation? Eur Respir J. 2012;40(3):794–5. doi:10.1183/09031936.00114311.
25. Rogliani P, Calzetta L, Cavalli F, Matera MG, Cazzola M. Pirfenidone, nintedanib and N-acetylcysteine for the treatment of idiopathic pulmonary fibrosis: A systematic review and meta-analysis. Pulm

Pharmacol Ther. 2016;40:95–103. doi:10.1016/j.pupt.2016.07.009.

26. Li P, Chen J, Zhang W, Li H, Wang W, Chen J. Network pharmacology based investigation of the effects of herbal ingredients on the immune dysfunction in heart disease. Pharmacol Res. 2019;141:104–13. doi:10.1016/j.phrs.2018.12.016.
27. Xu TF, Li SZ, Sun YF, Pi ZF, Liu S, Song FR, et al. Systematically characterize the absorbed effective substances of Wutou Decoction and their metabolic pathways in rat plasma using UHPLC-Q-TOF-MS combined with a target network pharmacological analysis. J Pharmaceut Biomed. 2017;141:95–107. doi:10.1016/j.jpba.2017.04.012.
28. Kapanci Y, Desmouliere A, Pache JC, Redard M, Gabbiani G. Cytoskeletal protein modulation in pulmonary alveolar myofibroblasts during idiopathic pulmonary fibrosis. Possible role of transforming growth factor beta and tumor necrosis factor alpha. Am J Respir Crit Care Med. 1995;152(6 Pt 1):2163–9. doi:10.1164/ajrccm.152.6.8520791.
29. Sime PJ, Marr RA, Gauldie D, Xing Z, Hewlett BR, Graham FL, et al. Transfer of tumor necrosis factor-alpha to rat lung induces severe pulmonary inflammation and patchy interstitial fibrogenesis with induction of transforming growth factor-beta1 and myofibroblasts. Am J Pathol. 1998;153(3):825–32. doi:10.1016/s0002-9440(10)65624-6.
30. Li B, Pozzi A, Young PP. TNFalpha accelerates monocyte to endothelial transdifferentiation in tumors by the induction of integrin alpha5 expression and adhesion to fibronectin. Mol Cancer Res. 2011;9(6):702–11. doi:10.1158/1541-7786.MCR-10-0484.
31. Verjee LS, Verhoekx JS, Chan JK, Krausgruber T, Nicolaidou V, Izadi D, et al. Unraveling the signaling pathways promoting fibrosis in Dupuytren's disease reveals TNF as a therapeutic target. Proc Natl Acad Sci U S A. 2013;110(10):E928-37. doi:10.1073/pnas.1301100110.
32. Gui X, Qiu X, Tian Y, Xie M, Li H, Gao Y, et al. Prognostic value of IFN-gamma, sCD163, CCL2 and CXCL10 involved in acute exacerbation of idiopathic pulmonary fibrosis. Int Immunopharmacol. 2019;70:208–15. doi:10.1016/j.intimp.2019.02.039.
33. Habiel DM, Hogaboam C. Heterogeneity in fibroblast proliferation and survival in idiopathic pulmonary fibrosis. Front Pharmacol. 2014;5:2. doi:10.3389/fphar.2014.00002.
34. Millar AB. IL-10: Another therapeutic target in idiopathic pulmonary fibrosis? Thorax. 2006;61(10):835–6. doi:10.1136/thx.2006.060772.
35. Yu G, Tzouvelekis A, Wang R, Herazo-Maya JD, Ibarra GH, Srivastava A, et al. Thyroid hormone inhibits lung fibrosis in mice by improving epithelial mitochondrial function. Nat Med. 2018;24(1):39–49. doi:10.1038/nm.4447.
36. Yu G, Tzouvelekis A, Wang R, Herazo-Maya JD, Ibarra GH, Srivastava A, et al. Thyroid hormone inhibits lung fibrosis in mice by improving epithelial mitochondrial function. Nature medicine. 2018;24(1):39.
37. Liu G, Zhai H, Zhang T, Li S, Li N, Chen J, et al. New therapeutic strategies for IPF: Based on the “phagocytosis-secretion-immunization” network regulation mechanism of pulmonary macrophages. Biomed Pharmacother. 2019;118:109230.

38. Xu Y, Mizuno T, Sridharan A, Du Y, Guo M, Tang J, et al. Single-cell RNA sequencing identifies diverse roles of epithelial cells in idiopathic pulmonary fibrosis. JCI insight. 2016;1(20).
39. Lee J, Arisi I, Puxeddu E, Mramba LK, Amicosante M, Swaisgood CM, et al. Bronchoalveolar lavage (BAL) cells in idiopathic pulmonary fibrosis express a complex pro-inflammatory, pro-repair, angiogenic activation pattern, likely associated with macrophage iron accumulation. PloS one. 2018;13(4).
40. O'Dwyer DN, Norman KC, Xia M, Huang Y, Gurczynski SJ, Ashley SL, et al. The peripheral blood proteome signature of idiopathic pulmonary fibrosis is distinct from normal and is associated with novel immunological processes. Scientific reports. 2017;7:46560.
41. Nethery DE, Moore BB, Minowada G, Carroll J, Faress JA, Kern JA. Expression of mutant human epidermal receptor 3 attenuates lung fibrosis and improves survival in mice. J Appl Physiol (1985). 2005;99(1):298–307. doi:10.1152/japplphysiol.01360.2004.
42. JA F, DE N, EF K, FJ RE, CL J. A, et al. Bleomycin-induced pulmonary fibrosis is attenuated by a monoclonal antibody targeting HER2. J Appl Physiol. 2007;103(6):2077–83. doi:10.1152/japplphysiol.00239.2007.
43. Buckley S, Shi W, Xu W, Frey MR, Moats R, Pardo A, et al. Increased alveolar soluble annexin V promotes lung inflammation and fibrosis. Eur Respir J. 2015;46(5):1417–29.
44. Polosa R, Prosperini G, Leir SH, Holgate ST, Lackie PM, Davies DE. Expression of c-erbB receptors and ligands in human bronchial mucosa. Am J Respir Cell Mol Biol. 1999;20(5):914–23. doi:10.1165/ajrcmb.20.5.3308.
45. Hikawa S, Kobayashi H, Hikawa N, Kusakabe T, Hiruma H, Takenaka T, et al. Expression of neurotrophins and their receptors in peripheral lung cells of mice. Histochem Cell Biol. 2002;118(1):51–8.
46. García-Suárez O, Pérez-Pinera P, Laurà R, Germanà A, Esteban I, Cabo R, et al. TrkB is necessary for the normal development of the lung. Respir Physiol Neurobiol. 2009;167(3):281–91.
47. Ricci A, Felici L, Mariotta S, Mannino F, Schmid G, Terzano C, et al. Neurotrophin and neurotrophin receptor protein expression in the human lung. Am J Respir Cell Mol Biol. 2004;30(1):12–9.
48. Groneberg DA, Peiser C, Eynott PR, Welker P, Erbes R, Witt C, et al. Transcriptional down-regulation of neurotrophin-3 in chronic obstructive pulmonary disease. Biological chemistry. 2005;386(1):53–9.
49. Avcuoglu S, Wygrecka M, Marsh LM, Günther A, Seeger W, Weissmann N, et al. Neurotrophic tyrosine kinase receptor B/neurotrophin 4 signaling axis is perturbed in clinical and experimental pulmonary fibrosis. Am J Respir Cell Mol Biol. 2011;45(4):768–80.
50. Richardson HE, Portela M. Tissue growth and tumorigenesis in Drosophila: cell polarity and the Hippo pathway. Curr Opin Cell Biol. 2017;48:1–9.
51. Pfleger CM. The Hippo pathway: a master regulatory network important in development and dysregulated in disease. Current topics in developmental biology. Elsevier; 2017. p. 181–228.
52. Gokey JJ, Sridharan A, Xu Y, Green J, Carraro G, Stripp BR, et al. Active epithelial Hippo signaling in idiopathic pulmonary fibrosis. JCI insight. 2018;3(6).

53. Zhao X, Sun J, Su W, Shan H, Zhang B, Wang Y, et al. Melatonin protects against lung fibrosis by regulating the hippo/YAP pathway. Int J Mol Sci. 2018;19(4):1118.
54. Strieter RM, Mehrad B. New mechanisms of pulmonary fibrosis. Chest. 2009;136(5):1364–70. doi:10.1378/chest.09-0510.
55. Feng XH, Derynck R. Specificity and versatility in tgf-beta signaling through Smads. Annu Rev Cell Dev Biol. 2005;21:659–93. doi:10.1146/annurev.cellbio.21.022404.142018.
56. Massague J. TGFbeta signalling in context. Nat Rev Mol Cell Biol. 2012;13(10):616–30. doi:10.1038/nrm3434.
57. Silva SR, Bowen KA, Rychahou PG, Jackson LN, Weiss HL, Lee EY, et al. VEGFR-2 expression in carcinoid cancer cells and its role in tumor growth and metastasis. Int J Cancer. 2011;128(5):1045–56. doi:10.1002/ijc.25441.
58. Tang J, Wang J, Kong X, Yang J, Guo L, Zheng F, et al. Vascular endothelial growth factor promotes cardiac stem cell migration via the PI3K/Akt pathway. Experimental cell research. 2009;315(20):3521–31.
59. Kobayashi T, Liu X, Wen F-Q, Kohyama T, Shen L, Wang XQ, et al. Smad3 mediates TGF-β1-induced collagen gel contraction by human lung fibroblasts. Biochem Biophys Res Commun. 2006;339(1):290–5.
60. Yan Z, Kui Z, Ping Z. Reviews and prospectives of signaling pathway analysis in idiopathic pulmonary fibrosis. Autoimmun Rev. 2014;13(10):1020–5. doi:10.1016/j.autrev.2014.08.028.

Additional Files

Additional file 1: Table S1 (xls): active components and targets of JHF

Additional file 2: Table S2 (xls): IPF-associated genes in DisGeNET database

Additional file 3: Table S3 (xls): up-regulated and down-regulated genes in IPF patients for GEO database

Additional file 4: Table S4 (xls): anti-IPF drugs and their targets in KEGG database

Additional file 5: Table S5 (xls): anti-IPF drugs and their targets in DrugBank database

Additional file 6: Table S6 (xls): Cluster analysis of diseases related to JHF targets

Additional file 7: Table S7 (xls): DAVID-KEGG analysis of pathways involved in targets

Figures

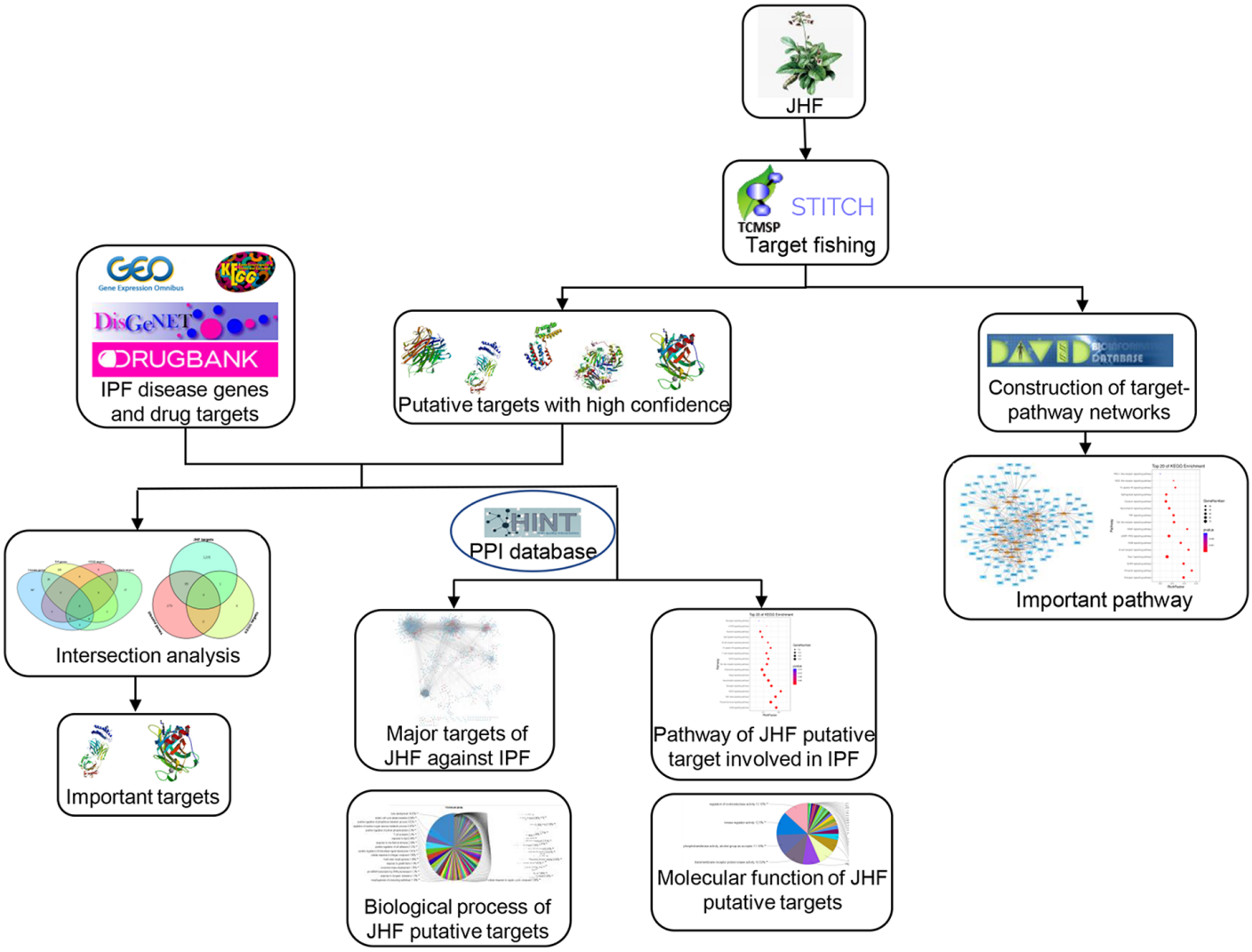

Figure 1

A comprehensive workflow for illustrating the mechanisms of JHF in the treatment of IPF.

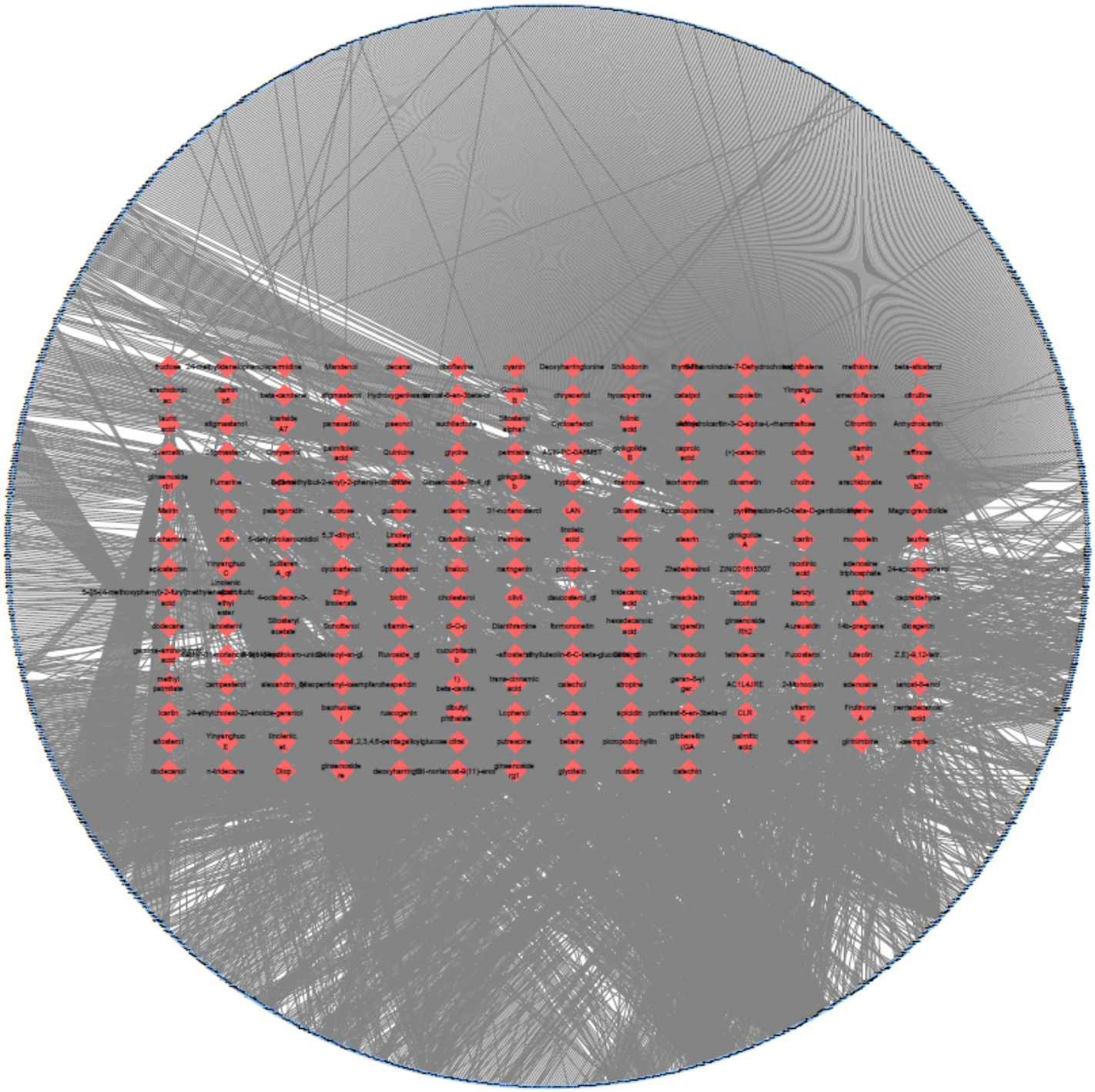

Figure 2

Drug-target network for active JHF compounds. Orange Diamonds represent JHF compounds, and blue nodes represent targets.

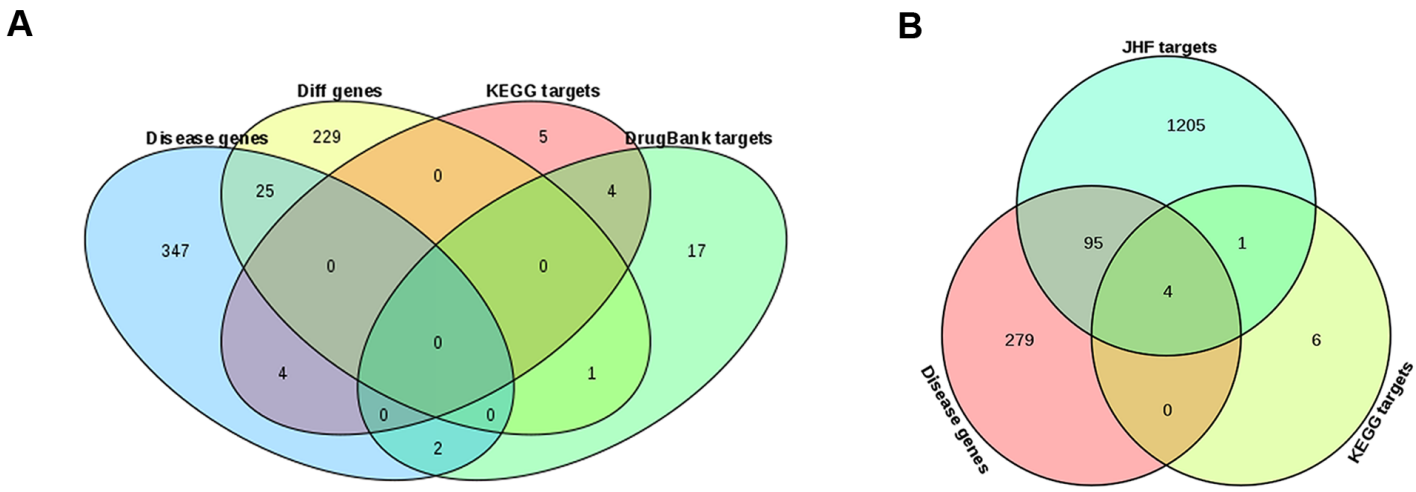

Figure 3

Overlapping of different gene sets. (A) The overlap of disease genes in DisGeNET(Disease genes), differential expression genes in GSE2052 dataset(Diff genes), drug target genes for anti-IPF drugs in KEGG and DrugBank database (KEGG targets, Drugbank targets). (B) Overlaps of disease genes from DisGeNET, drug target genes of anti-IPF drugs in KEGG database and potential JHF targets.

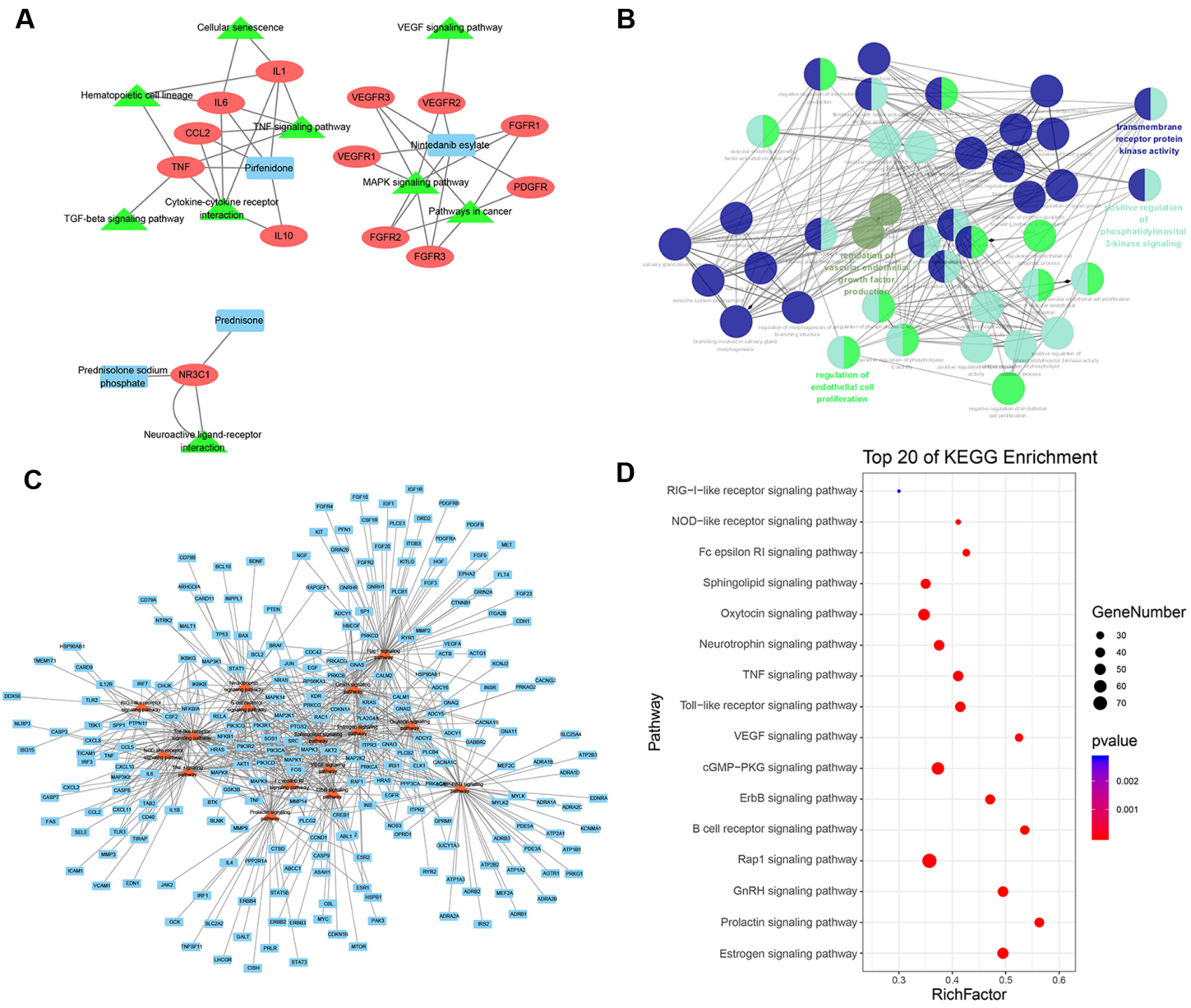

Figure 4

Identification of targets and pathways for anti-IPF drugs and JHF. (A) Drug-target-pathway network of KEGG's anti-IPF drugs. Orange ellipses represent drugs, blue rectangles represent targets, green triangles represent pathways. (B) Biological processes involved in KEGG's anti-IPF drug targets. (C) Target-pathway network of putative JHF targets. Blue rectangles represent targets and orange triangles represent the pathways. (D) Bubble diagram of main enrichment pathways of putative JHF targets.

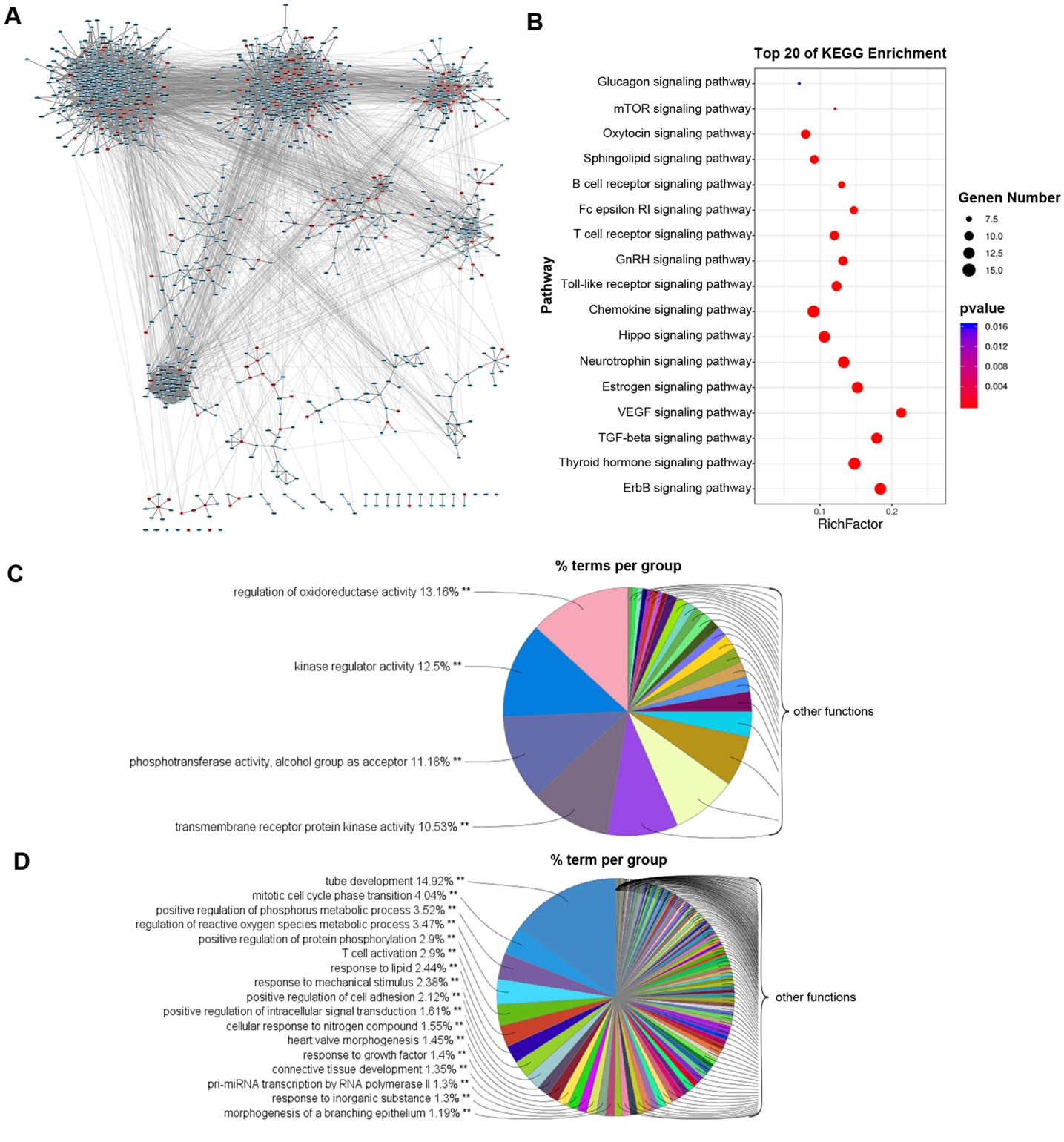

Figure 5

Target, pathway and gene ontology analysis of IPF differential expression gene targeted in JHF. (A) Analysis of disease genes differentially expressed in IPF regulated by JHF. Blue represents the differentially expressed genes in IPF, red represents the overlap of potential targets of JHF and the differentially expressed genes in IPF disease. (B) Pathway analysis of overlapping genes between

potential targets of JHF and differentially expressed genes in disease. Molecular function analysis (C) and biological process analysis (D) of overlapping genes.

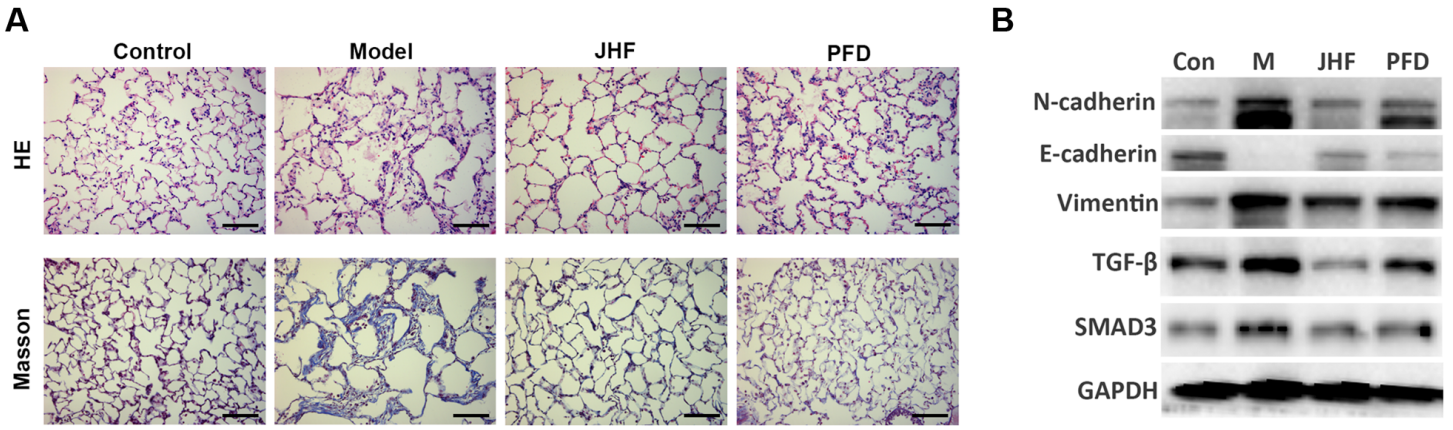

Figure 6

Experimental verification of important targets and effects. (A) Pulmonary fibrosis is ameliorated by JHF (magnification 200×). (B) Effects of JHF on epithelial-mesenchymal transition related proteins and TGF-β signaling pathway proteins.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.xls
- TableS7.xls
- TableS6.xls
- TableS5.xls
- TableS4.xls
- TableS3.xls
- TableS1.xls
- TableS2.xls
- TableS5.xls
- TableS4.xls
- TableS7.xls
- TableS6.xls
- TableS2.xls
- TableS3.xls