European Journal of Integrative Medicine 32 (2019) 101003

Contents lists available at ScienceDirect

European Journal of Integrative Medicine

journal homepage: www.elsevier.com/locate/eujim

Research paper

Pharmacological mechanism of Astragalus and Angelica in the treatment of idiopathic pulmonary fibrosis based on network pharmacology

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ARTICLE INFO

Keywords:
Astragalus
Angelica
Network pharmacology
Protein protein interaction network
Multiple component
Multiple targets

ABSTRACT

Introduction: Herbal medicine is varied and complex, and research on multi-component, multi-target and multi-pathways of Astragalus and Angelica is lacking. This study aimed to study the pharmacological mechanism of Astragalus and Angelica in the treatment of idiopathic pulmonary fibrosis (IPF) using on network pharmacology.
Methods: The main active components, corresponding targets and target genes of Astragalus and Angelica were searched by TCMSP and UniProt database. The target genes of IPF were obtained by GeneCards database and the target genes of active components were intersected with IPF target genes to obtain predictive targets of Astragalus and Angelica acting on IPF. The medicine-compound-target-disease network was constructed by Cytoscape3.6.0 software. The protein protein interaction network was constructed by STRING database to select the key target genes. The DAVID database and KEGG PATHWAY Database were used to analysis the gene ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment.
Results: Sixty-nine overlapping genes were obtained by intersecting 100 compound target genes with 2231 IPF target genes, corresponding to 17 effective compounds, including 15 compounds from Astragalus and two compounds from Angelica. GO enrichment showed the main biological functions of potential genes of Astragalus and Angelica in the treatment of IPF. KEGG pathway enrichment showed the main pathways of Astragalus and Angelica in the treatment of IPF.
Conclusion: In this study, the target and mechanism of the components of Astragalus and Angelica in the treatment of IPF have been systematically discussed, and have provided ideas for future clinical research.

1. Introduction

Idiopathic pulmonary fibrosis (IPF) is a kind of interstitial lung disease with unknown etiology, characterized by chronic and progressive pulmonary fibrosis. Usual interstitial pneumonia (UIP) is its characteristic manifestation [1]. IPF cannot be cured at present. In recent years, herbal medicine plays an obvious role in the treatment of IPF. Astragalus (Astragalus membranaceus, whose Latin name is *Hedysarum Multijugum Maxim.*) and Angelica (Angelica sinensis, whose Latin name is *Angelicae Sinensis Radix*) are two kinds of traditional herbal medicine which are commonly used. DangGuiBuXue Decoction, which is composed of Astragalus and Angelica, has the effect of supplementing qi and activating blood, and has a history of nearly 800 years. At present, based on the association rules of the traditional Chinese medicine literature, the treatment of pulmonary fibrosis with herbal medicine is mainly based on the method of supplementing qi and activating blood circulation, among which Astragalus and Angelica are the most commonly selected herbal medicine, and have achieved certain curative effects [2,3]. Astragalus and Angelica are the main herbal medicines used in the treatment of IPF. Basic studies have also shown

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https://doi.org/10.1016/j.eujim.2019.101003
Received 16 October 2019; Received in revised form 3 November 2019; Accepted 3 November 2019

that Astragalus and Angelica can significantly improve pulmonary fibrosis in mice [4].

Network pharmacology together with the research method of multi-component therapy and employing the characteristics of the multi-component, multi-target and multi-pathway is widely used in pharmacology research in the herbal medicine [5]. The herbal medicine is various and complex, and at present, the pharmacological research of Astragalus and Angelica is mainly based on the single chemical composition, and is limited to the mechanism, the pathway for explaining the action mechanism and the pathway aiming at one target gene, while the research on the multi-component, multi-target and multi-pathway of Astragalus and Angelica is lacking.

In this study, the mechanism of Astragalus and Angelica in the treatment of IPF was analyzed by exploring the multi-level network of "molecule-target-pathway-disease" through the connection and relationship of biological network nodes, and finally the mechanism of Astragalus and Angelica in the treatment of IPF was clarified in order to point out the direction of future experimental and clinical research.

2. Materials and methods

2.1. The active components and targets of Astragalus and Angelica

The chemical constituents of Astragalus and Angelica were obtained by using Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) (http://tcmspw.com/tcmsp.php). According to the oral bioavailability (OB) and drug-likeness (DL), set condition (OB ≥ 30 %, DL ≥ 0.18), the qualified candidate compounds and their corresponding targets were selected [6]. Using UniProt knowledge base (http://www.uniprot.org/) retrieval, search format: (Homo sapiens [organism]), the human gene codes were screened out, and all the searched genes were corrected to their official names.

2.2. IPF related targets acquisition

Using "idiopathic pulmonary fibrosis" as the key word, the related targets of IPF were searched in GeneCards database (https://www.genecards.org/).

2.3. Construction and analysis of "medicine-compound-target-disease" network

The targets of herbal medicine compound were intersected with the targets of IPF, and these targets were the predictive targets of Astragalus and Angelica acting on IPF. The medicine-compound-target-disease network was constructed, and the "Network Analyzer" function of Cytoscape 3.6.0 software was used to analyze the network. Node represented Astragalus and Angelica, compounds, potential targets and disease, while edge represented the relationship between Astragalus and Angelica, compounds, potential targets and disease. According to the connection between the compound and the target, the key compounds of Astragalus and Angelica acting on IPF were screened out.

2.4. Construction of protein protein interaction (PPI) network and selection of key targets

The genes corresponding to the drug-disease intersection target proteins were introduced into STRING database (https://string-db.org/) to construct a PPI network, the research species was defined as "Homo sapiens", the lowest interaction score was set to 0.4, the rest of the parameters were set to the default setting to obtain the PPI network of Astragalus and Angelica on the IPF. By means of the "Network Analyzer" of the Cytoscape 3.6.0 software, the PPI network was subjected to the topology analysis, and the targets whose point of Degree were ahead were the key targets.

Table 1

Basic information on candidate compounds.

MOL ID	Molecule Name	OB(%)	DL
MOL000211	mairin	55.38	0.78
MOL000239	jaranol	50.83	0.29
MOL000296	hederagenin	36.91	0.75
MOL000033	(3*S*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*)-10,13-dimethyl-17-[(2*R*,5*S*)-5-propan-2-yloctan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1*H*-cyclopenta[*a*]phenanthren-3-ol	36.23	0.78
MOL000354	isorhamnetin	49.6	0.31
MOL000371	3,9-di-*O*-methylnissolin	53.74	0.48
MOL000374	5'-hydroxyiso-muronulatol-2',5'-di-*O*-glucoside	41.72	0.69
MOL000378	7-*O*-methylisomucronulatol	74.69	0.3
MOL000379	9,10-dimethoxypterocarpan-3-*O*-β-ᴅ-glucoside	36.74	0.92
MOL000380	(6*aR*,11*aR*)-9,10-dimethoxy-6*a*,11*a*-dihydro-6*H*-benzofurano[3,2-*c*]chromen-3-ol	64.26	0.42
MOL000387	bifendate	31.1	0.67
MOL000392	formononetin	69.67	0.21
MOL000398	isoflavanone	109.99	0.3
MOL000417	calycosin	47.75	0.24
MOL000422	kaempferol	41.88	0.24
MOL000433	FA	68.96	0.71
MOL000438	(3*R*)-3-(2-hydroxy-3,4-dimethoxyphenyl) chroman-7-ol	67.67	0.26
MOL000439	isomucronulatol-7,2'-di-*O*-glucosiole	49.28	0.62
MOL000442	1,7-Dihydroxy-3,9-dimethoxy pterocarpene	39.05	0.48
MOL000098	quercetin	46.43	0.28
MOL000358	beta-sitosterol	36.91	0.75
MOL000449	stigmasterol	43.83	0.76

2.5. Gene ontology (GO) functional enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

The drug-disease intersection genes whose names were modified to the official gene symbols were introduced into DAVID 6.8 database (https://david.ncifcrf.gov/home.jsp) and KEGG PATHWAY Database (https://www.genome.jp/kegg/pathway.html), the species was defined as "Homo sapiens", the threshold was set $P < 0.05$, and GO functional enrichment analysis and KEGG pathway enrichment analysis were carried out.

3. Results

3.1. Screening results of candidate compounds and targets

A total of 87 compounds of Astragalus and 125 compounds of Angelica were obtained in TCMSP. Setting condition OB ≥ 30 % and DL ≥ 0.18, 20 candidate compounds of Astragalus and two candidate compounds of Angelica were selected. The basic information of candidate compounds is shown in Table 1. The corresponding targets of 22 compounds were obtained in TCMSP. In the UniProt knowledge base, the corresponding human gene codes were screened by Homo sapiens search, and 100 target genes were obtained. Among them, the compounds numbered MOL000374, MOL000398, MOL000438, MOL000439 and MOL000442 did not find targets or human gene codes.

3.2. Results of IPF target gene acquisition

2231 IPF related target genes were found from GeneCards database.

3.3. Network construction and analysis results

Sixty-nine overlapping genes were obtained by intersecting 100 compound target genes with 2231 IPF target genes, corresponding to 17 effective compounds, including 15 compounds from Astragalus and two compounds from Angelica. The interaction network of medicine-compound-target-disease was constructed by Cytocsape 3.6.0 software. There were 89 nodes (17 compound nodes, 69 target gene nodes, two

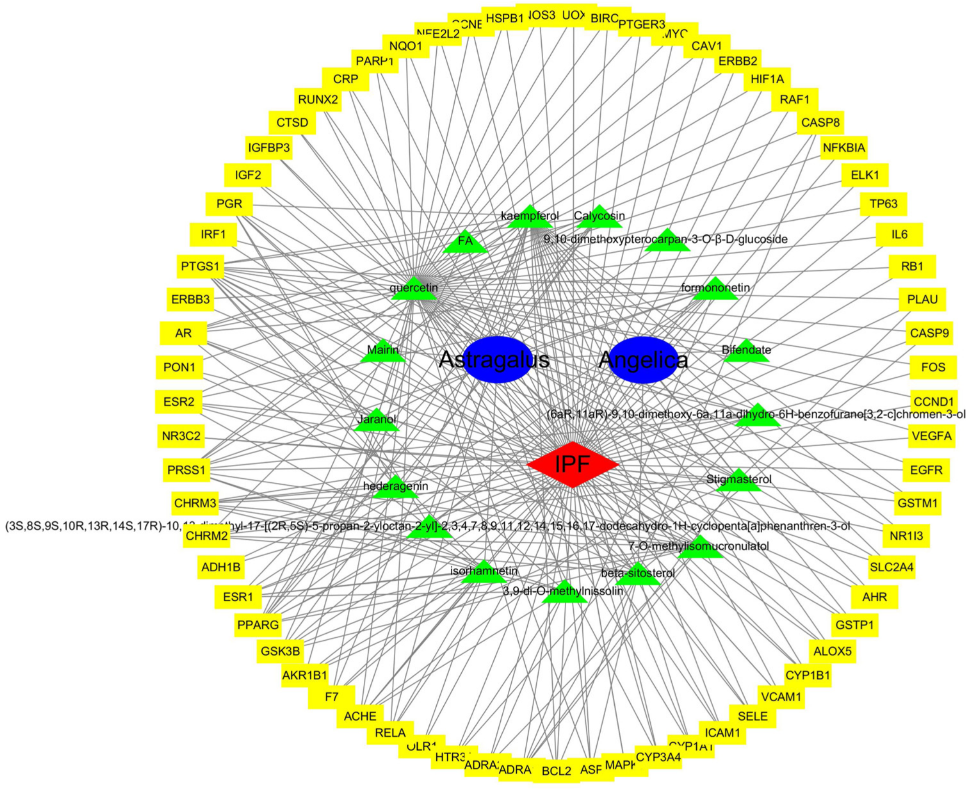

Fig. 1. Medicine-compound-target-disease network.
There were 89 nodes (17 compound nodes, 69 target gene nodes, two herbal medicine nodes and one disease node) and 352 edges in the network.

Table 2
Key compounds of Astragalus and Angelica in the treatment of IPF.

Herbal medicine	Compound	Degree
Astragalus	quercetin	58
Astragalus	kaempferol	26
Astragalus	isorhamnetin	13
Astragalus	7-*O*-methylisomucronulatol	12
Astragalus	formononetin	11
Angelica	β-sitosterol	11
Angelica	stigmasterol	9
Astragalus	3,9-di-*O*-methylnissolin	8
Astragalus	calycosin	8

herbal medicine nodes and one disease node) and 352 edges in the network (Fig. 1). The compounds with the highest value in the figure are quercetin, kaempferol, isorhamnetin, 7-*O*-methylisomucronulatol, formononetin, β-sitosterol, stigmasterol, 3, 9-di-*O*-methylnissolin and calycosin as shown in Table 2. These compounds with high value may be the key compounds in the treatment of IPF with Astragalus and Angelica.

3.4. Construction of PPI network and results of key target screening

In order to further study the mechanism of Astragalus and Angelica in the treatment of IPF, 69 overlapping target genes were analyzed by PPI network through STRING database and the PPI network was obtained. When the lowest interaction score was set to 0.4, 68 targets in the network can have protein interaction (one target does not have protein interaction), 605 edges represent the interaction between proteins (Fig. 2). The targets with high degree in the figure were Interleukin (IL) 6, Vascular endothelial growth factor (VEGF) A, Mitogen-activated protein kinase (MAPK) 8, Caspase (CASP) 3, Proto-oncogene myc (MYC), Estrogen receptor 1 (ESR1), Cyclin D1 (CCND1), c-Fos proto-Oncogenes (FOS) and Androgen receptor (AR) (Table 3, Fig. 3). It is speculated that these targets may be the key targets for Astragalus and Angelica in the treatment of IPF.

3.5. GO functional enrichment

The 69 intersection target genes affected 87 biological processes (p value < 0.05, q value < 0.05), and the function information of the top 20 of the p-value ranking was selected. The intersection genes of Astragalus and Angelica in the treatment of the IPF are mainly enriched in DNA-binding transcription activator activity, RNA polymerase II-specific, nuclear receptor activity, transcription factor activity, direct ligand regulated sequence-specific DNA binding, steroid hormone receptor activity, protein heterodimerization activity, proximal promoter sequence-specific DNA binding, steroid binding, RNA polymerase II proximal promoter sequence-specific DNA binding, ubiquitin-like protein ligase binding, RNA polymerase II transcription factor binding, ubiquitin protein ligase binding, G protein-coupled amine receptor activity, heme binding, activating transcription factor binding, tetrapyrrole binding, neurotransmitter binding, transcription cofactor binding, kinase regulator activity, ammonium ion binding and E-box binding (Table 4, Fig. 4).

3.6. KEGG pathway enrichment

KEGG pathway enrichment analysis showed that 69 intersecting target genes were significantly enriched in 44 pathways (p value < 0.05, q value < 0.05). Among them, the most significant 20 pathways include Kaposi sarcoma-associated herpesvirus infection, Hepatitis B, Human cytomegalovirus infection, AGE-RAGE signaling pathway in diabetic complications, Proteoglycans in cancer, Apoptosis, TNF signaling pathway, Prolactin signaling pathway, Endocrine resistance, Measles, Hepatitis C, Epstein-Barr virus infection, Platinum drug resistance, PI3K-Akt signaling pathway, Apoptosis-multiple species, EGFR

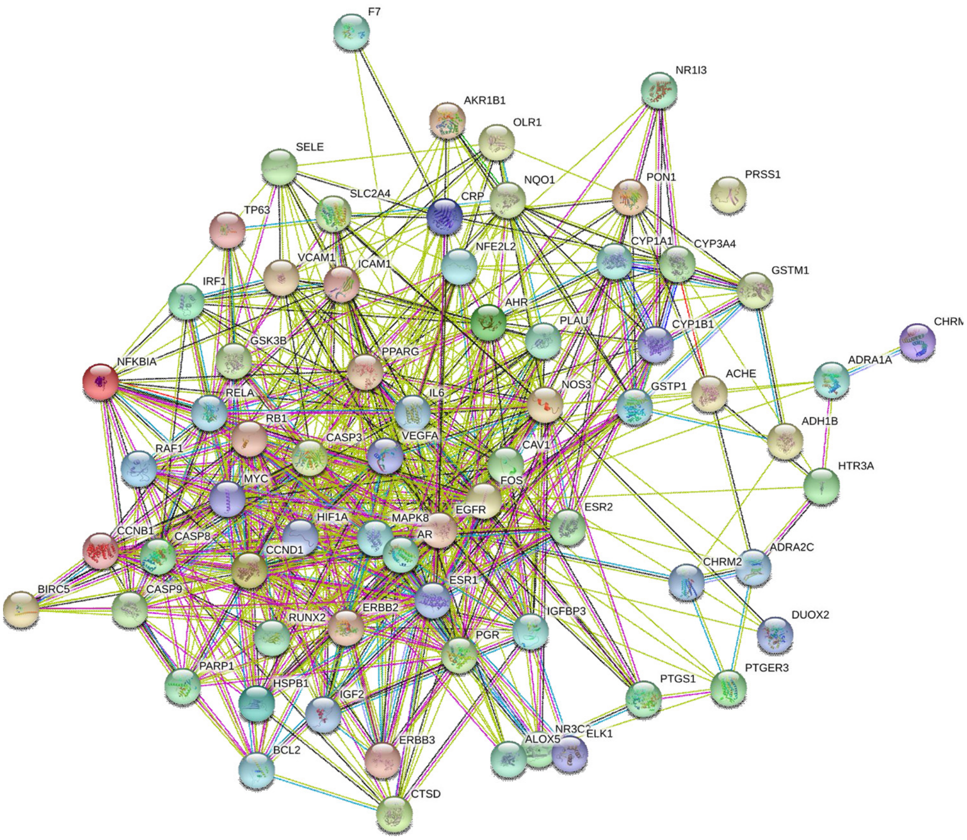

Fig. 2. PPI network of Astragalus and Angelica in the treatment of IPF.
When the lowest interaction score was set to 0.4, 68 targets in the network can have protein interaction (one target does not have protein interaction), 605 edges represent the interaction between proteins.

Table 3
Key genes of Astragalus and Angelica in the treatment of IPF.

Gene	Full name	Degree
IL6	Interleukin 6	48
VEGFA	Vascular endothelial growth factor A	45
MAPK8	Mitogen-activated protein kinase 8	44
CASP3	Caspase 3	42
EGFR	Epithelial growth factor receptor	42
MYC	proto-oncogene myc	40
ESR1	Estrogen receptor α	39
CCND1	Cyclin D1	37
FOS	proto-oncogene c-Fos	35
AR	Androgen receptor	32

tyrosine kinase inhibitor resistance, ErbB signaling pathway, MAPK signaling pathway, IL-17 signaling pathway and Estrogen signaling pathway, suggesting that Astragalus and Angelica plays an important role in the treatment of IPF by acting on the above multiple pathways (Table 5, Fig. 5). The main pathways were shown in Figs. 6 and 7.

4. Discussion

IPF is a type of interstitial lung disease characterized by its chronic, progressive and fibrotic nature [1]. It is a rare disease, which can occur in the elderly. In European and North America, the incidence of IPF ranges between 2.8–9.3 in 100,000 people. There is little epidemiological data in China, but there has been a significant increase in the number of IPF cases in recent years [7]. The chest high resolution CT or lung histology of IPF is characterized by UIP. The main clinical characteristics are progressive dyspnea, restrictive ventilation dysfunction and gas exchange disorder, hypoxia and even respiratory failure [8]. IPF cannot be cured at present. The purpose of treatment is to delay the progress of the disease, improve the quality of life and prolong survival. As antifibrotic drugs, pirfenidone and nintedanib have been used in the treatment of IPF, and have achieved certain curative effect, but the price is expensive and has certain side effects, which is often difficult for patients to tolerate [9]. In recent years, herbal medicine has played an active role in the treatment of IPF. Many studies have shown that herbal medicine can improve the clinical symptoms, quality of life, exercise tolerance and delay the decrease of pulmonary function in patients with IPF [10,11].

As a means of traditional Chinese medicine (TCM) in the treatment of diseases, herbal medicine is used in view of the complex pathogenesis of IPF and the common pathogenesis syndrome of patients. According to the diagnostic criteria of TCM syndromes of diffuse interstitial lung disease (2012 Edition), pulmonary fibrosis is divided into lung qi deficiency syndrome, yin deficiency and internal heat syndrome, lung and kidney qi deficiency syndrome, lung and kidney qi deficiency syndrome, phlegm heat obstruction of lung syndrome, phlegm turbid obstruction of lung syndrome, blood stasis syndrome and so on [12]. The main principle of clinical medication is to invigorate qi and promote blood circulation, invigorate qi and nourish yin, and promote blood circulation and remove blood stasis [13]. At present, the study of herbal medicine treatment of pulmonary fibrosis based on the rules of literature association shows that herbal medicine treatment of pulmonary fibrosis is mainly drugs of invigorating qi and activating blood, among which Astragalus and Angelica are the most selected and the most important drugs [2,3].

Astragalus and Angelica are used as the main herbal medicine for treating IPF. Astragalus and Angelica are used for treating the IPF mouse model, and inhibiting VEGF can obviously relieve the inflammation of the lung tissue of the mouse and Astragalus and Angelica

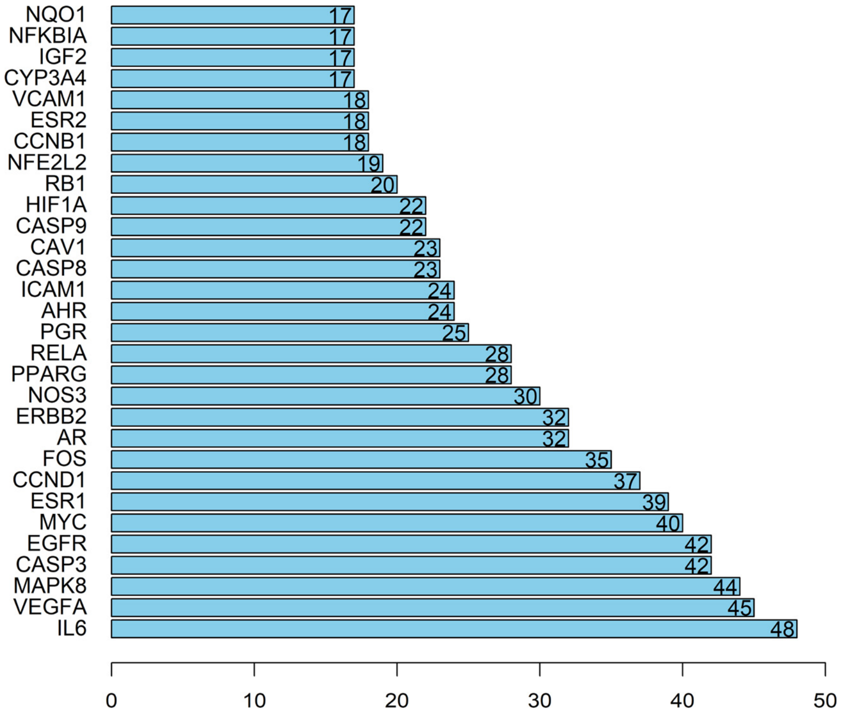

Fig. 3. Key genes in PPI network.
The targets with high degree in the figure were Interleukin (IL) 6, Vascular endothelial growth factor A (VEGFA), Mitogen-activated protein kinase (MAPK) 8, Caspase (CASP) 3, Proto-oncogene myc (MYC), Estrogen receptor 1 (ESR1), Cyclin D1 (CCND1), c-Fos proto-Oncogenes (FOS) and Androgen receptor (AR).

Table 4
Functional information of drug-disease intersection genes.

ID	Description	*p* value	Count
GO:0001228	DNA-binding transcription activator activity, RNA polymerase II-specific	4.46E-10	14
GO:0004879	nuclear receptor activity	6.35E-10	7
GO:0098531	transcription factor activity, direct ligand regulated sequence-specific DNA binding	6.35E-10	7
GO:0003707	steroid hormone receptor activity	2.05E-09	7
GO:0046982	protein heterodimerization activity	1.01E-08	14
GO:0000987	proximal promoter sequence-specific DNA binding	2.85E-08	13
GO:0005496	steroid binding	4.37E-08	7
GO:0000978	RNA polymerase II proximal promoter sequence-specific DNA binding	1.51E-07	12
GO:0044389	ubiquitin-like protein ligase binding	2.24E-07	10
GO:0001085	RNA polymerase II transcription factor binding	3.70E-07	7
GO:0031625	ubiquitin protein ligase binding	1.41E-06	9
GO:0008227	G protein-coupled amine receptor activity	4.09E-06	4
GO:0020037	heme binding	4.49E-06	6
GO:0033613	activating transcription factor binding	6.07E-06	5
GO:0046906	tetrapyrrole binding	7.56E-06	6
GO:0042165	neurotransmitter binding	1.19E-05	4
GO:0001221	transcription cofactor binding	1.66E-05	4
GO:0019207	kinase regulator activity	3.79E-05	6
GO:0070405	ammonium ion binding	4.26E-05	4
GO:0070888	E-box binding	4.62E-05	4

can inhibit the expression level of transforming growth factor (TGF)-βand VEGF, and improve pulmonary fibrosis in mice [4,14].

The composition of herbal medicine is diverse and complex. At present, there are still some problems in the study of herbal medicine, such as complex composition and unclear mechanism of action. Most of the existing studies are limited to the mechanism and pathway of explaining herbal medicine for a certain target gene, and lack of macro-whole research on multi-component, multi-target and multi-pathway of herbal medicine [5]. With the development of network pharmacology, network pharmacology advocates the research method of network goal and multi-component therapy, which is consistent with the characteristics of multi-component, multi-target and multi-pathway of herbal medicine, and is now widely used in the study of pharmacology of herbal medicine. Through the connection and relationship of biological network nodes, this paper analyzes the multi-level network of "molecule-target-pathway-disease", clarifies the mechanism of action of herbal medicine, realizes the comprehensive network analysis of molecular action, and finally understands the interaction between herbal medicine and the disease from the overall point of view [15,16].

This research uses the TCM holistic view and the syndrome differentiation science system and the network pharmacology analysis method union, with the corresponding database and the software, takes the network target, the multicomponent therapy as the research method, constructs the network and has carried on the pathway enrichment to the target, systematically, the whole has discussed the action mechanism of Astragalus and Angelica in the treatment IPF.

The study shows that quercetin, kaempferol, isorhamnetin, 7-*O*-methylisomucronulatol, formononetin, beta-sitosterol, stigmasterol, 3,9-di-*O*-methylnissolin and calycosin are the key compounds in the treatment of IPF. There have been research reported that quercetin can effectively inhibit the pulmonary fibrosis process of the rat and promote the self-healing of the lung tissue, can inhibit the oxidative stress to relieve the pulmonary fibrosis, reduce the collagen deposition, promote the degradation of the extracellular matrix, improve the pulmonary fibrosis, induce the apoptosis of the pulmonary fibrosis cells and inhibit the treatment of pulmonary fibrosis by inhibiting the sphingosine kinase (SphK)-1/sphingosine-1-phosphate (S1P) [17–20]. The kaempferol can inhibit the epithelial-mesenchymal transition (EMT) and fibrosis of the mouse airway induced by endotoxin [21]. Isorhamnetin can protect bleomycin-induced mouse pulmonary fibrosis by inhibiting endoplasmic reticulum stress and EMT [22]. Formononetin can improve the pulmonary fibrosis by inhibiting the signal pathway of myocyte enhancer factor-2c (MEF2c) [23]. Beta-sitosterol can inhibit the treatment of pulmonary fibrosis by inhibiting the TGF-β1 pathway and inhibiting the EMT [24]. The results of this study are in accordance with the previous study, which indicates that the treatment of IPF with Astragalus and Angelica are the effect of multiple compounds.

GO functional enrichment analysis suggests that Astragalus and Angelica can act as multiple targets in the treatment of IPF. Key target genes, such as IL6, VEGFA, MAPK8, CASP3, EGFR, MYC, ESR1, CCND1,

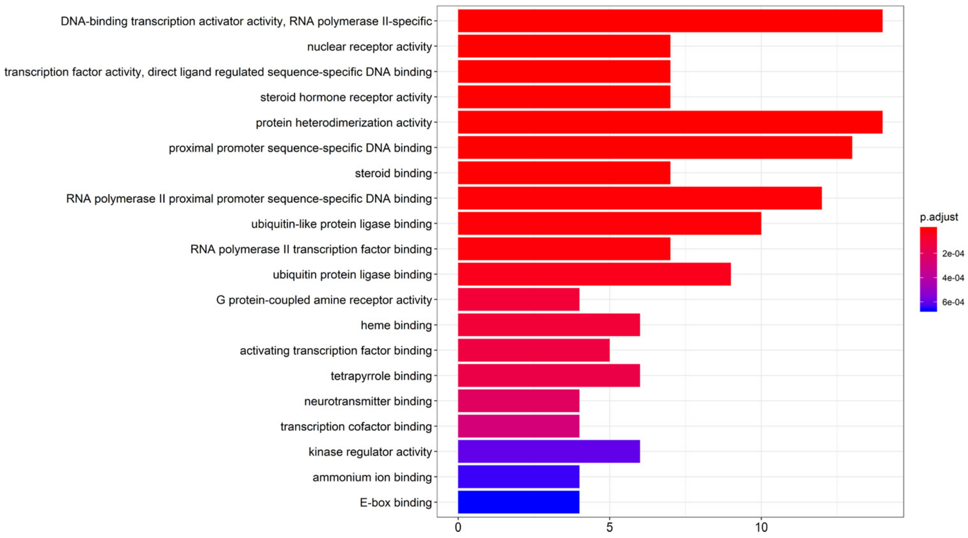

Fig. 4. Histogram of GO functional enrichment analysis.
The intersection genes of Astragalus and Angelica in the treatment of the IPF are mainly enriched in DNA-binding transcription activator activity, RNA polymerase II-specific, nuclear receptor activity, transcription factor activity, direct ligand regulated sequence-specific DNA binding, steroid hormone receptor activity, protein heterodimerization activity, proximal promoter sequence-specific DNA binding, steroid binding, RNA polymerase II proximal promoter sequence-specific DNA binding, ubiquitin-like protein ligase binding, RNA polymerase II transcription factor binding, ubiquitin protein ligase binding, G protein-coupled amine receptor activity, heme binding, activating transcription factor binding, tetrapyrrole binding, neurotransmitter binding, transcription cofactor binding, kinase regulator activity, ammonium ion binding and E-box binding.

Table 5
Pathway information of drug-disease intersection genes.

ID	Description	*p* value	Count
hsa05167	Kaposi sarcoma-associated herpesvirus infection	1.23E-12	16
hsa05161	Hepatitis B	3.58E-11	14
hsa05163	Human cytomegalovirus infection	2.67E-10	15
hsa04933	AGE-RAGE signaling pathway in diabetic complications	4.18E-10	11
hsa05205	Proteoglycans in cancer	7.86E-10	14
hsa04210	Apoptosis	8.32E-10	12
hsa04668	TNF signaling pathway	1.43E-09	11
hsa04917	Prolactin signaling pathway	4.54E-09	9
hsa01522	Endocrine resistance	5.89E-09	10
hsa05162	Measles	1.33E-08	11
hsa05160	Hepatitis C	4.49E-08	11
hsa05169	Epstein-Barr virus infection	7.05E-08	12
hsa01524	Platinum drug resistance	1.25E-07	8
hsa04151	PI3K-Akt signaling pathway	1.29E-07	15
hsa04215	Apoptosis-multiple species	1.96E-07	6
hsa01521	EGFR tyrosine kinase inhibitor resistance	2.34E-07	8
hsa04012	ErbB signaling pathway	4.16E-07	8
hsa04010	MAPK signaling pathway	6.53E-07	13
hsa04657	IL-17 signaling pathway	8.35E-07	8
hsa04915	Estrogen signaling pathway	1.72E-06	9

FOS and AR, play an important role in inflammatory response, aging, cell proliferation, cell migration and apoptosis.

KEGG enrichment pathway analysis showed that many pathways were closely related to the pathogenesis of IPF. The main pathways included Kaposi Sarcoma-associated herpesvirus infection (Fig. 6), hepatitis B, human cytomegalovirus infection (Fig. 7), AGE-RAGE signaling pathway, proteoglycan, TNF signaling pathway, prolactin signaling pathway, endocrine resistance, measles, hepatitis C, Epstein-Barr virus infection and so on. The relationship between many pathways and IPF has been found or confirmed by basic clinical studies, such as human Epstein-Barr virus (EBV) and Kaposi's sarcomas-associated herpesvirus (KSHV)-related murine gamma-herpesvirus (MHV) infection can cause IPF [25], and human herpesvirus antibody can be detected in patients with interstitial lung disease [26]. Liver cirrhosis in patients with hepatitis B and C is closely related to the development and treatment of IPF. Hepatitis C may play a role in the development or aggravation of idiopathic pulmonary fibrosis [27–29]. Potential mouse cytomegalovirus (MCMV) infection can aggravate pulmonary fibrosis by activating TGF- β 1. The activation of Nod-like receptor protein (NLRP) 1 and NLRP3 inflammatory pathways is related to pulmonary fibrosis induced by potential MCMV infection [30,31]. Advanced glycation end products(AGE) is the result of non-enzymatic reaction of lipid and protein with a variety of oxidants during aging. Receptor for AGE (RAGE) is related to pulmonary fibrosis and alveolar homeostasis. The abnormal repair ability of IPF epithelial stroma is related to the aging process. The increase of the proportion of AGE-RAGE in IPF may be related to the repair of epithelial stroma in the process of pulmonary fibrosis [32,33]. Proteoglycan can control the stable level of TGF- β 1 by binding its core protein to TGF- β 1. Proteoglycan has a significant therapeutic effect on pulmonary fibrosis in rats [34]. Mitochondrial mediated apoptosis plays an important role in the occurrence and development of pulmonary fibrosis diseases. Apoptosis of alveolar macrophages in pulmonary fibrosis model mice is obvious. MicroRNA-29c can prevent pulmonary fibrosis by regulating the regeneration and apoptosis of epithelial cells [35–38]. Inhibition of nuclear factor-kappa B (NF- κ B) signaling pathway can provide protective effect on pulmonary fibrosis. By up-regulating the expression of Bcl-2 and decreasing the contents of fibrosis cytokines TGF- β 1 and TNF- α, the oxidative stress injury of pulmonary fibrosis can be regulated [39,40]. Abnormal prolactin was occasionally found in patients with IPF [41], and the expression of prolactin-induced protein(PIP) in pulmonary fibrosis complicated with pulmonary hypertension was increased. PIP expression was also up-regulated in rat fibrosis model [42].

The relationship between viral infection and IPF has been concerned all the time. This study shows that the main pathways are closely related to the virus, including Kaposi Sarcoma-associated herpesvirus infection, hepatitis B, human cytomegalovirus infection, measles, hepatitis C, EBV infection. It is found that in patients with respiratory tract virus infection, pulmonary fibrosis often occurs after the control of viral infection. The essence of lung injury caused by virus infection may be the false injury of the body to autoimmune response, and a variety of immune cells are activated, and then secrete a large number of cytokines, proteolytic enzymes and so on, which can directly damage the lung tissue, promote the release of other cytokines from macrophages and cause pulmonary fibrosis [43,44]. It has been confirmed that herpesvirus infection is significantly related to IPF, and herpesvirus infection is related to fibrosis after chronic allogenic solid organ transplantation [45]. In addition to respiratory infections, the study also found that chronic hepatitis C virus (HCV) is also associated with lung fiber. Chronic HCV infection has been found to be associated with IPF, which may be one of the causes of IPF [46]. Compared with HCV-Ab negative population, HCV-Ab positive patients may have subclinical alveolitis and / or interstitial lung disease, the pathological process of which is that HCV initiates lymphoid alveolitis, then causes organic pneumonia, and finally develops into pulmonary fibrosis [47].

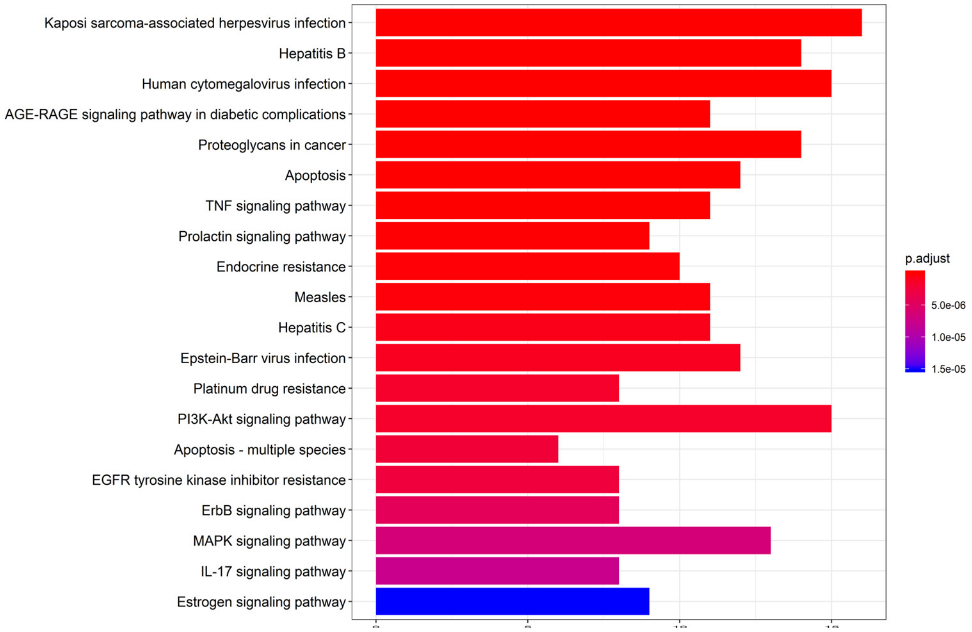

Fig. 5. Histogram of KEGG pathway enrichment analysis.
The most significant 20 pathways include Kaposi sarcoma-associated herpesvirus infection, Hepatitis B, Human cytomegalovirus infection, AGE-RAGE signaling pathway in diabetic complications, Proteoglycans in cancer, Apoptosis, TNF signaling pathway, Prolactin signaling pathway, Endocrine resistance, Measles, Hepatitis C, Epstein-Barr virus infection, Platinum drug resistance, PI3K-Akt signaling pathway, Apoptosis-multiple species, EGFR tyrosine kinase inhibitor resistance, ErbB signaling pathway, MAPK signaling pathway, IL-17 signaling pathway and Estrogen signaling pathway.

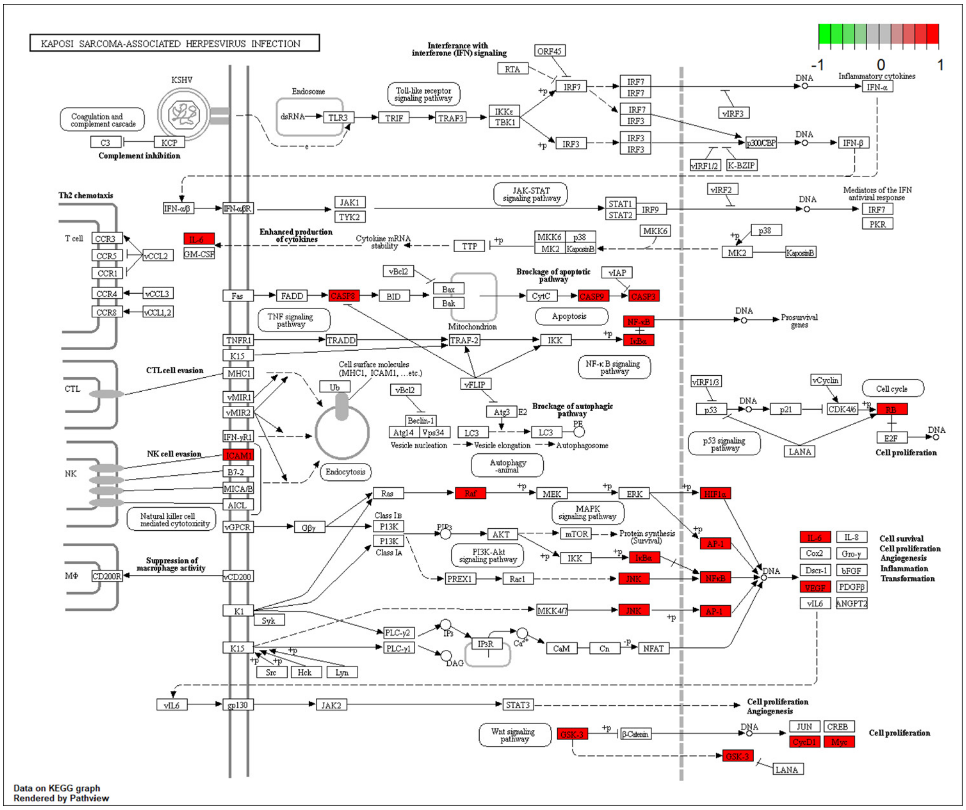

Fig. 6. Kaposi sarcoma-associated herpesvirus infection pathway.

Therefore, it is not respiratory tract virus infection that is the cause of IPF, and there may be latent virus infection in vivo, which may lead to related pulmonary fibrosis.

The pathogenesis between virus infection and IPF is not completely clear. Exposure to various risk factors, latent infection of herpesvirus can aggravate pulmonary fibrosis. It has been confirmed that the injury and necrosis of alveolar epithelial cells may play an important role in the pathogenesis of IPF [48]. In mouse model, pulmonary fibrosis can be induced by using diphtheria toxin to injure alveolar epithelial cells. When combined with certain genetic factors or environmental exposure, herpesvirus infection may lead to serious and persistent injury. EBV latent infection does not significantly change the proliferation or survival ability of alveolar epithelial cells in vitro, but it can aggravate the degree of pulmonary fibrosis under a specific inducing factor [49]. Herpesvirus can cause pulmonary fibrosis by affecting lung inflammation. The latent infection of herpesvirus is related to the increase of pro-inflammatory cytokines in human or animal models [50]. Compared with wild type mice infected with mouse herpesvirus MHV-68, mice with interferon gamma deficiency infected with mouse herpesvirus MHV-68 were more likely to develop pulmonary fibrosis,

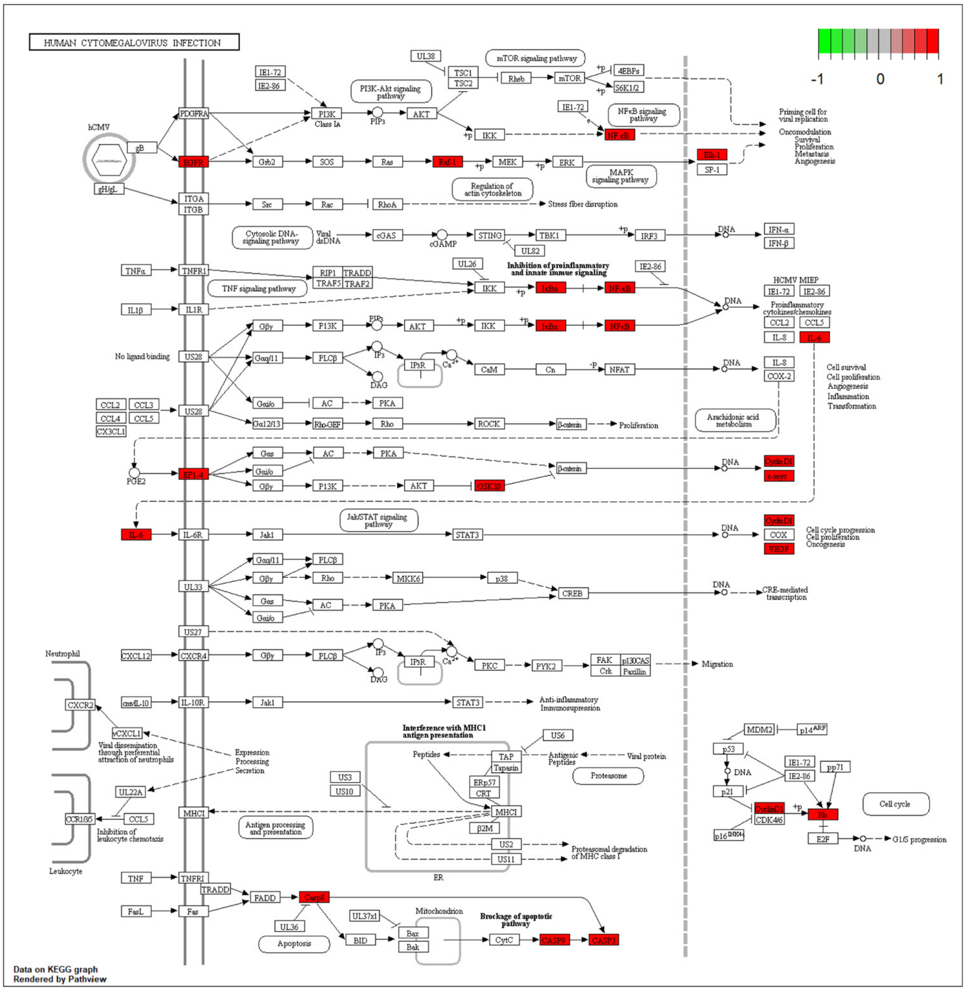

Fig. 7. Human cytomegalovirus infection pathway.

suggesting that pulmonary fibrosis is more likely to occur in immunocompromised mice [51,52]. In this model, herpesvirus infection increased the number of activated macrophages and participated in tissue remodeling, resulting in changes in the stroma of some columns [53]. Many studies have confirmed that CMV infection can induce fibroblasts, epithelial cells, glioma cells and osteosarcoma cells to secrete TGF- β 1. TGF- β 1 induced by CMV infection can be activated by other cells or cytokines in the infected area [54,55].

There are some shortcomings in this study, such as network pharmacology is based on data, whether the data collection is comprehensive or not and the establishment of screening criteria for active components cannot be completely accurate. In addition, the therapeutic effect of other component, effects of dosage, dosage form on the treatment results were not considered in this study.

5. Conclusion

To sum up, based on the methods and techniques of network pharmacology, this study expounded the relationship among the active components, targets and pathways of Astragalus and Angelica in the treatment of IPF, and found the characteristics of Astragalus and Angelica which were multi-component, multi-target and multi-pathway. The results of pathway enrichment suggest that the mechanism of Astragalus and Angelica in the treatment of IPF may be by inhibiting virus, inflammatory factor secretion, participating in anti-inflammatory reaction and inhibiting oxidative stress. Regulation of regeneration and apoptosis pathway slow down epithelial cell transformation, aging, apoptosis, promote epithelial cell regeneration, promote lung tissue self-repair and reduce pulmonary fibrosis. The mechanism of Astragalus and Angelica in the treatment of IPF was analyzed by the connection and relationship of biological network nodes, and the comprehensive network analysis of molecular action was realized. Finally, the mechanism of Astragalus and Angelica in the treatment of IPF was clarified from the overall point of view, which provided a new direction for the mechanism of action of TCM and provided more ideas and theoretical basis for further experimental and clinical research.

Funding

This study was partially supported by the National Natural Science Foundation of China (Grant 81200049 and Grant 81670059 to Dr Cao and Grant 81302768 to Dr Qi) and the Nanjing Medical Science and Technique Development Foundation (Grant ORX17005 to Dr Cao).

CRediT authorship contribution statement

Yufeng Zhang: Data curation, Software, Supervision, Writing - original draft, Writing - review & editing. **Weilong Jiang:** Project administration, Writing - original draft. **Qingqing Xia:** Supervision, Writing - original draft. **Jia Qi:** Formal analysis, Funding acquisition, Writing - original draft, Writing - review & editing. **Mengshu Cao:** Data curation, Funding acquisition, Methodology, Software, Project administration.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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