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Network Pharmacology-Based Analysis of the Mechanism of Action of Yiqi Resolving Stasis and Detoxification Formula in COPD

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Abstract: *To investigate the molecular biological mechanism of Yiqi Huayu Xieju Fang in chronic obstructive pulmonary disease (COPD) by using network pharmacology and bioinformatics. The TCM systematic pharmacology platform (TCMSP) was used to screen the active ingredients and targets of TCM in Yi-qi Huayu Huayu Xiehu Fang, the UniProt database was used to query the genes of the target proteins corresponding to the active ingredients, and the GeneCards database was used to screen the targets of hepatocellular carcinoma (HCC); and the online Venny was used to obtain the common targets of Yi-qi Huayu Huayu Xiehu Fang and COPD. The obtained results were imported into Cytoscape software for visualization and analysis, and the PPI network was constructed to screen the core targets. Metascape database was used to analyze the GO enrichment and KEGG-related pathway enrichment of the core targets; Autodock Tools and Pymol software were used to validate the molecular docking of the core target proteins with the core active ingredients. A total of 181 active ingredients, 478 potential targets, and 15,256 COPD targets were obtained from Yiqi Huayu Detoxification Formula, and 400 common targets were obtained after intersection. The analysis of the targets and active ingredients of Yiqi Huayu Xiehu Xiehu Fang for COPD prevention and treatment showed that the key active ingredients, mainly quercetin, luteolin, kaempferol, skullcapflavone II, isorhamnetin, etc., as well as MAPK3, AMPK3, AMPK3, and AMPK3 were obtained. The GO function enrichment analysis yielded 1154 biological processes (BP), 137 cellular components (CC), 135 molecular functions (MF), and 166 KEGG signaling pathways, indicating that they were mainly involved in AGE-RAGE, cAMP, and NF-κB signaling pathways, NF-κB signaling pathways, which are closely related to inflammation, oxidative stress, and metabolism; the molecular docking results all showed that the binding required lower energy, higher affinity, and could form stable conformations. Through network pharmacology and molecular docking technology, it was found that Yiqi Huayu Detoxification Formula was anti-COPD through multi-targets and multiple pathways, indicating that the formula has the characteristics of multi-components, multi-targets, and multi-pathways of efficacy, which will provide new ideas and bases for the future related research.*

Keywords: *chronic obstructive pulmonary disease; qi-benefiting and stasis-eliminating formula; network pharmacology; bioinformatic technology*

1. Introduction

Chronic Obstructive Pulmonary Disease (COPD) is a major respiratory disease that seriously jeopardizes people's lives and health, with an extremely high morbidity and mortality rate, causing a heavy economic burden on patients, families, and the community; it is one of the world's diseases with the highest disability and mortality rates, and it has become the third leading cause of death worldwide [1]. With the increase in the number of smokers, the aggravation of air pollution and the aging of the population, the incidence of COPD will continue to rise [2-3].COPD is a pulmonary emphysema and chronic bronchial inflammatory disease characterized by airflow obstruction, with typical symptoms such as coughing up sputum, coughing up wheeze, and shortness of breath, etc., and with the characteristic of recurrent episodes and difficult to cure, it seriously affects the life of patients. Modern medicine has made it clear that this disease is mainly caused by the inflammatory reaction induced by harmful gases and particles in daily life, and it is mainly treated conservatively with medications, including bronchodilators, inhaled glucocorticoids, cough suppressants, expectorants, and antioxidants, etc., which can achieve a certain effect, but the recurrence rate is relatively high [4]. Traditional Chinese

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medicine is characterized by multi-components and multi-targets, and in recent years, the search for effective therapeutic agents with good efficacy and low adverse effects from traditional Chinese medicine has been favored by more and more researchers. Prof. Du Huaitang was the former head of the Northern Heat Disease Group and assisted Academician Dong Jianhua in formulating the "Three Stages and Twenty-One Symptoms" of exogenous heat disease, and has rich experience in the identification and treatment of acute and chronic pulmonary diseases. Prof. Du Huaitang's experienced formula for COPD, which consists of Astragalus, Scutellaria baicalensis, Honeysuckle, Panax ginseng, Radix Paeoniae Alba, Citrus aurantium, Drabanemerosa hebecarpa, and Sangbaek Pi, is a combination of additives and subtractions, which is clinically effective in relieving respiratory symptoms in COPD patients. In order to better investigate the molecular mechanism and mechanism of action of Yiqi Huayu Xietu Formula for COPD, this study was conducted to refine the study of "Yiqi Huayu Xietu Formula for Chronic Obstructive Lung Disease" through bioinformatics techniques, such as network pharmacology, molecular docking, and the GEO database, which can be used to provide a reference for the research and development of new drugs for the treatment of COPD, and can also assist in the interpretation of traditional Chinese medicine formulas for the treatment of diseases. It can also help to elucidate the synergistic mechanism of traditional Chinese medicine formulas in treating diseases.

2. Materials and Methods

2.1 Query for active ingredients and their related targets of the traditional Chinese medicine of Yiqi Resolving Blood Stasis and Detoxification Formula

The Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) was used to analyze the active ingredients of Astragalus, Honeysuckle, Scutellaria baicalensis, Sang Bai Pi, Draba hebecarpa, Citrus aurantium, Panax ginseng, and Radix panax quinquefolii in the formula of Beneficent Qi, Resolving Blood Stasis, and Removing Toxins. And Paeonia lactiflora. Oral bioavailability (OB) ≥30% and drug likeness (DL) ≥0.18 were used to predict the target genes corresponding to the active ingredients. SMILES expression of drugs was converted using PubChem software (http://pubchem.ncbi.nlm.nih.gov/). Drug target screening was performed using SwissTar-getPrediction (http://www.swisstargetprediction.ch/) and STITCH (http://stitch.embl.de/) with humans defined as species.

2.2 COPD-related targets

GeneCards (http://www.genecards.org), PharmGKB (https://www. pharmgkb. org/), and OMIM (https://www. omim.org/) were searched for COPD targets using the search term "Chronic Obstructive Pulmonary Disease". PharmGKB (http://www. pharmgkb. org/), and OMIM (http://www. omim.org/) for COPD targets.

2.3 Prediction of active ingredient-common targets in chronic lung disease

The database-predicted targets of action of Yiqi Huayu Xietong and the disease targets related to COPD were uploaded to the Jvenn (http://jvenn.toulouse.inra.fr/) platform to obtain the intersecting targets of Yiqi Huayu Xietong and COPD, and the intersecting targets were plotted as Wayne diagrams.

2.4 Protein-protein interaction (PPI) network construction and key target screening

The common targets of Yiqi Resolving Blood Stasis and Detoxification Formula for Chronic Obstructive Pulmonary Diseases (COPD) were imported into the STRING database (https://string-db.org/), and a PPI network was created. Species were labeled with the species designation "chronic obstructive pulmonary diseases", the minimum required interaction score was selected as 0.9, and the Cluster was set to 3 in the Clusters module to obtain a PPI network. In the Clusters module, the Cluster was set to 3, and the PPI network was obtained. The information was input into Cytoscape 3.7.2 software, and the PPI network topology was analyzed by using the function of "Analyze Network", and the target proteins were screened according to the degree value of each node, and the key targets were obtained. The top 10% of target proteins are the core targets according to the degree value of the targets.

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2.5 Key target enrichment analysis and pathway analysis

The key targets were entered into Metascape (https://metascape.org/gp/index.html#/main/step1) for online analysis, Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Genes and Genomes (KEGG) pathway enrichment analysis, the species was "H. Sapiens", the analysis mode was "Custom", the setting was "P≤0.1", and the other values were default. "And other default values were used as the significance screening criteria to obtain the potential pathways of Yiqi Resolving Blood Stasis and Detoxification Formula for the treatment of chronic obstructive pulmonary disease (COPD).

2.6 Molecular docking

Core targets were selected for molecular docking validation. The PDB format of the above core targets was downloaded from the PDB database (https://www.rcsb.org/), and the principles of selection were as follows: human proteins, high resolution (≤2.5 A), having original ligands, and crystallization PH should be as close as possible to the normal physiological range of the human body. At the same time, the chemical structures of the core compounds were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), and then the 3D structural formula of the small molecules was drawn using chendraw, and finally the protein receptors and small molecule ligands were processed into docking using AutoDock vina, and the results were visualized using PyMOL for the model. Visualization.

3. Results

3.1 Enquiry: The active ingredients of in the formula for promoting qi and resolving blood stasis and detoxification and their potential targets action

Searching in TCMSP, the 8-flavored drug ingredients contained in Yiqi Resolving Stasis and Detoxification Formula were able to obtain 181 active ingredients, 19, 26, 59, 25, 11, 26, 20 and 15 existed in Astragalus, Honeysuckle, Scutellaria baicalensis, Sang Bai Pi, Scabrachia hebecarpa, Citrus medica, Panax quinquefolii and Paeonia lactiflora, respectively, and there were a total of 176 active ingredients queried for their corresponding target proteins after de-emphasizing them, and using the Uniprot Using Uniprot database, the names of the genes corresponding to the target proteins were queried, and the results of verified and species origin as human were selected, corresponding to a total of 1,774 targets, and after de-emphasis, 478 targets of traditional Chinese medicine were obtained.

3.2 Access to COPD targets

GeneCards, TTD, DrugBank, OMIM, DisGeNET, TCMIP disease target databases were searched with the keyword "chronic obstructive pulmonary disease" and 4050 disease targets related to COPD were obtained. COPD-related disease targets were obtained. By analyzing the GSE130928 chip, 51 differentially expressed genes were obtained, including 18 up-regulated genes and 33 down-regulated genes; 13062 differentially expressed genes were obtained from the GSE5058 chip, 12995 up-regulated genes and 67 down-regulated genes. After combining and de-emphasizing the 13113 differentially expressed genes obtained from GEO with the disease genes obtained from the above six databases in Excel software, a total of 15256 COPD disease targets were obtained.

3.3 Construction of "Drug-Active Ingredient-Common Target-Disease" Network Diagrams

The active ingredient targets in Yiqi Resolving Stasis and Detoxification Formula were obtained by TCMSP, and 253 targets remained after deleting the duplicates. The database was searched with the keyword "chronic obstructive pulmonary diseases", and the relevant targets of COPD were obtained, and 19526 targets remained after deleting the duplicates. After deleting the duplicates, 19526 targets remained. After that, all the drug and disease targets were annotated according to their target information, and their GeneSymbol was obtained. After uploading the results to the VENN online website, about 400 intersected targets were obtained, and the VENN diagram was obtained, see Figure 1. After uploading the protein genes common to drugs to the STRING website, Cytoscape 3.7.2 software and Cytohubba plug-in were run respectively, and the top 5 proteins with the highest degree were obtained automatically. They are quercetin, luteolin, kaempferol, skullcapflavone II, isorhamnetin.

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Among them, the larger Degree is, the more likely it is the key target of Yiqi Resolving Stasis and Detoxification Formula for COPD, see Figure 2.

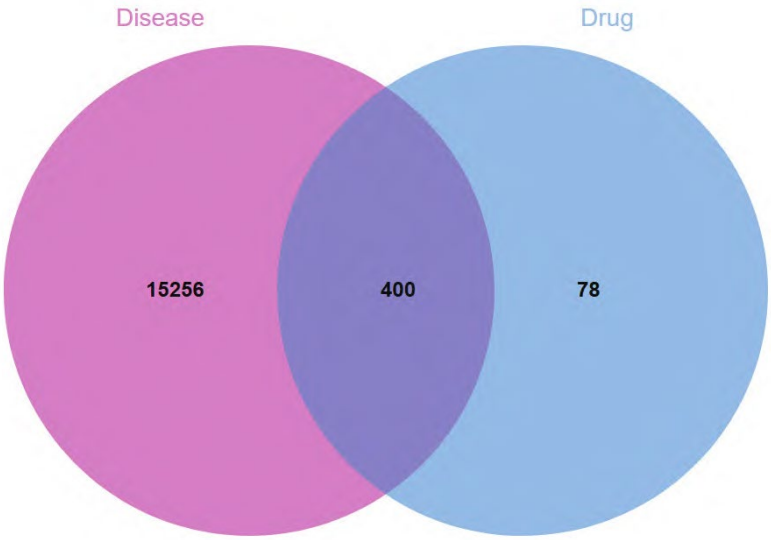
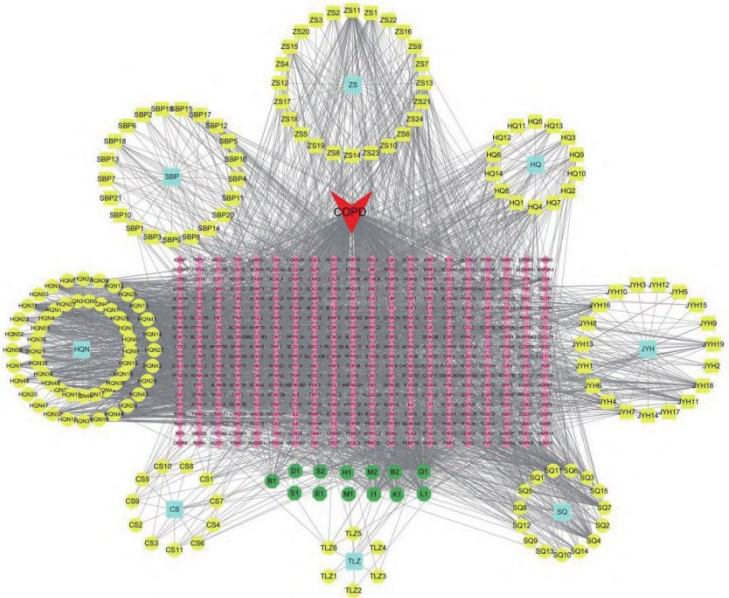


Figure 1: Wayne's diagram of intersecting targets of Benefiting Qi, Resolving Blood Stasis and Detoxification Formula and COPD



Note: Blue square nodes are drug names, yellow round nodes are active ingredients, pink diamond nodes are potential targets, red inverted triangles are diseases, and a line between two nodes means that there is an action relationship between them

Figure 2: Integrated network diagram of "drug-active ingredient-common target-disease".

3.4 PPI construction

The common action targets of Yiqi Resolving Stasis and Detoxification Formula and COPD were imported into STRING database for protein interactions, and the obtained results were imported into Cytoscape 3.9.1 software for visualization and analysis, and the PPI network was constructed, and the network diagrams of 126nodes and 2591edges were obtained, as shown in Figure 3 and Figure 4.

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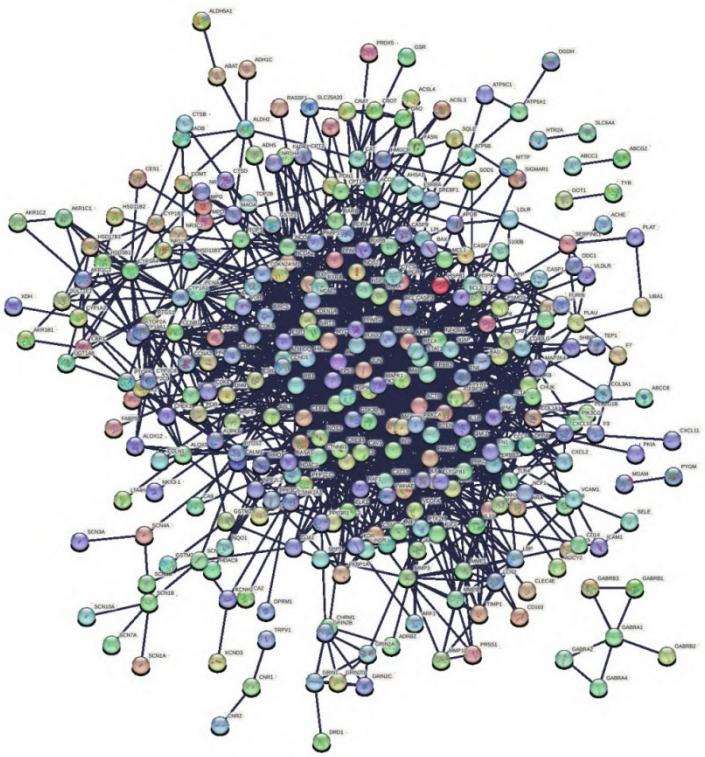

Figure 3: PPI network diagram

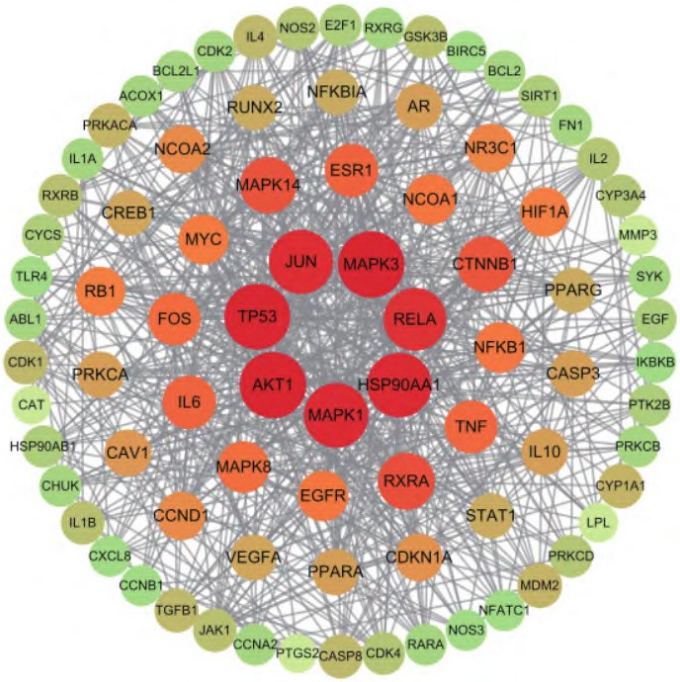

Figure 4: Core target PPI network diagram

4. Conclusions

According to the clinical manifestations of COPD, such as coughing up sputum, wheezing and even dyspnea, COPD belongs to the category of "lung distension" and "wheezing syndrome" in Chinese medicine. The name of "lung distension" was first appeared in "Yellow Emperor's Classic of Internal Medicine", it is the recurrence of various chronic lung diseases, which leads to distension of lung qi and can not be converged and downward, and the nature of the pathology is mostly deficiency of the original and the standard, with deficiency of qi as the root, phlegm and stasis as the standard, mixed

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with deficiency and solid, and difficult to be cured. The formula of "Benefiting Qi, Resolving Blood Stasis and Relieving Toxins" is a common prescription used by Prof. Du Huaitang, former head of the Northern Heat Disease Group, in the treatment of exacerbation of chronic lung diseases, which is based on the treatment method of supporting the positive and dispelling the evil, with the core medicines of Astragalus, Scutellariae baicalensis, Honeysuckle, Panax quinquefolii, Paeonia lactiflora, fried Citrus aurantium, Drabanemerosae hebecarpa, and Sangbaekpi. It was found that benefiting qi, resolving blood stasis and detoxifying formula could reduce the levels of pro-inflammatory factors interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) and elevate the levels of anti-inflammatory factors IL-4 and IL-10, reduce inflammatory infiltration of lung tissues, and significantly improve pathological injury in rats [5]. However, there are fewer studies on the related therapeutic mechanisms.

In this study, we found that the main active ingredients in Yiqi Resolving Stasis and Detoxification Formula were quercetin, lignans, kaempferol, Scutellariae flavonoid II, and isorhamnetin through network pharmacology. Quercetin is a natural flavonoid with anti-inflammatory, immunosuppressive, antioxidant, antimicrobial, antitumor, nutritional supplementation, and antiparasitic effects [6]; studies have shown that quercetin effectively reduces the incidence of upper respiratory tract infections [7] and improves pulmonary fibrosis [8]; Farazuddin et al. [9] found that Quercetin reduced the rate of acute exacerbation of COPD in rhinovirus-infected mice by decreasing lung inflammation and changes in emphysema; Yang et al. [10] found that quercetin was effective in ameliorating airway inflammation and oxidative stress status in COPD rats. Lignans are flavonoid substances with anti-inflammatory, antioxidant, antitumor, and antimyocardial injury effects. Lignans are flavonoid substances with anti-inflammatory, antioxidant, antitumor, and antimyocardial injury effects. Chen et al. found that lignans can reduce tumor necrosis factor (TNF)-α, interleukin (IL)-6 and other inflammatory factors release to improve the symptoms of COPD mice; Li Yuanhang found that lignans can reduce the levels of TNF-α, IL6, IL-12 in COPD patients with acute exacerbation, alleviate the patients' dyspnea, and improve the lung function. Kaempferol is a natural flavonol with a variety of pharmacological properties, including antibacterial, anti-inflammatory, antioxidant, antitumor, cardioprotective, neuroprotective, and antidiabetic activities. It can reduce the expression of multiple inflammatory mediators and effectively reduce the body's inflammatory burden by regulating pro-inflammatory enzyme activities, inhibiting the activation of MAPK (ERK, p38 and JNK) pathways in LPS-induced lung-injured mice, and blocking the inflammatory process mediated by the Tyk-STAT signaling pathway and NF-κB, etc.; it can also reduce the inflammatory burden of the body by inhibiting the LPS-induced NF-κB pathway in macrophages related protein expression in macrophages to reduce the production of inflammatory mediators, while up-regulating the expression of VE-calmodulin and β-linker protein in lung tissues to ameliorate pulmonary vascular endothelial barrier damage. Studies have shown that Scutellaria baicalensis flavonoid II and isorhamnetin both have strong antioxidant and anti-inflammatory effects, which can delay inflammation, airway remodeling and other pathological processes in COPD.

In conclusion, this paper describes the active ingredients and possible targets of action of Yiqi Huayu Xietoxin Relief Formula for the treatment of COPD and the mechanism of action of the related pathways by network pharmacology methods, which helps to analyze the effective value of Yiqi Huayu Xietoxin Relief Formula for the treatment of COPD from a new perspective and provides directions for further experimental studies.

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