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Network Pharmacology Analysis on the Mechanism of Beimu Gualou Formula in Treating Chronic Obstructive Pulmonary Disease

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Abstract: Beimu Gualou Formula (BMGLF) is a traditional Chinese medicine (TCM) formula with the potential to treat chronic obstructive pulmonary disease (COPD). However, the main components and potential mechanisms of BMGLF remain unclear. This study aimed to explore the active ingredients and potential action mechanisms of BMGLF for treating COPD by utilizing network pharmacological analysis. In this study, The TCMSP database was searched for the medicinal chemistry of BMGLF to screen its active ingredients and action targets; the GeneCards database was searched to obtain COPD targets; drug targets and disease targets were intersected and a PPI network was constructed using the String database and Cytoscape 3.9.1 software to obtain key targets.; GO enrichment analysis and KEGG pathway analysis of the intersected targets were performed using David database. A total of 47 active ingredients of BMGLF were found, among which the core components were luteolin, naringenin, beta-sitosterol, and acacetin; 112 intersecting targets of BMGLF for COPD treatment were obtained, among which the core targets were TP53, TNF, CASP3, AKT1, and ESR1, etc. GO analysis yielded 328 entries, and KEGG analysis yielded 151 signals, involving Pathways in cancer, PI3K-Akt signaling pathway, Lipid and atherosclerosis, and so on. This study revealed that BMGLF may act synergistically via multi-components, multi-targets, and multi-pathways for COPD treatment, providing a theoretical basis for further mechanistic studies.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a commonly occurring chronic airway disease that usually develops as a result of exposure to high levels of harmful particles or gases. The prevalence and mortality rates of COPD are on the rise, which has a serious impact on the patients' quality of life and workforce [1]. According to statistics, by 2019 COPD was already ranked as the third highest death reason in the world, leading to 3.23 million deaths [2].

Western drug therapies for COPD primarily include inhaled bronchodilators and glucocorticoids,

which have proven effective in reducing the risk of exacerbations and relieving symptoms. However, treatment of COPD remains challenging. Despite aggressive maintenance therapy with medications, symptoms and airway inflammation persist in some COPD patients[3],[4]. Nowadays, traditional Chinese medicine (TCM) offers a promising prospect for the systematic treatment and prevention of COPD and other complex diseases for its reliable treatment efficacy and fewer side-effects. [5][6].

Beimu Gualou Formula (BMGLF), from "Yi Xue Xin Wu" by Peng Guocheng of the Qing Dynasty, is a classic prescription in TCM. It consists of Zhebeimu (Fritillariae Thunbrgii Bulbus), Gualou (Trichosanthes Kirilowii Maxim), Tianhuafen (Trichosanthis Radix), Fuling (Poria Cocos), Juhong (Citri Exocarpium Rubrum), and Jiegeng (Platycodon grandiforus). TCM believes that BMGLF has the efficacy of moistening the lungs and clearing heat, regulating qi and resolving phlegm, therefore, BMGLF has been commonly used in ttreating lung diseases in the past two hundred years. In addition, relevant studies in modern pharmacology have found that BMGLF has anti-inflammatory [7][8][9][10], anti-infectious [11][12], expectorant, and anti-asthmatic effects [13][14][15]. BMGLF is valuable for COPD treatment in clinical practice, but the nature of the pharmacodynamic effects of its active ingredients and the action mechanism are still unclear.

Network pharmacology is a new type of research approach that is based on the theoretical foundation of systems biology, combined with the analysis method of bioinformatics, which reveals the interaction between drug components, targets, and disease treatment from the perspective of pharmacology in a more comprehensive way. It has unique advantages over TCM, which is characterized by miscellaneous components, multiple targets, and wide pathways. Therefore, in this study, we screened the main components of BMGLF, predicted the key targets and related pathways of drug action through network pharmacology analysis, and preliminarily explored the possible action mechanism of BMGLF in COPD treatment, to provide scientific basis for the subsequent in-depth basic research as well as clinical application.

2. Materials and Methods

2.1. Screening of BMGLF for Active Ingredients and Related Targets

The components of Fritillariae Thunbrgii Bulbus, Trichosanthes Kirilowii Maxim, Trichosanthis Radix, Poria Cocos, Citri Exocarpium Rubrum, and Platycodon grandiforus were searched in the TCMSP database [16] (https://tcmsp-e.com/), and the active ingredients and their corresponding targets were screened out for each flavor in the formula (OB ≥30%, DL ≥0.18). The String database [17] (https://cn.string-db.org/) was used to standardize the target names obtained above. In addition, a "Drug-Active Ingredient-Drug Target" network diagram was constructed with Cytoscape 3.9.1 software [18].

2.2. Prediction of COPD targets

In GeneCards database [19] (https://www.genecards.org/), "chronic obstructive pulmonary disease" was used as the search term to obtain the relevant COPD disease targets. The BMGLF active ingredient targets were intersected with COPD targets, and the intersected targets were visualized by drawing a Venn diagram using the Venny 2.1.0 platform.

2.3. Acquisition of Potential Therapeutic Targets and Construction of PPI Network

The intersecting targets were imported into the String database (the species was "Homo Sapiens", hiden disconnected nodes in the network, and the confidence level of target association was 0.90),

and constructed the protein-protein interaction (PPI) network. After importing the PPI network into Cytoscape 3.9.1 software, the top 10 core targets were filtered based on Degree value from the PPI network.

2.4. GO Functional Enrichment Analysis and KEGG Pathway Analysis

The intersecting targets were subjected to GO functional enrichment analysis and KEGG pathway analysis utilizing the David database [20][21] (https://david.ncifcrf.gov/), evaluated at P <0.05 and FDR <0.05, and the online mapping platform was used to generate GO analysis diagrams as well as KEGG pathway analysis bubble plots.

2.5. Construction of "Disease-Pathway-Target-Component-Drug" Network

The data of KEGG pathway analysis were used to map the "Disease-Pathway-Target-Component-Drug" network diagram of BMGLF for COPD treatment using Cytoscape 3.9.1 software.

3. Results

3.1. Collection of BMGLF Active Ingredients and Action Targets

Through TCMSP database, we screened 7 active ingredients in Fritillariae Thunbrgii Bulbus, 11 active ingredients in Trichosanthes Kirilowii Maxim, 2 active ingredients in Trichosanthis Radix, 15 active ingredients in Poria Cocos, 9 active ingredients in Citri Exocarpium Rubrum, 7 active ingredients in Platycodon grandiforus, and after de-emphasizing the weights, we obtained 47 BMGLF active ingredients in total. Furthermore, we gained 291 targets corresponding to the active ingredients and finally got 124 drug targets after de-emphasis. The "Drug-Active Ingredient-Drug Target" network was created by using Cytoscape 3.9.1 (Figure 1). In this network, there were 177 nodes and 312 edges (the larger the graph, the larger the Degree value). The results showed that luteolin, naringenin, beta-sitosterol, acacetin, etc. had a high Degree value (Degree value >20) and were the core components of BMGLF.

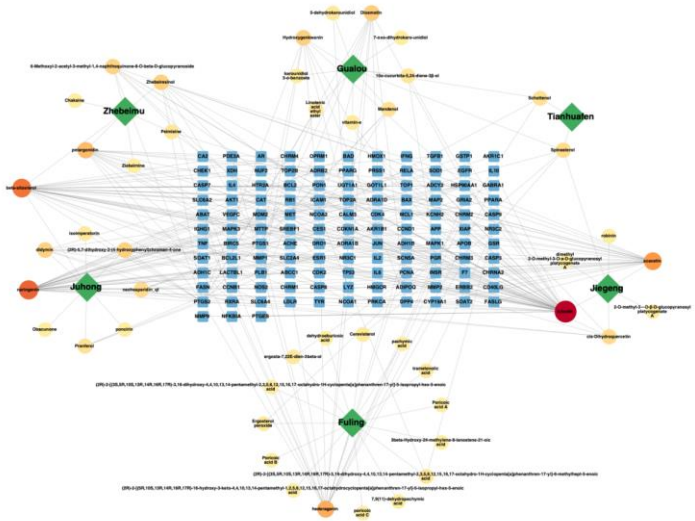

Figure 1: The "Drug-Active Ingredient-Drug Target" Network Diagram.

3.2. Acquisition of Disease Targets for COPD

From the GeneCards database, we obtained a total of 9230 COPD disease targets from the GeneCards database. Using the Venny platform to generate a Venn diagram of drug target intersections with COPD targets, we obtained 112 intersecting targets (Figure 2).

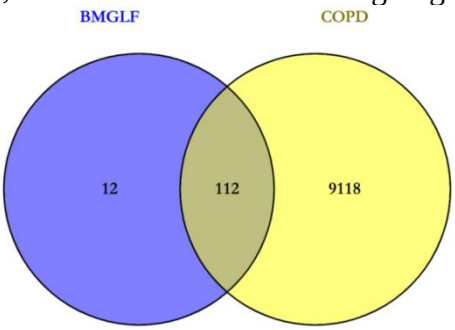

Figure 2: The "Drug-Active Ingredient-Drug Target" Network Diagram.

3.3. Construction of PPI Network

We uploaded the intersection targets to the String database and used Cytoscape software to constructed the PPI network. In this network diagram, there were 78 nodes and 248 edges (Figure 3). The Degree value and node area are positively proportional to each other (Degree value increases, node area increases), the larger the Degree value, the darker the color, and the higher the probability of being a core target point. Among them, the Degree value of TP53 (Degree value = 32) was much higher than that of other protein nodes, reflecting the key role of this protein in the network as a bridge to other nodes in the network. We predicted that the core targets for the top 10 were: TP53, TNF, CASP3, AKT1, ESR1, JUN, BCL2, IL6, HSP90AA1, CCND1 (Figure 4).

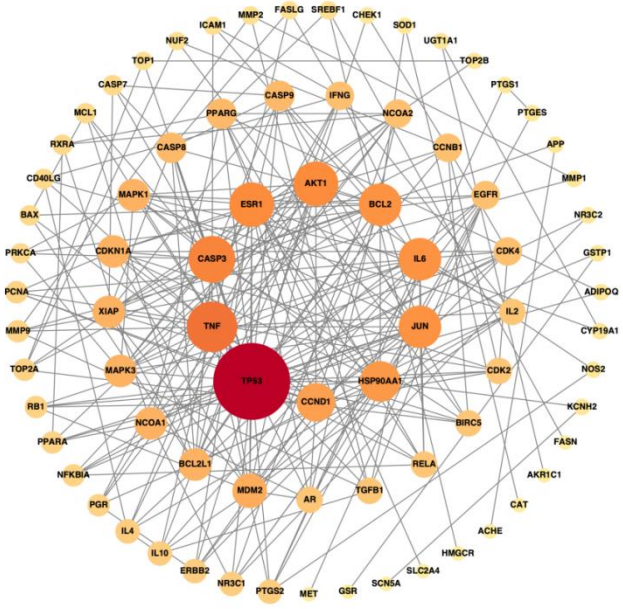

Figure 3: Core Targets PPI Network Diagram.

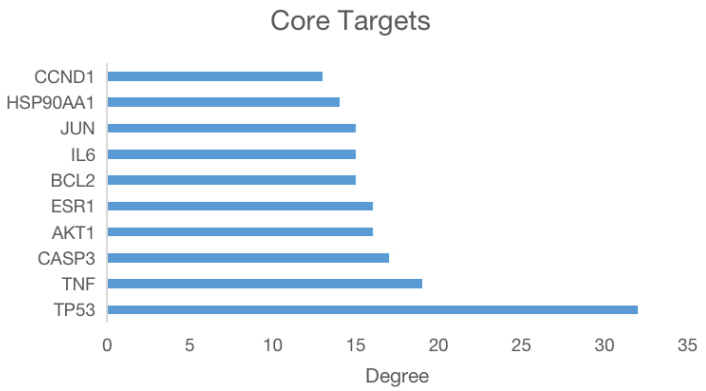

Figure 4: Core Targets Bar Chart.

3.4. GO Functional Enrichment Analysis

In the GO analysis, we obtained altogether 234 biological process (BP), 46 cellular component (CC), and 48 molecular function (MF). The BP, CC, and MF were sorted by the Count value, and taken the top 10 respectively to plot the GO enrichment analysis diagram (Figure 5). The enrichment analysis showed that BMGLF treatment of COPD was strongly associated with positive regulation of transcription from RNA polymerase II promoter, response to xenobiotic stimulus, positive regulation of apoptotic process, cytosol, nucleus, plasma membrane, protein binding, identical protein binding, enzyme binding, etc.

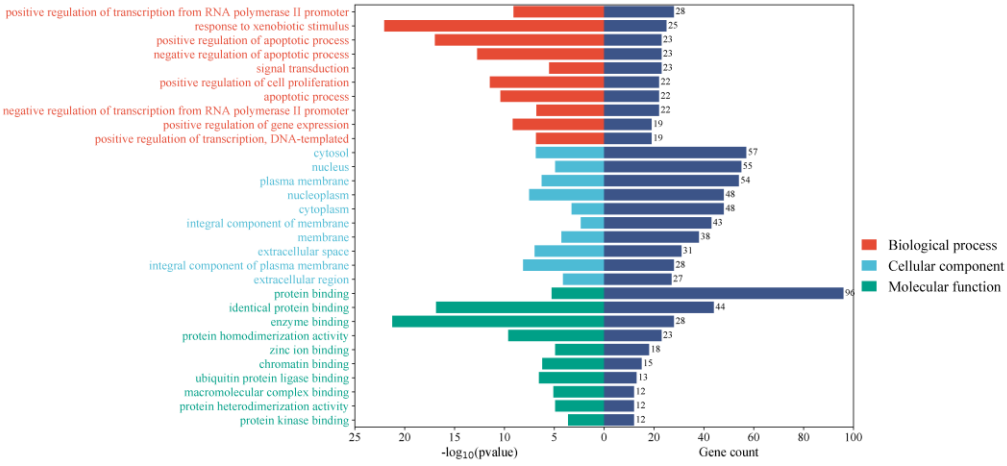

Figure 5: GO Analysis Diagram.

3.5. KEGG Pathway Analysis

In KECG pathway analysis, we gained 151 signaling pathways in total, which were mainly enriched in Pathways in cancer, PI3K-Akt signaling pathway, Lipid and atherosclerosis, Hepatitis B, and so on. The pathways were sorted by the Count value (Figure 6). The "Disease-Pathway-Target-Ingredient-Drug" network diagram connected the drug, the main active ingredient, the disease, the key target, and the corresponding pathway. In this study, this network diagram included 158 nodes and 540 edges (Figure 7).

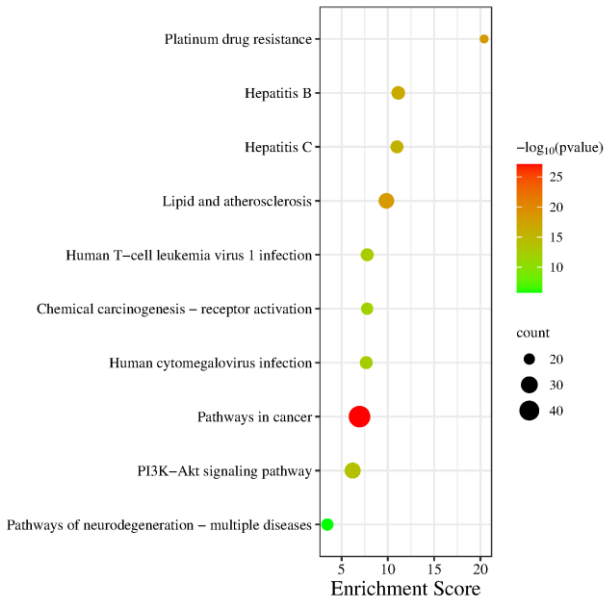

Figure 6: KECG Pathway Analysis Bubble Diagram.

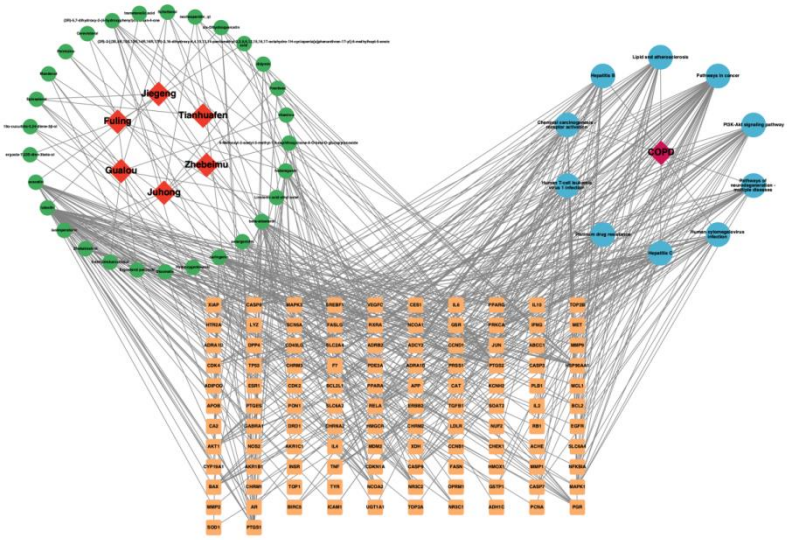

Figure 7: The "Disease-Pathway-Target-Ingredient-Drug" Network Diagram

4. Conclusions

Network pharmacology can provide a novel model of multi-targets, multi-pathways, and multi-links to analyze the correlation between the main components of therapeutic drugs and diseases at the level of systemic biological networks, which can provide new research methods for the study of the TCM action mechanisms and the law of drug compounding. In this study, we analyzed the primary components in BMGLF by network pharmacology, and the results can provide a basis of reference for further research on the action mechanism of COPD.

We obtained a total of 47 active ingredients from 6 flavors in BMGLF and screened the core ingredients by the PPI network. Among them, luteolin, naringenin, beta-sitosterol, and acacetin had the greatest Degree value, indicating that they may be the active ingredients in BMGLF for COPD

treatment and achieve the therapeutic effect through synergistic action on multiple targets. Luteolin is a representative flavonoid that has antioxidant, anti-inflammatory, and immunomodulatory effects. Luteolin is a representative flavonoid with antioxidant, anti-inflammatory, and immunomodulatory effects [22]. Li et al. found that luteolin could relieve COPD mice from inflammation and oxidative stress and attenuate lung injury via the NOX4-mediated NF-κB signaling pathway [23]. Naringenin, a citrus flavonoid with multiple biological activities, may improve chronic lung diseases such as asthma via its anti-inflammatory, anti-angiogenic biological, and antioxidant biological actions [24][25]. Moreover, Liu et al. found that naringenin could dramatically improve lung function, reduce the level of inflammatory cells in serum and alveolar lavage fluid, as well as inhibit the generation of pro-inflammatory cytokines in COPD mice models, which is a potential drug for treating COPD-associated inflammation [26]. Beta-sitosterol belongs to the group of alcohol compounds, and beta-sitosterol can exert anti-inflammatory effects through increasing the activity of SHP-1 in J774A to downregulate the STAT1 and NF-κB pathways, and reducing the expression of pro-inflammatory cytokines and chemokines [27]. Acaciachin significantly reduces the number of proteins, inflammatory cytokines, and inflammatory cells in the bronchoalveolar lavage fluid of septic mice, and reduces myeloperoxidase activity in the lungs, which in turn attenuates lung injury and pulmonary edema [28]. To summarize, the chemical components contained in BMGLF mainly have complex effects such as anti-inflammatory, antioxidant, immunomodulatory, and so on.

In this study, we obtained core targets such as TP53, TNF, CASP3, AKT1, and ESR1 by PPI analysis. These targets are widely involved in various aspects of inflammation, cellular value-addition, and apoptosis. TP53 dysfunction is one of the major genetic variants in the coexistence of lung cancer and COPD [29], and activation of the nicotinic acetylcholine receptor (nAChR) in COPD can down-regulate expression of signaling pathways associated with the key tumor-suppressor gene TP53 [30]. TNF, especially TNF-α, is one of the most studied cytokines in COPD [31]. TNF-α is a relatively well-recognized COPD susceptibility gene, which activates macrophages and increases cytotoxicity, and also stimulates the secretion of IL-6, which induces the accumulation of inflammatory cells, and further induces oxidative stress, tissue remodeling, etc., to promote the development of COPD [32]. CASP3 plays a key role in the regulation of apoptosis. Increased expression of CASP3 may inhibit tissue repair in COPD patients' airways, leading to permanent airway destruction [33]. AKT1 is a crucial protein downstream of the PI3K/AKT pathway, activation of which can activate mTOR gene expression, induce endothelial cell differentiation and cell proliferation, and promote epithelial cell mesenchymal transition [34]. Meanwhile, some studies have shown that AKT1 is involved in processes such as lung injury, lung fibrosis, and viral infection [35]. ESR1 can encode an estrogen receptor [36], and it has been found that estrogen signaling may be crucial in lung disease, and that ESR1 mRNA overexpression was strongly associated with the prognosis of non-small cell lung cancer [37]. In additio, ESR1 was found to be expressed in lung tissues of COPD patients, but the exact mechanism is unknown.

Further, from GO analysis, it can be seen that the treatment of COPD with BMGLF goes through cellular, tissues, and various biological processes, which is a complex and large process. And from KEGG analysis, we found that BMGLF treatment of COPD mainly involves Pathways in cancer, PI3K-Akt signaling pathway, Lipid and atherosclerosis, and so on. BMGLF involves pathways that are associated with cancer, and there is experimental evidence that COPD is one of the risk factors for developing lung cancer and that the same mechanisms are present in inflammation, oxidative stress, repair of genetic damage, and immunity [38]. During COPD, BMGLF may be able to act on Pathways in cancer to curb the further progression of COPD to cancer. PI3K/Akt pathway can regulate cell proliferation, differentiation, apoptosis, etc [39]. After entering the human body, nicotine and other oxidants in cigarettes, which are common causes of COPD, can induce the

secretion of large amounts of inflammatory mediators in the lungs via the PI3K/Akt pathway, exacerbate the degradation of collagen and elastase, and destroy the proteins in the airway tissues under the joint action of oxidized molecules (methionine and cystine), and impede the repair of airway epithelium, which is one of the major reasons of the inflammation and remodeling of the airway in COPD [40]. Therefore, modulation of the PI3K/Akt pathway has emerged as an effective COPD treatment. The macrolides commonly used in clinical practice may regulate the PI3K/Akt pathway to reduce pulmonary and systemic inflammation in patients with COPD, thus achieving satisfactory therapeutic effects [41]. COPD is often associated with extrapulmonary co-morbidities like cardiovascular disease (CVD) and metabolic syndrome, besides pulmonary manifestations [42][43]. There is a complex cardiopulmonary interaction in COPD combined with CVD, and the mechanism involves systemic inflammation [44], hypoxia [45], oxidative stress [46], and cell death [47], etc. Atherosclerosis (As) is a major pathologic basis for CVD development. Khedoe et al. found that hyperlipidemia increased lung inflammation and caused systemic inflammation when analyzing the relationship between COPD and as in mice models. In addition, diets rich in cholesterol and fat increase the development of non-bacterial inflammation and emphysema in the lungs, thus increasing the incidence of as [48]. The use of BMGLF for COPD treatment may also have a therapeutic effect on As. Therefore, based on the pathway enrichment results, BMGLF most likely alleviated the chronic inflammation, oxidative stress, and tissue cell damage present in COPD by modulating the signal transduction process of pathways related to inflammation, oxidative stress, and cellular metabolism.

In summary, BMGLF may regulate Pathways in cancer, PI3K-Akt signaling pathway, Lipid and atherosclerosis, etc. via active ingredients such as luteolin, naringenin, beta-sitosterol, acacetin, etc. based on the targets of TP53, TNF, CASP3, etc. to treat COPD. This study confirmed the synergistic therapeutic effects of BMGLF in a multi-component, multi-target, and multi-pathway manner, which was useful for investigating the basis of its efficacy and for new drug development. At the same time, this study still had shortcomings. The research data from major databases may lack certain components and targets, and the results of this study were predicted. Therefore, the action mechanism of BMGLF in COPD treatment needs to be verified by further basic and clinical experiments.

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