

Study on Mechanism of Guomin Jian in Treatment of ALRH based on Network Pharmacology

Xin Tao, Xile Liu*

School of Medicine, Xinjiang University of Science and Technology, Korla 841000, China

Abstract

Based on the network pharmacology, this paper discussed the action mechanism of Guomin Jian(GMJ) in the treatment of allergic rhinitis(ALRH). Method: TCMSP database was used to search and screen the active components of Guomin Jian. The Drugbank, GeneGards and OMIM databases were used to predict and screen the related targets of ALRH. TCM regulation network was constructed by adopting Cytoscape 3.6.0 software, and Gene Ontology (GO) and KEGG enrichment analysis were carried out to construct GMJ-active ingredients-target genes-disease pathway networks via DAVID database. Results: In this study, 141 active ingredients, 95 disease targets and 161 pathways of GMJ were screened. Conclusion: This study preliminarily clarified the mechanism of GMJ treating ALRH through multi-components, multi-targets and multi-pathways.

Keywords

Allergic Rhinitis; Network Pharmacology; Pathway; Mechanism; Guomin Jian.

1. Introduction

ALRH is a common otorhinolaryngological disease in clinic, which is characterized by watery nasal mucus, nasal itching, nasal obstruction and sneezing. The treatment of ALRH is difficult, and long-term repeated attacks can cause sinusitis, asthma, and even lead to malformation of the jaw skeleton in pediatric [1]. ALRH belongs to the category of "Bi Qiu" in traditional Chinese medicine [2]. ALRH is mostly caused by the consumptive disease of viscera, insufficient body resistance and taking advantage of wind-cold. It was documented in the medical prescription that " the nose is known to belong to the lungs, while nasal belongs to spleen ", and in Miraculous Pivot " lung qi deficiency can cause nasal congestion"[3].

GMJ is a famous prescription created by Zhu Zhanyu, a famous veteran doctor of traditional Chinese medicine, which is often used to treat many allergic diseases, such as ALRH, allergic dermatitis, allergic asthma, irritable bowel syndrome etc. In the prescription, Radix Saposhnikoviae can relieve exterior syndrome by dispersion, dispel the wind and cure wind diseases. Radix Stellariae has effect in clearing heat and cooling blood and can be cold, Schisandra taste acid, warm in nature, Licorice tonifying spleen yiqi. The whole formula is consistent with the pathogenesis of ALRH in traditional Chinese medicine.

Due to the complexity of the compound ingredients of traditional Chinese medicine and the unclear mechanism of action, it is still quite difficult to explore the potential molecular mechanism. With the proposal of network pharmacology, the study of single target has gradually become a holistic and systematic regulatory study. Through the biological database, the visual Chinese medicine regulatory network of "Chinese medicine-active ingredient-target gene-disease" is constructed, furthermore, the mechanism of action of traditional Chinese medicine compounds is clarified, and the comprehensive network analysis of molecular effects is realized[4, 5]. Therefore, network pharmacological methods were used to study the mechanism of action of GMJ in the treatment of ALRH, from single to whole, from part to system. The results of the study point the way for future experimental research.

2. Organization of the Text

2.1. Method

2.1.1. Acquisition of Effective Component and Target

The chemical compositions of GMJ were searched through The traditional Chinese medicine systems pharmacology database and analysis platform(TCMSP) and screen the active ingredient of the drug according to the drug-likeness ($DL > 0.18$) and oral bioavailability ($OB > 30\%$). Through the Uniprot database, the names of the screened targets are standardized and the drug active ingredient target dataset Drug.txt is established.

2.1.2. Acquisition of Disease-related Genes

Genecards(<https://www.genecards.org/>) and OMIM(<https://omim.org/>) were searched by using the search term "Allergic asthma". The above predicted target genes were combined as disease-related gene data for Disease.txt

2.1.3. Acquisition of Possible Targets for Drug Therapy of the Disease

Adopt an online program(<http://bioinformatics.psb.ugent.be/webtools/Venn/>), the Disease and Drug target genes are intersected to obtain the common target of the single medicine drug and the disease, and this collections were the possible targets of the single herb to treat the disease, and the Drug_Disease.txt file was generated.

2.1.4. Drawing Network Diagram of Drugs, Disease and Target Genes

Drawing network diagram of drugs, disease and target genes by using Cytoscape3.7.1.

2.1.5. PPI Network Building

Submit the genes in the Drug_Disease.txt to the String website(<https://string-db.org/>), and set "minimum required interaction score" to "highest confidence(0.900)". PPI interacting network diagrams and string_interactions.tsv files were generated.

2.1.6. Generate Statistical Chart

Using the perl language, the key gene connectivity protein statistical chart(ranked in the top 30) is extracted in the string_interactions.tsv file, and the statistical results of all node connectivity were shown in the count.xls file.

2.1.7. Convert the Gene Names to the Gene IDs

Using R3.6.0 software, install the RSQLite package and org.Hs.eg.db package to convert the gene names in the Drug_Disease.txt to gene IDs, prepare for(gene ontology, GO) enrichment analysis and(kyoto ency-clopedia of genes and genomes, KEGG) analysis, and generate id.txt files.

2.1.8. GO Analysis

Using R3.6.0 software, install Bioconductor's colorspace, stringi, DOSE, clusterProfiler, pathview, ggplot2 and other process collection packages. GO enrichment analysis based on id.txt files to obtain bar and bubble charts(only the most statistically significant results of the top 20 are displayed); Convert gene IDs to gene symbols to obtain all statistically significant results in the form of statistics file GO.xls. The BP, CC, and MF classification output of GO are carried out by using the ggplot2 package, and the bar chart and bubble chart are stored in the GO2 folder.

2.1.9. KEGG Analysis

Using R3.6.0 software, install Bioconductor's colorspace, stringi, DOSE, clusterProfiler, pathview and other process collection packages. KEGG enrichment analysis based on id.txt files to obtain bar and bubble charts(only the most statistically significant results of the top 20 are displayed); Convert the gene IDs to the gene symbol to obtain all statistically significant results in Excel form of the statistics file KEGG.xls and pathway map files.

2.2. Result

2.2.1. Active Ingredients of GMJ

OB and DL were used as search criteria through TCMSP database to search, and a total of 141 active ingredients were obtained. Compounds mainly include: Wuweizisu C, beta-sitosterol, kaempferol, Stigmasterol, isorhamnetin etc.

Table 1. OB and DL values of active ingredients of GMJ (top 30)

Compound	Compound name	OB%	DL
MOL004624	Longikaurin A	87.00	0.53
MOL005317	Deoxyharringtonine	68.83	0.81
MOL008956	Angeloylgomisin O	59.65	0.85
MOL008957	Schizandrer B	50.24	0.83
MOL008968	Gomisin-A	49.69	0.78
MOL008974	Gomisin G	48.87	0.83
MOL008978	Gomisin R	48.57	0.86
MOL008992	Wuweizisu C	47.72	0.84
MOL011730	11-hydroxy-sec-o-beta-d-glucosylhamaudol_qt	46.43	0.27
MOL011732	anomalin	46.27	0.66
MOL011737	divaricatacid	45.46	0.32
MOL011740	divaricatol	43.83	0.38
MOL001941	Ammidin	43.83	0.22
MOL011747	ledebouriellol	43.39	0.51
MOL011749	phelloptorin	42.98	0.28
MOL011753	5-O-Methylvisamminol	42.36	0.25
MOL002644	Phellopterin	42	0.28
MOL000359	sitosterol	41.88	0.75
MOL000173	wogonin	40.19	0.23
MOL000358	beta-sitosterol	39.67	0.75
MOL001494	Mandenol	39.27	0.19
MOL001942	isoimperatorin	39.27	0.23
MOL003588	Prangenidin	38.8	0.22
MOL007514	methyl icoso-11,14-dienoate	37.99	0.23
MOL013077	Decursin	37.87	0.38
MOL001040	(2R)-5,7-dihydroxy-2-(4-hydroxyphenyl) chroman-4-one	37.58	0.21
MOL000358	beta-sitosterol	37.42	0.75
MOL000422	kaempferol	36.91	0.24
MOL000449	Stigmasterol	36.91	0.76
MOL005043	campest-5-en-3beta-ol	36.91	0.71

Note: Only the top 30 were displayed.

2.2.2. Predict Active Ingredients of GMJ-therapeutic Targets

A total of 1241 targets related to ALRH were obtained by integrating TCMSP and DrugBank databases through OMIM and Gene-cards database screening. 95 active ingredients of the drug - disease-related targets were obtained by intersecting the GMJ active ingredients and the targets associated with ALRH. See picture1.

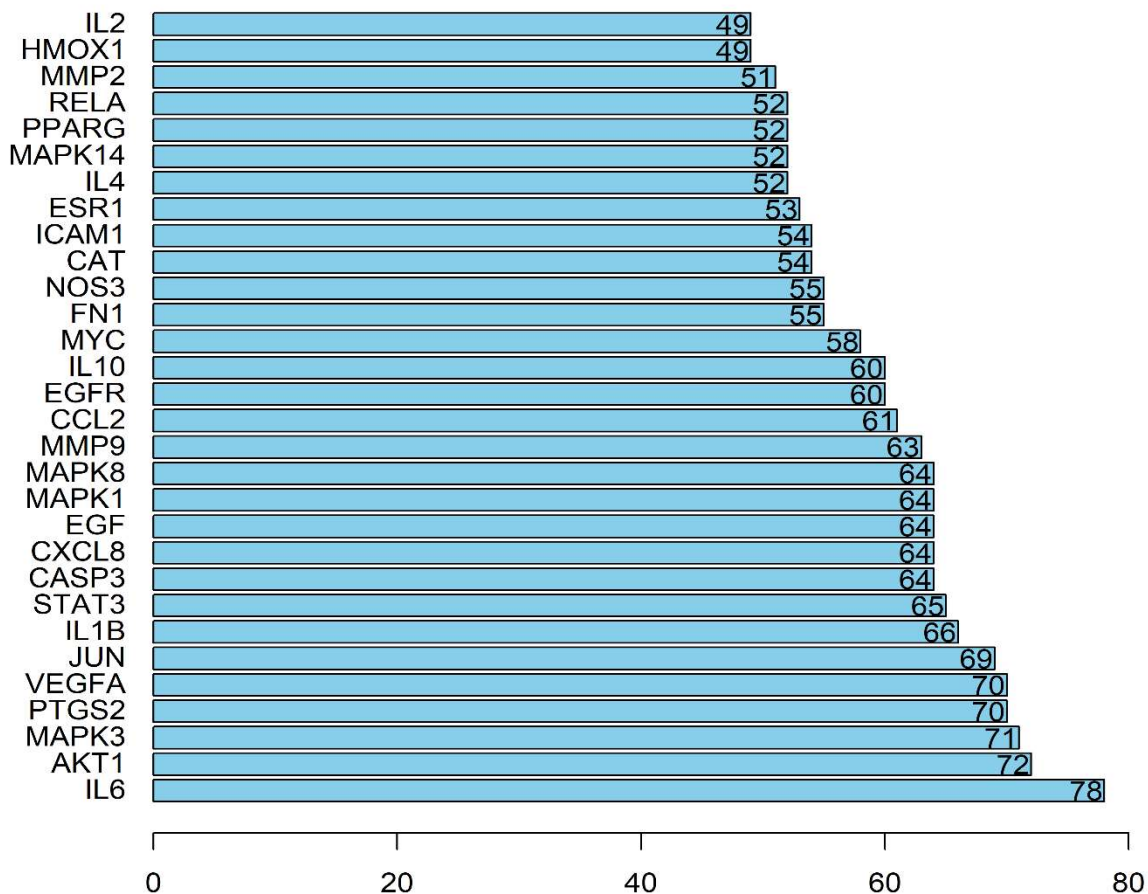


Figure 1. Target action map of GMJ component target and ALRH

2.2.3. Networks Construction and Analysis Results

The above prediction and screening results are constructed by Cytoscape3.2.1 software: GMJ of active compounds for the treatment of allergic asthma target network diagram. See Figure 2.

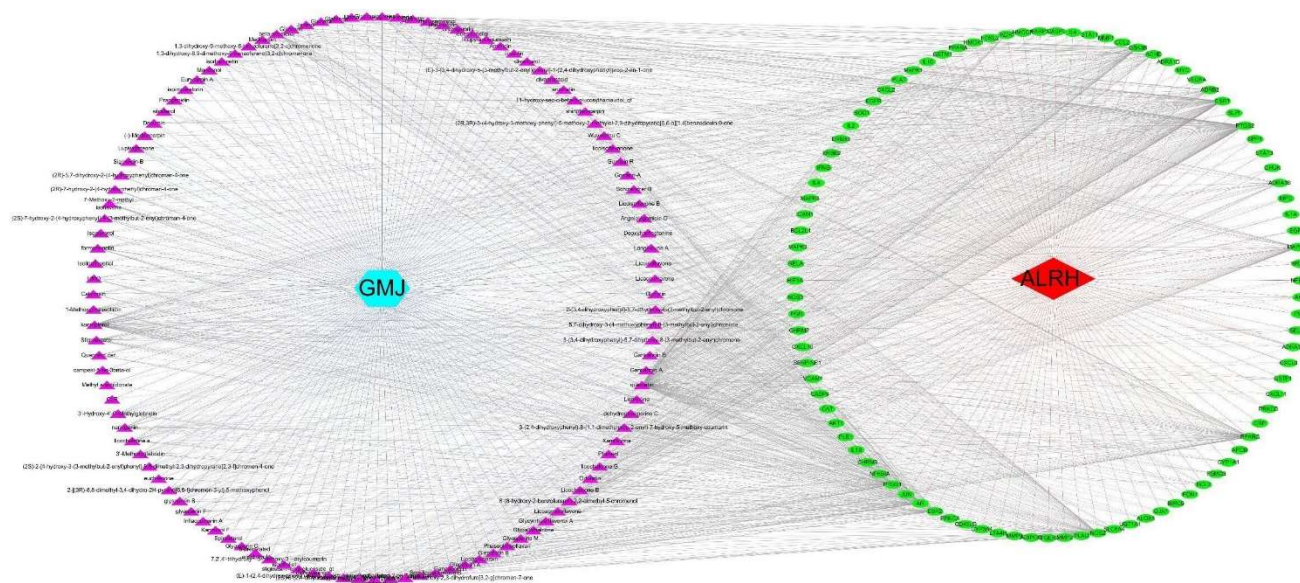


Figure 2. GMJ active ingredient targets and ALRH action targets map

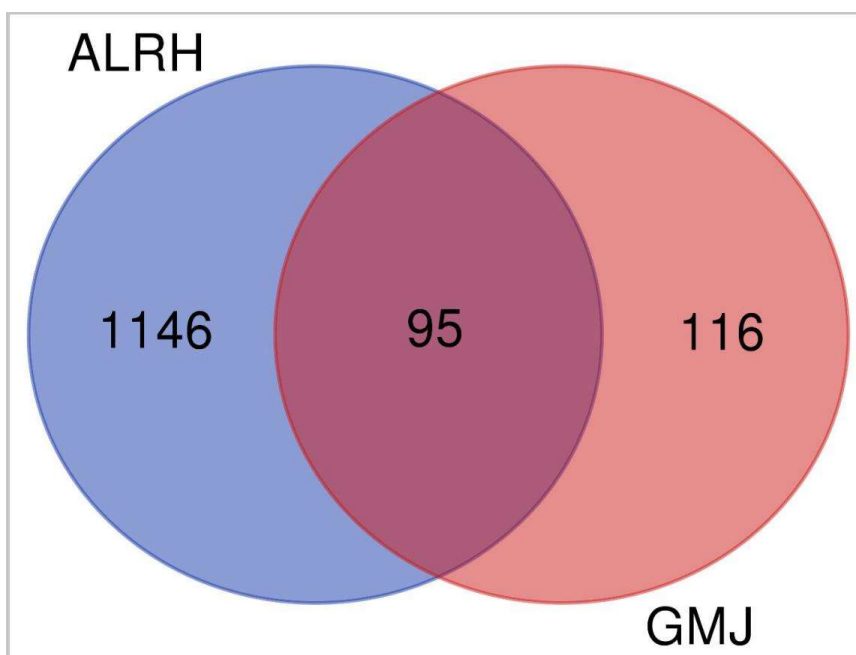


Figure 3. Histogram of important targets for GMJ treatment of ALRH(number of intersections)

From Figure 3, we can know that the core targets of GMJ therapy are IL2, HMOX1, RELA, PPARG, MAPK14, IL4, etc. In order to further understand the therapeutic effect of GMJ on ALRH, 95 intersection gene targets obtained by Wayne Diagram were analyzed by PPI network through STRING database, and the results were shown in Figure 4.

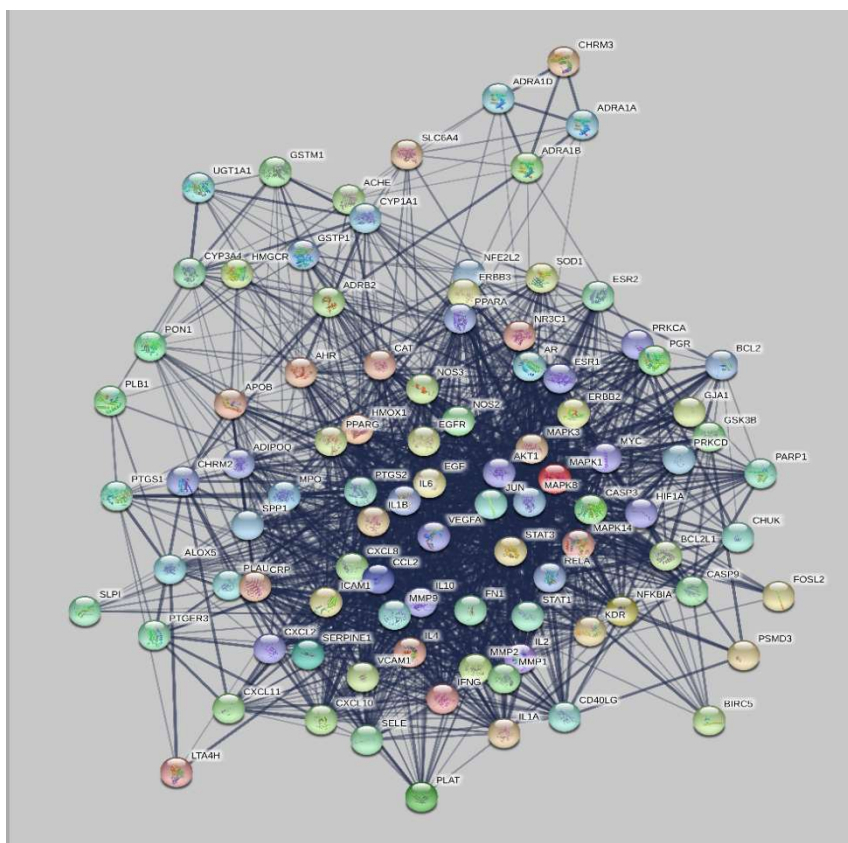


Figure 4. Interaction network diagram among potential targets of GMJ treatment of ALRH

2.2.4. GO Classification Enrichment Analysis

After DAVID database analysis, a total of 127 enrichment results were obtained, and the top 20 P-values were selected for GO classification enrichment analysis, the results were shown in Figure 5. The results showed that the main biological processes of GMJ in the treatment of ALRH were mainly cytokine receptor binding, cytokine activity, protein phosphatase activity and binding.

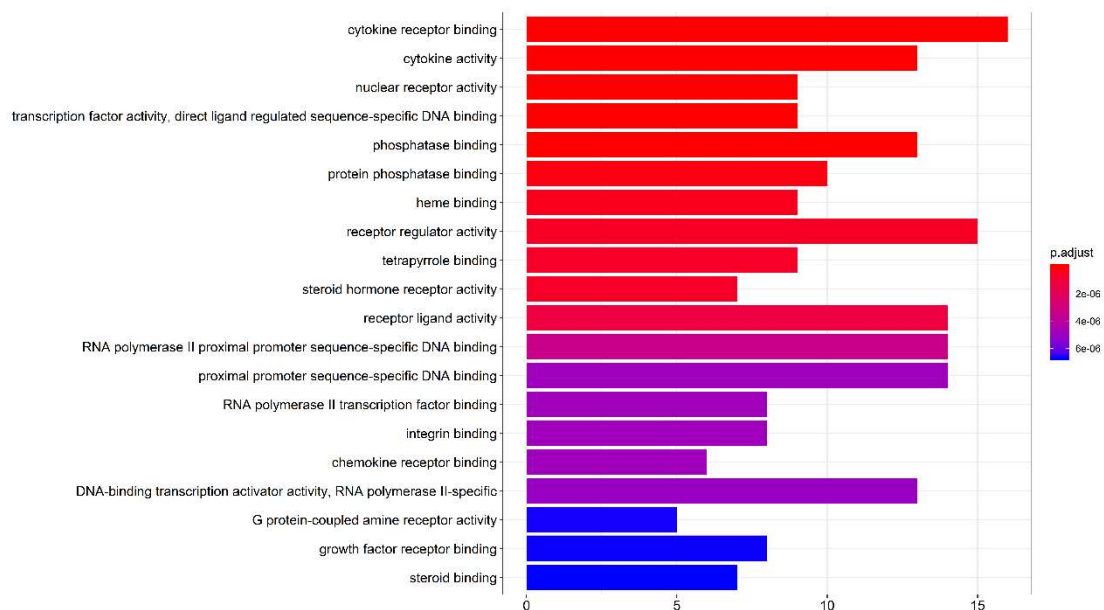


Figure 5. Classification and enrichment analysis of GO in the treatment of ALRH

2.2.5. KEGG Classification Enrichment Analysis

The KEGG pathway enrichment analysis of GMJ genes was performed by using the DAVID database, and a total of 161 pathways were obtained which was shown in Figure 6. Top 20 which were screened as enrichment results were mainly AGE-RAGE signal pathway, IL-17 signaling pathway, TNF signaling pathway, HIF-1 signaling pathway, Toll-like receptor signaling pathway, PI3K-Akt signaling pathway, etc.

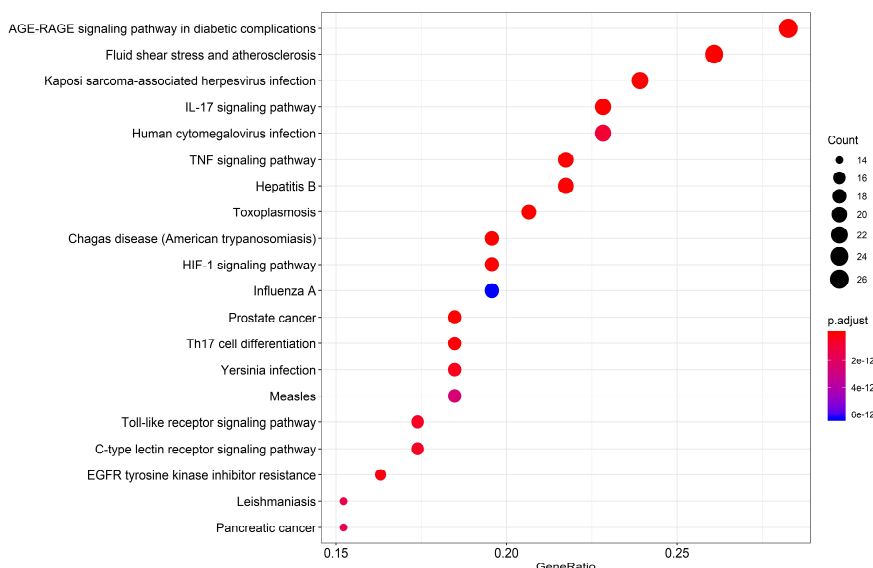


Figure 6. KEEGG bubble chart of GMJ treatment of ALRH

2.3. Discussion

Traditional Chinese medicine has the characteristics of complex compositions and flexible compatibility, which makes it advantageous compared with Western Medicines in the treatment of allergic diseases like allergic asthma, allergic rhinitis, urticaria etc. However, the limitation of traditional Chinese medicine is that the effect is slow and the mechanism of action is not clear. With the rise of network pharmacology, the wide application of big data analysis and "multi-component-multi-target" research methods has provided new methods for the study of the mechanism of action of traditional Chinese medicine[6]. 141 active ingredients, 95 disease targets and 161 pathways of GMJ were screened which were Selected from Radix Saposhnikoviae, Umeboshi, Silver Chaihu, Schizandra and Licorice. It is shown that GMJ treatment of ALRH is the result of combined action of multiple components and multiple targets. The results of this study show that isorhamnetin in the compound target network can inhibit the growth of A549 in lung tumor cells and inhibit the protein expression of MMP2 and MMP9[7]. Beta-sitosterol has a good protective effect on acute lung injury in mice caused by LPS, and its protective mechanism may be related to preventing the release of inflammatory factors(TNF- α , IL-6) and downregulating the activation of NF-KB signaling pathways[8]. Kaempferol has a very pronounced anti-inflammatory effect and is able to alleviate oxidized LDL-induced human venous endothelial cell damage, which is associated with kaempferol's promotion of the activation of the AMPK/Nrf2/HO-1 signaling pathway[9, 10, 11].

The results of GO functional enrichment analysis show that the efficacy of GMJ involves a number of biological processes, mainly based on cytokine receptor binding, cytokine activity, protein phosphatase activity and binding, etc., and the cytokine interleukin-17(IL-17) is mainly secreted by Th17 cells, which can promote the migration and activation of white blood cells, and plays an important role in autoimmune diseases, tumors and other diseases[12].

A large number of studies discovered that Th17 cells are a powerful class of pro-inflammatory cells that can mobilize, recruit and activate neutrophils, and together with the cytokines such as IL -17A, IL -17F, etc., which are involved in the pathological progression of autoimmune diseases such as RA, asthma, SLE, psoriasis, MS and other diseases[13, 14]. The NF-KTHFa pathway Peimine and TNF- α can downregulate the phosphorylation level of MAPKs in the inflammatory response signaling pathway and reduce the transcription intensity of the B gene, thereby reducing the expression of pro-inflammatory cytokines such as TNF- α and IL-6[15]. Therefore, the IL-17 signaling path and the TNF signaling pathway are the main pathways we predicted. Both BML-111 or IL-1 β antibody therapy significantly prevented inflammatory changes in the toll receptor model of ALRH mice induced with ovalbumin[16].

In summary, the paper analyzes the active ingredients, targets and signal pathways of allergic asthma treatment based on the methods and techniques of network pharmacology, and secondly, important signal nodes are obtained by multiple screenings of important active ingredients from multiple levels and angles[17].

This study provides ideas for future exploration of GMJ treatment, and provides a basis for further experimental research by analyzing the mechanism of action of GMJ in the treatment of allergic rhinitis from the perspective of data network.

Acknowledgments

This work is supported by National Nature Science Foundation of China(81560755).

References

- [1] M. Wang, M. Zheng, X.D. Wang, et al. Epidemiological research progress of allergic rhinitis in China, Chin Arch Otolaryngol Head Neck Surg, vol. 29(2019) No.8, p.415-420.

- [2] Y.Y. Zhong, Q. Wang, Y.Z. Jia, et al. Study on Mechanism of Magnoliae Flos in Treatment of Allergic Rhinitis Based on Network Pharmacology, Chinese Journal of Information on Traditional Chinese Medicine, vol. 28(2012) No.3, p.33-39.
- [3] B.Q. Duan, Y.D. Sun. Research progress in the treatment of allergic rhinitis in traditional Chinese medicine, The Medical Forum, vol. 20(2016) No.1, p.107-108.
- [4] Y.Q. Liu, A.S. Wang, S. Jiang, et al. Mechanism of Prunellae Spica on Non-small Cell Lung Cancer Based on Network Pharmacology, China Journal of Experimental Traditional Medical Formulae, vol. 25(2019) No.24, p.159-165.
- [5] Yu, J.H. Jian, T.T. Lou. Network Pharmacology-based Study on Material Basis and Mechanism of Liujunzi Decoction for Gastric Cancer Treatment, Chinese Journal of Modern Applied Pharmacy | Chin J Mod Appl Pharm, vol. 36(2019) No.12, p.1508-1515.
- [6] S. Li. Network target: a starting point for traditional Chinese medicine network pharmacology, China Journal of Chinese Materia Medica, vol. 36(2011) No.15, p.2017-2020.
- [7] Veldhoen M. Interleukin 17 is a chief orchestrator of immunity, Nature Immunology, vol. 18(2017) No.6, p. 612-621.
- [8] W. Luo, Q.B. Liu, J. Nan, et al. Isorhamnetin inhibited migration and invasion via suppression of Akt/ERK-mediated epithelial-to-mesenchymal transition(EMT) in A549 human non-small-cell lung cancer cells, Bioscience Reports, vol. 39(2019) No.9.
- [9] F. Yao, Q.Y. Zhou, Y. Xiong, et al. Protective Effects of β -Sitosterol on Acute Lung Injury Induced by Lipopolysaccharide in Mice, Chinese Agricultural Science Bulletin, vol. 31(2015) No.2, p.55-61.
- [10] Devi K P, Malar D S, Nabavi S F, et al. Kaempferol and inflammation: From chemistry to medicine, Pharmacological Research, vol. 99(2015) p.1-10.
- [11] LI W, CHEN Z, YAN M, et al. The protective role of isorhamnetin on human brain microvascular endothelial cells from cytotoxicity induced by methylglyoxal and oxygen glucose deprivation, Journal of Neurochemistry, vol. 136(2016) No.3, p.651-659.
- [12] G.L. Kang, Z.X. Jing. Kaempferol alleviates ox-LDL-mediated endothelial cell injury via regulating AMPK / Nrf2 / HO-1 signaling pathway, Chinese Journal of Immunology, vol. 34(2018) No.4, p.51-56.
- [13] Wang Y, Xing F, Ye S, et al. Jagged-1 signaling suppresses the IL-6 and TGF- β treatment-induced Th17 cell differentiation via the reduction of ROR γ t/IL-17A/IL-17F/IL-23a/IL-12rb1, Scientific Reports, vol. 5(2015) No.1, p.1-10.
- [14] Zhu S, Qian Y. IL-17/IL-17 receptor system in autoimmune disease: mechanisms and therapeutic potential, Clinical science, vol. 122(2012) No.11, p.487-511.
- [15] G.X. Gui. Effect of myrtin A on TNF- α signaling pathway in mice with endotoxin acute lung injury, Chinese Manipulation & Qi Gong Therapy, vol.8(2017) No.4, p.75-78.
- [16] X. Kong, S.H. Wu, L. Zhang, et al. Regulation Effects of Lipoxin A4 Receptor Agonist and Interleukin-1 β in Toll-Like Receptors/Myeloid Differentiation Factor 88/Nuclear Factor- κ B Signal Pathway on Asthma Mice with Airway Inflammation, Journal of Pediatric Pharmacy, vol. 24(2018) No.7, p. 1-6.
- [17] J. Xie, S. Gao, L. Li, et al. Research progress and application strategy on network pharmacology in Chinese materia medica, Chinese Traditional and Herbal Drugs, vol. 50(2019) No.10, p.2257-2265.