ORIGINAL RESEARCH

Differential Gene Analysis of Ferroptosis in the Treatment of Allergic Rhinitis with Bu-Zhong-Yi-Qi-Decoction Based on GEO Using Network Pharmacology and Molecular Docking

Jingtu Cen, PhD; Lihua Wang, MD; Haopeng Zhang, PhD; Lin Ji, MD; Yu Guo, BD

ABSTRACT

Objective • The differential gene analysis of ferroptosis inreating allergic rhinitis with Bu-Zhong-Yi-Qi-Decoction based on GEO using network pharmacology and molecular docking .

Method • This study used databases such as TCMSP to search for traditional Chinese herbal medicine's active ingredients and targets in Bu-Zhong-Yi-Qi-Decoction in treating allergic rhinitis. GeneCards, OMIM, TTD, and PharmaGkb were used to obtain disease targets for allergic rhinitis, and R language was used to screen Bu-Zhong-Yi-Qi-Decoction as the main target for treating allergic rhinitis. Retrieve the gene dataset of allergic rhinitis using the GEO database, analyze ferroptosis-related genes, and select the intersection of effective targets of Bu-Zhong-Yi-Qi-Decoction for treating allergic rhinitis and ferroptosis-related genes of allergic rhinitis, draw protein interaction networks using the STRING database, use Cytoscape software to construct the target regulatory network of Bu-Zhong-Yi-Qi-Decoction for treating allergic rhinitis and ferroptosis related genes, and then use the CytoNCA plugin to screen key targets. Using R language, Gene ontology, and the biological pathway enrichment analysis were performed on the predicted targets related to the treatment of allergic rhinitis and ferroptosis with Bu-Zhong-Yi-Qi-Decoction. Selecting key targets and active ingredients for molecular docking to explore the potential mechanism of Bu-Zhong-Yi-Qi-Decoction in treating ferroptosis in allergic rhinitis.

Result • After searching the TCMSP database, a total of 182 active ingredients were obtained from 8 traditional Chinese medicines of Bu-Zhong-Yi-Qi-Decoction, such as naringenin, kaempferol, Isorhamnetin, corresponding to 3023 targets and 2025 targets related to allergic rhinitis. There are 30 remarkably enriched Go analyses for biological function of potential target genes of Bu-Zhong-Yi-Qi-Decoction in allergic rhinitis, such as regulation of apoptotic signaling pathway, cellular response to peptide, wound healing, etc. Among them, there are 7 key genes related to the treatment of allergic rhinitis and ferroptosis with Bu-Zhong-Yi-Qi-Decoction, namely TP53, MAPK1, MAPK14, HIF1A, AR, CAV1, GSK3B.

Conclusion • The treatment of allergic rhinitis with Bu-Zhong-Yi-Qi-Decoction is a process involving multiple divisions, targets, and pathways. These results indicated that oral Bu-Zhong-Yi-Qi-Decoction may effectively treat allergic rhinitis in clinical practice. (*Altern Ther Health Med.* 2024;30(1):366-373).

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INTRODUCTION

The objective of this article is the differential gene analysis of ferroptosis inreating allergic rhinitis with Bu-Zhong-Yi-Qi-Decoction based on GEO using network pharmacology and molecular docking. Allergic rhinitis (AR) is a serious allergic disease. It is a chronic inflammatory disease of nasal mucosa involving the release of IgE-mediated inflammatory mediators and the participation of a variety of immune active cells and cytokines, which is caused by the contact of atopic individuals with allergen. This disease is one of the most common in otolaryngology, affecting a large population. The incidence rate of this disease in European and American countries is about 10-30%, or even more.[1] At present, the treatment of allergic rhinitis mainly relies on antihistamines such as cetirizine. However there are adverse reactions such as dry mouth and drowsiness. Therefore, it is necessary to seek safer and less adverse drug reactions for the treatment of allergic rhinitis. Bu-Zhong-Yi-Qi-Decoction (BZYQD) is a traditional

Chinese medicine compound that includes 8 types of herbs: ginseng, licorice, atractylodes macrocephala, angelica sinensis, tangerine peel, astragalus membranaceus, cohosh, and bupleurum. Therefore, it contains a variety of active ingredients with anti-inflammatory, anti-allergic, and immunomodulatory effects. It's effective for various diseases. Research[2] has shown that as an adjuvant treatment for cancer patients after chemotherapy, BZYQD can significantly prolong their survival time. In addition, BZYQD can reduce the level of serum inflammatory factor IL-1β and TNF- α, then suppress nuclear transcription factor NF-κ Phosphorylation of B, and finally downgrade the inflammatory factor IL-1β and IL-6, thereby alleviating constipation. BZYQD in treating AR is effective. For example, the combination of BZYQD and sublingual administration of dust mite drops was more efficient compared to simple sublingual administration of dust mite drops.[3] The relieving effect of BZYQD in treating AR may be achieved by inhibiting the release of SP and the expression of SPR in the nasal mucosa, reducing the infiltration of mast cells, and alleviating tissue inflammatory reactions.[4] Ferroptosis is a new type of programmed cell death that is iron-dependent and distinct from cell apoptosis, necrosis, and autophagy.

Previous studies have shown that the OVA-induced ferroptosis program in B cells of AR mice can reduce the production of specific IgE, thereby alleviating allergic inflammatory reactions.[5] Network pharmacology is a new discipline based on the fundamental theories of systems biology, multi-directional pharmacology, and molecular network analysis.[6] Network pharmacology technology can be used to explore the interaction patterns and regulatory mechanisms of multiple components, targets, and pathways in treating allergic rhinitis with BZYQD to explore its pharmacological substance basis and potential targets of action. This study first screened the effective ingredients of BZYQD, and then analyzed and summarized the target effects of the effective ingredients in allergic rhinitis.

Then, analyze the ferroptosis genes associated with allergic rhinitis through the GEO database. Finally, the effective targets of BZYQD for treating allergic rhinitis were intersected with the genes related to ferroptosis in allergic rhinitis. The active component targets and target pathways related to ferroptosis were studied through gene network visualization analysis, key genes, and immune correlation analysis, Gene ontology (GO), and the biological pathway (KEGG) enrichment analysis. Based on the above results, molecular docking technology was used to analyze the key gene docking between the effective ingredients in BZYQD and ferroptosis, providing a theoretical basis for the molecular mechanism of BZYQD in treating allergic rhinitis. This study utilizes data mining methods for biological research , and computer programming language may shine brightly in future biological research.

MATERIALS AND METHODS

Screening of Drug Components and Their Action Targets

On the data platform and analysis platform of Systems pharmacology of traditional Chinese medicine (TCMSP), http://tcmspw.com/tcmsp.php, http://tcmsp-e.com,, Retrieve the drugs in BZYQD and collect chemical composition and target information of the drugs based on literature.[7] Oral bioavailability (OB) and Drug Likeness (DL) are important indicators for evaluating whether a compound can develop into a drug. According to relevant research, this study selected chemical components that meet both OB ≥ 30% and DL ≥ 0.18 as candidate active ingredient,[8] and used the Uniprot database[9] (https://www.uniprot.org/). Correct and unify the target name. Screen the effective ingredients of inseng, licorice, atractylodes macrocephala, angelica sinensis, tangerine peel, astragalus membranaceus, cohosh, and bupleurum.

Screening of targets for AR

Based on the GeneCards database[10] (https://www.genecards.org/), OMIM database (https://www.omim.org/), PharmGKB database[11] (https://www.pharmgkb.org/), TTD database[12] (https://db.idrblab.net/ttd/), DrugBank database (https://go.drugbank.com/) contains information and protein targets related to the treatment of diseases, using the above database and searching with the keyword "Allergic Rhinitis" to obtain targets related to AR. Use the relevant program package "Venn" in R language to intersect the disease targets retrieved from the above database and construct a Venn diagram.

Screening of drug-disease common targets

After collecting the targets of drug components and diseases,draw a Venn diagram of the intersection target of BZYQD and AR using the relevant program package "Venn" in R language, and obtain the common target of drugs and diseases.

Collect GEO gene dataset and differentially expressed genes related to ferroptosis

Gene Expression Omnibus database (*GEO*)[13,14] provides a large set of genes and clinical data, which can be used to study related gene changes and pathways that affect disease occurrence, progression, and prognosis. This study used the search term "allergic rhinitis," and the species' location was "Homo sapiens". The relevant dataset was searched in the GEO database as of June 2022. Using R language-related packages such as "pacman" to standardize and log 2 convert the microarray raw data of the allergic rhinitis-related gene set retrieved by the CEO, then merge the preprocessed data and perform the batch correction. Using the "Limma" package to screen differentially expressed genes related to iron death with FDR adjust $P < .05$ and | log Fold Change |>0.2 as the standard, and draw heat maps and volcano maps.

Construction of Gene Network and Key Target Screening for ferroptosis Related Genes in the Treatment of AR with BZYQD

Using Cytoscape 3.9.1 software, a "component target" interaction network diagram was constructed by intersecting the differentially expressed genes related to ferroptosis in allergic rhinitis with the active ingredients of BZYQD.

Subsequent analysis was conducted based on the visualization model-related network feature value data. Input the common targets related to BZYQD and ferroptosis in allergic rhinitis obtained from the above network visualization model into the STRING database (https://cn.string-db.org). Retrieve and select a minimum required interaction score>0.4 to construct a PPI network and obtain its target genes. Import the target gene into Cytoscape and perform topology analysis using the CytoNCAt plugin function. Select 6 indicators: betweenness centrality (BC), closeness centrality (CC), degree centrality (DC), eigenvector centrality (EC), local average connectivity (LAC) based on the local mean method, and network centrality (NC). Use R language-related program packages such as "Pacman" to calculate the correlation analysis between key genes, immune cells, and immune function.

KEGG enrichment analysis and GO annotation analysis

Using R language-related program packages such as "clusterProfiler", "enrichplot", "pathview", "org.Hs.eg.db" and "ggplot2". GO function and KEGG pathway enrichment analysis were performed on the key genes obtained ($P < .05$). The top 30 GO functions and top 30 KEGG pathways significantly enriched with GO were selected, and bubble and bar plots were drawn, respectively.

Molecular Docking

Download core compounds from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and 2D structures of target proteins from PDB database, perform preliminary processing using ChemDraw 20.0 software, and then download gene structures from Uniprot database (https://www.uniprot.org/). Docking of active ingredients with target genes through CB-Dock[15,16] (http://cao.labshare.cn/cbdock/) online molecular docking.

RESULT

Active ingredients and related targets of BZYQD

After searching the TCMSP database, a total of 182 active ingredients were obtained from 8 traditional Chinese medicines of BZYQD, corresponding to 3023 target points.

Screening of targets related to AR

Retrieved 1950 targets related to AR from the GeneCards database; Retrieved 3 targets related to AR from the OMIM database; Retrieved 9 targets related to AR from the PharmaGKB database; Retrieved 24 targets related to AR from TTD database; 306 targets related to AR were retrieved from the DrugBank database. After merging the search results of various databases and deleting duplicate targets, a total of 2025 targets related to AR were obtained. The Venn map is shown in Figure 1.

Screening of intersection targets between BZYQD and AR

The obtained drug targets were first corrected and unified through the Uniprot database, and a Venn diagram of the intersection targets of BZYQD and AR was drawn by

Figure 1. Venn map of targets related to AR

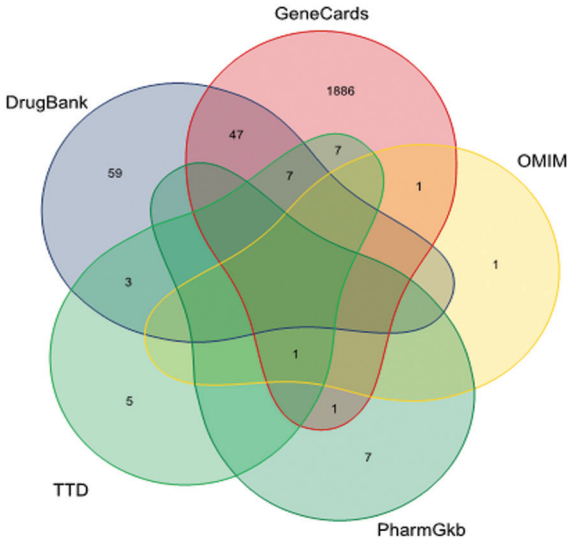


Figure 2. Venn diagram of the intersection target of BZYQD and AR

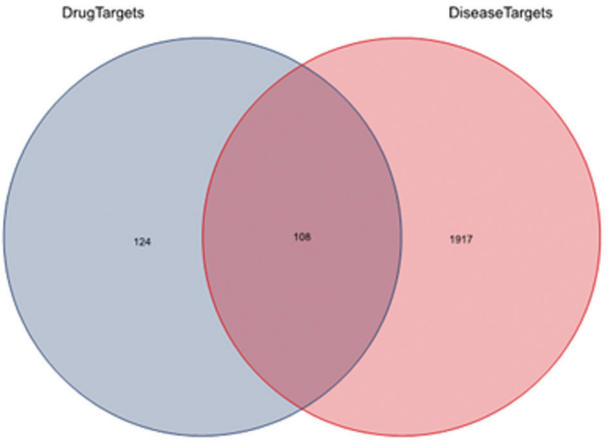


Figure 3. Differential expression gene heatmap associated with ferroptosis in AR

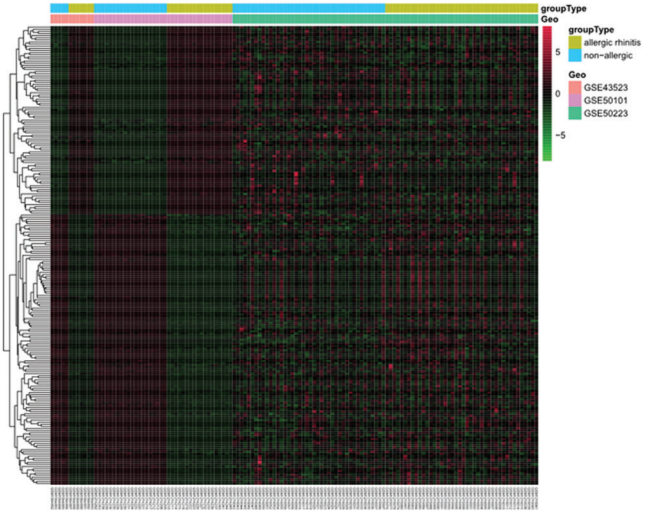


Figure 4. Volcano map of differentially expressed genes related to ferroptosis in AR

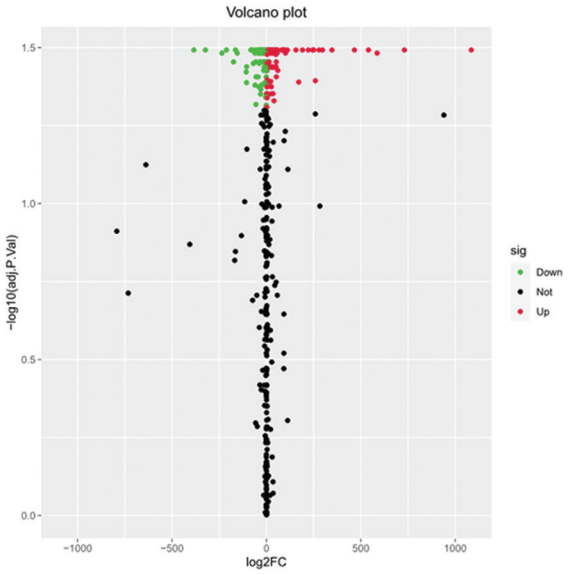


Figure 5. Venn diagram of ferroptosis related targets in the treatment of AR with BZYQD

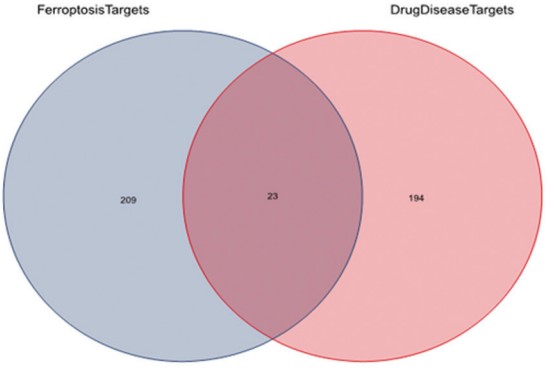


Figure 6. Network diagram of target points related to ferroptosis in AR treated with BZYQD

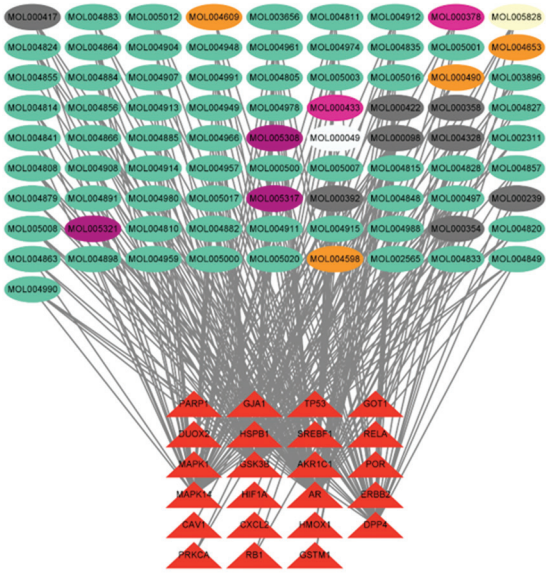
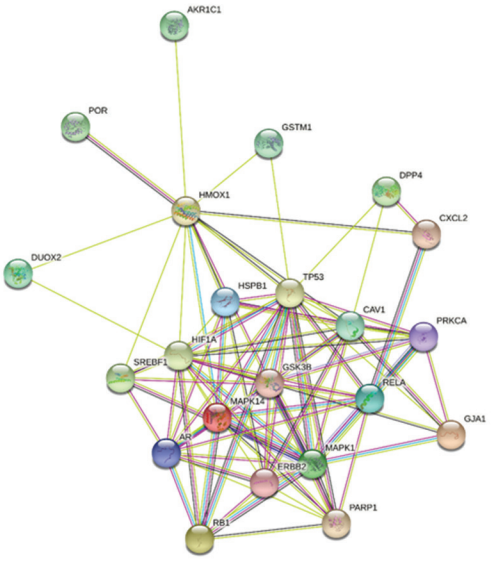


Figure 7. Intersection Target PPI Network Diagram



using R language. A total of 108 common targets were obtained, as shown in Figure 2.

Collect the GEO gene dataset

Retrieve and collect mRNA microarray research datasets, including GSE43523, GSE50101, and GSE50223. The heat maps of differentially expressed genes related to ferroptosis and volcano maps are shown in Figures 3 and 4.

Gene Network Diagram and Key Target Visualization Analysis of Ferroptosis-Related Genes in the Treatment of AR with BZYQD

The target of the active ingredient action of BZYQD in treating AR was intersected with the differential genes related to ferroptosis in AR obtained from the GEO database, and a Venn diagram was drawn, as shown in Figure 5. 23 intersected targets were obtained, corresponding to 82 active ingredients. A network diagram was constructed using Cytoscape 3.9.1 software, as shown in Figure 6. Upload 23 intersecting targets to STRING to construct a PPI network, as shown in Figure 7. Use R language to analyze the functional analysis of 23 intersection targets and immune cells, as shown in Figure 8. The CytoNCA plugin screened targets based on network node topology attributes, as shown in Figure 9. The screening criteria were 6 indicators for each target, including BC, CC, DC, EC, LAC, and NC, all exceeding the median value. A total of 7 key genes related to the treatment of AR and ferroptosis with BZYQD were selected, including TP53, MAPK1, MAPK14, HIF1A, AR, CAV1, GSK3B, as shown in Figure 10.

Enrichment Analysis of GO and KEGG Pathways of Ferroptosis-Related Genes in the Treatment of AR with BZYQD

By conducting GO and KEGG pathway analysis on 23 genes related to **ferroptosis** in the treatment of AR with

Figure 8. Analysis of intersection target and immune cell functional analysis

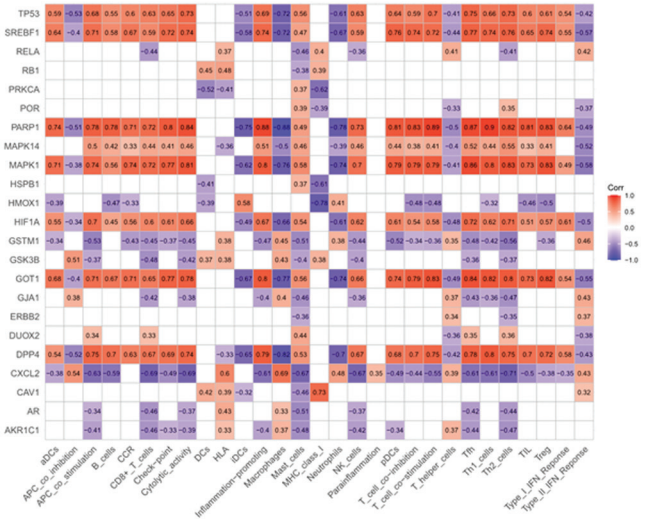
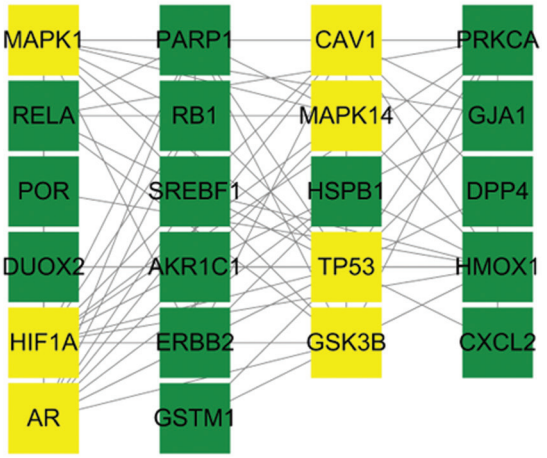


Figure 9. Topological Analysis of the Network Related to Ferroptosis in the Treatment of AR with BZYQD



BZYQD, 1482 biological processes, and 148 KEGG signaling pathways were obtained. There are a total of 30 remarkably enrichment analyses of KEGG pathways of BZYQD in treating AR, such as IL-17 signaling pathway, T cell receptor signaling pathway ,Th17 cell differentiation, etc. Partial results are shown in Figures 11 and 12.

Molecular Docking

As lower vina scores indicate a stronger and stable interaction between the compound and receptor. Selecting three active components from the network target genes, naringenin, kaempferol, and Isorhamnetin, can bind to key genes TP53, MAPK1, and HIF1A to varying degrees, as shown in Figures 12, 13, and 14. This may point the way for further experimental research.

Figure 10. Network Diagram of Key Genes Related to Ferroptosis in the Treatment of AR with BZYQD

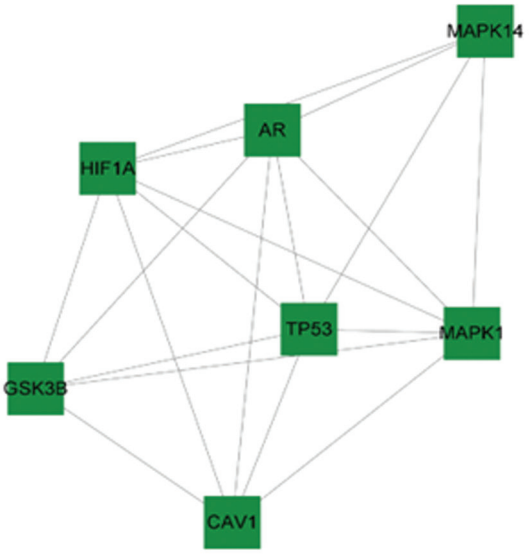


Figure 11. GO Analysis Bubble Chart

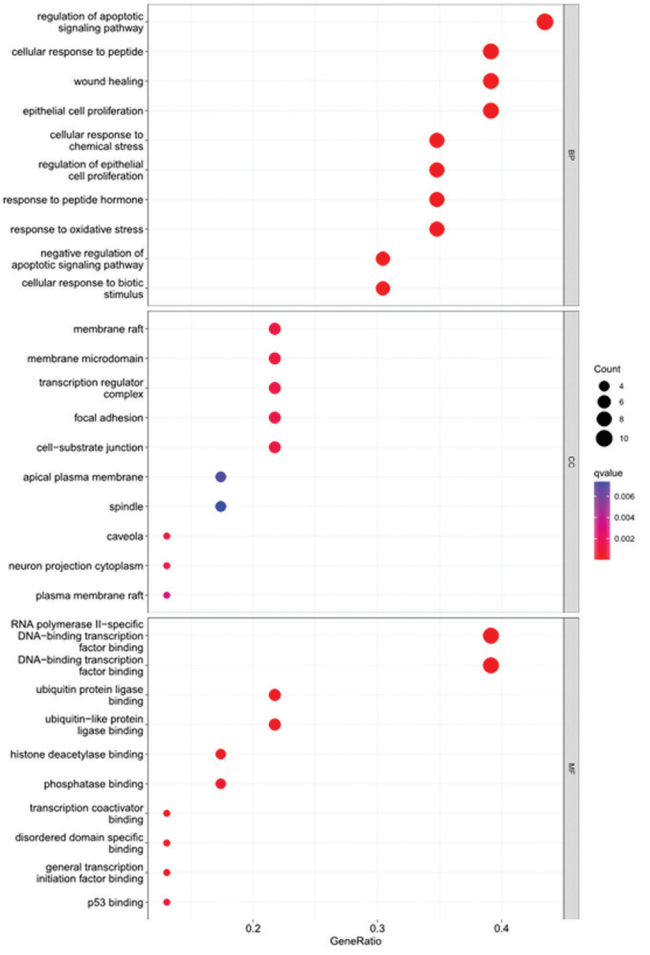


Figure 12. KEGG Analysis Histogram

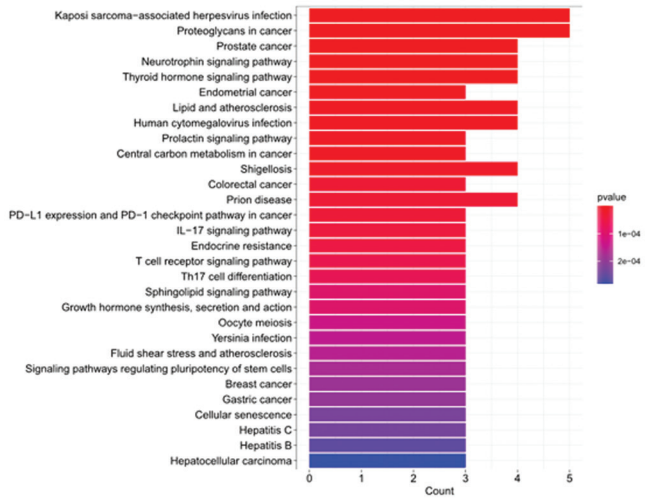


Figure 13. Naringenin-TP53 Molecular Docking

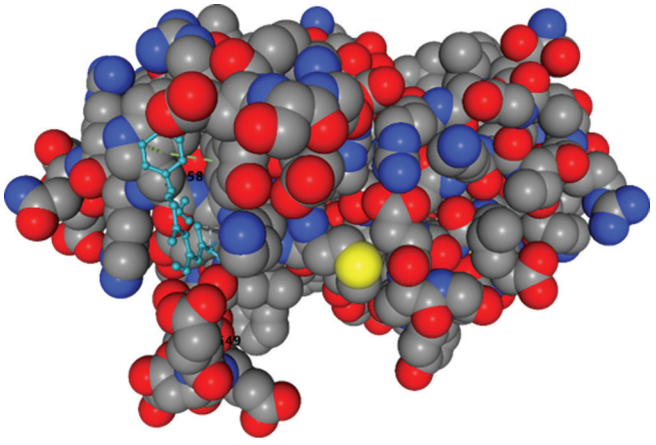


Figure 14. Kaempferol- MAPK1 Molecular Docking

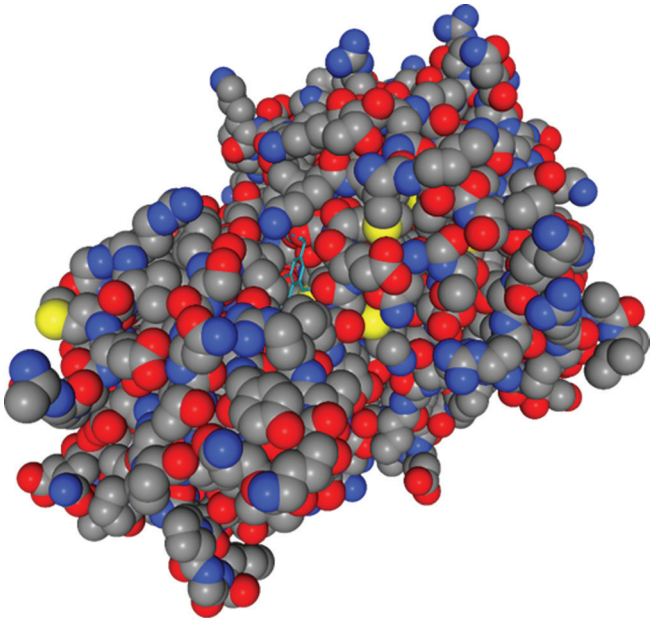
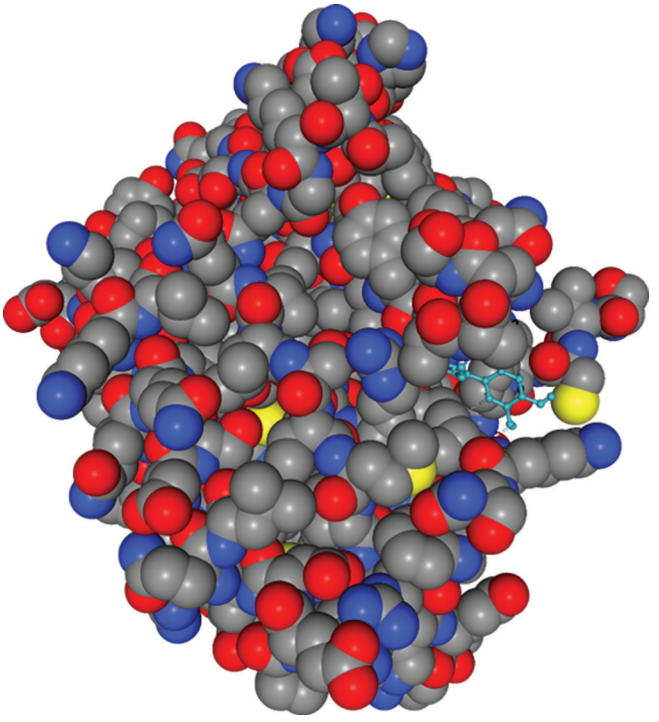


Figure 15. Isorhamnetin-HIF1A Molecular Docking



DISCUSSION

This study combined network pharmacology methods with GEO database to analyze the molecular mechanism of ferroptosis-related targets in the treatment of AR with BZYQD. From a micro perspective, it explained the mechanism of action of traditional Chinese medicine prescriptions, which has certain innovation. Currently, no similar research has been seen.

Ferroptosis is an iron-dependent, new type of programmed cell death, which is different from apoptosis, necrosis, and autophagy.[17] The main mechanism of ferroptosis[18,19] is that under the action of divalent iron or ester oxygenase, it catalyzes the unsaturated fat acid highly expressed on the cell membrane to produce lipid peroxidation, thus inducing cell death. In addition, it is also manifested as a decrease in the expression level of antioxidant systems (glutathione GSH and glutathione peroxidase 4-GPX4). Research has found that ferroptosis is closely related to respiratory diseases, such as chronic obstructive pulmonary disease,[20] and asthma.[21] In the study on ferroptosis and asthma, Nan Yang et al.[22] constructed an asthma mouse model using OVA and established IL-3 induced cell types in bronchial epithelial cells. It was found that treatment with ferrostatin-1 and 3-methyladenine reduced iron deposition in BEAS-2B cells and lung tissue of IL-13 induced asthma mice, thereby improving asthma in vivo and in vitro. Allergic rhinitis and asthma are both allergic diseases. Yutaka Nakamura et al.[5] found that dietary iodine alleviates allergic rhinitis by inducing activated B cells to remove iron. Weijing Gu et al.[23] found that ferroptosis is involved in acute nasal epithelial injury induced by PM2.5 through AMPK mediated autophagy. These all indicate that ferroptosis is

involved in the nasal inflammatory response. Therefore, this study attempts to explore the role of ferroptosis genes through network pharmacology research on the treatment of AR with BZYQD.

Through key gene analysis, it can be seen that a total of 7 genes, including TP53, MAPK1, and HIF1A, are associated with iron death. TP53 is generally located on human chromosome 17P13, mainly composed of 11 exons and 11 introns. It is an important tumor suppressor gene that can control cell growth and proliferation in the human body to maintain normal life activities. If certain factors stimulate or carcinogenic factors affect, leading to the loss of monitoring of tumor suppressor genes, it is prone to carcinogenesis.[24] At present, there is no report on its role in allergic diseases. This study is the first to discover its role in allergic rhinitis, which may be related to its ability to inhibit inflammatory reactions. However, further research and verification are needed. MAPK1 is a member of the mitogen-activated protein kinase family. Most MAPK actually participate in the response to potentially harmful abiotic stress stimuli (high osmotic pressure, oxidative stress, DNA damage, low osmotic pressure), which indicates that MAPK can be a potential therapeutic target for inflammatory diseases.[25] Hypoxia-inducible factor-1 was first discovered by Semenza and Wang in 1992 subsequently establishing the structure of HIF-1 and demonstrating its cDNA encoding sequence. HIF-1 is a nuclear protein with transcriptional activity with a wide range of target gene profiles, including nearly 100 target genes related to hypoxia adaptation, inflammatory development, and tumor growth.[26-28] Inducing abnormal HIF under normoxic conditions may lead to diseases containing chronic inflammatory components. Because of the abnormal microenvironment, transcription factors are activated abnormally, leading to changes in the balance of growth factors, chemokines, cytokines, and active oxygen balance in cells, which is not enough to provide growth needs, causing inflammation.

This study found through network pharmacology and molecular docking that Naringenin-TP53, Kaempferol MAPK1, and Isorhamnetin HIF1A have strong binding ability and relatively stable binding. In the screening of active ingredients in drugs, we predicted that naringenin, kaempferol, isorhamnetin, and others may play important roles in the treatment of allergic rhinitis. Naringenin, kaempferol, and isorhamnetin are flavonoids that have various pharmacological effects, such as immune regulation, anti-tumor, antioxidant, and anti-allergic effects.

After heat treatment with naringenin, Maatouk et al.[29] found that the cytotoxicity of mouse T cells was inhibited, while the killing activity of NK cells was significantly increased. In addition, flavonoids can also participate in regulating cellular immunity by enhancing macrophage phagocytic activity and inhibiting dendritic cell activation pathways.[30,31] It is found that kaempferol can simultaneously inhibit leukotriene B4 and TNF- α Release of; β- Sitosterol has a strong inhibitory effect on IgE mediated PGE2 release in RBL-2H3 cells.[32] Isorhamnetin[33] has a wide range of pharmacological activities, which can protect cardiovascular and cerebrovascular systems, anti-tumor, anti-inflammatory, antioxidant, organ protection, and prevent obesity. Its related mechanisms involve PI3K/AKT/PKB、NF-κB、MAPK. The regulation of signaling pathways such as MAPK and the expression of related cytokines and kinases.

In further analysis of GO functional enrichment, the results showed that the treatment of AR with BZYQD was related to the function of Epithelial cell proliferation. Nasal mucosal epithelial function plays an important role in the pathogenesis of allergic rhinitis. Studies have shown that toll-like receptors 9 (TLR9) in nasal mucosal epithelial cells of patients with allergic rhinitis is higher than that of the control group.[34] In addition, during KEGG pathway analysis, the results showed that the main biological processes related to treating AR with BZYQD were the IL-17 signaling pathway and Th17 cell differentiation pathway. T helper cell 17 (Th17) is a newly discovered subset of T cells that can secrete interleukin 17 (IL-17), playing an important role in autoimmune diseases and body defense responses. Some studies have shown that IL-17 deficient mice reduced recruitment of eosinophils, neutrophils and mast cells after ovalbumin challenge, indicating that IL-17A recruited the above immune cells in the nasal mucosa.[35] In addition, IL-17A can also inhibit mast cell degranulation and regulate Th2 cytokines (IL-4, IL-5 and IL-13), including TNF- α And IL1 β The expression of pro-inflammatory cytokines regulates the pathogenesis of allergic rhinitis.[36] Therefore, IL-17A has a two-way regulatory role in allergic rhinitis, which can be considered as a neoplasm marker of disease progression and disease severity in AR, and it can also provide a therapeutic target to overcome clinical complications related to AR.[37]

CONCLUSION

In conclusion, the treatment of AR with BZYQD is a process involving multiple divisions, targets, and pathways. BZYQD exerts its therapeutic effect on allergic rhinitis through multiple key targets and pathways related to iron death, such as TP53, MAPK1, and HIF1A. At the same time, this study uses molecular docking technology to verify the interaction between the active ingredients in BZYQD and the key targets related to iron death in AR, providing a basis for further exploration of its molecular mechanism. However, due to the diversity of traditional Chinese medicine ingredients and the complexity of their mechanisms of action, relying solely on network pharmacology and bioinformatics prediction has certain limitations. Therefore, further validation through in vitro and in vivo experiments is needed to better explore the iron death related mechanism of BZYQD in treating AR. In addition, as the small sample size of clinical researches on the treatment of AR with BZYQD, evidence from multicenter, large sample randomized controlled trials is still needed. And the results of this study can be integrated to extract the effective ingredients of BZYQD so as to treat AR more accurately in the future.

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