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The Mechanism of Gancao Xiexin Decoction in the Treatment of Behçet's Disease Based on GEO Differential Analysis and Network Pharmacology

Xin Zhang*

Department of Ophthalmology, The First Affiliated Hospital of Zhejiang Chinese Medical University (Zhejiang Provincial Hospital of Chinese Medicine) Hangzhou, China

*Corresponding Author

Xin Zhang, Department of Ophthalmology, The First Affiliated Hospital of Zhejiang Chinese Medical University (Zhejiang Provincial Hospital of Chinese Medicine), E-mail: jangwoohyukhot@163.com

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Abstract

Background: To analyze the main zcomponents and possible mechanisms of Gancao Xiexin decoction in the treatment of Behçet's disease (BD) using network pharmacology combined with GEO database.

Methods: The GSE and GPL files of Behçet's disease were downloaded from the GEO database, and the differential genes were analyzed R software to obtain potential disease targets. The TCMSP database was used to search for compounds in Glycyrrhiza radix, Pinellia pinellia, Scutellaria radix, Rhizoma coptidis, dried ginger, jujube and ginseng in Gancao xiexin decoction. The oral bioavailability (OB)≥30% and drug-like properties (DL)≥0.18 were used as the criteria to screen the active components and find their corresponding targets. The intersection of drug target genes and disease differential genes was taken, and the "drug-compound - target" regulatory network and protein-protein interaction network were constructed by Cytoscape3.8.0 software, and the core targets were screened by topological analysis. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed using R software.

Results: A total of 3769 differential genes were obtained, including 201 active components of Gancao Xiexin decoction, corresponding to 1916 target genes. There were 46 proteins in the PPI core network, which were mainly involved in NTRK1, EGFR, CUL3, APP, TP53, MCM2, XPO1, ESR1, FN1, etc. GO enrichment analysis showed that 825 pathways were obtained from biological process,

19 pathways were obtained from cellular components, and 41 pathways were obtained from molecular function. They were mainly enriched in biological processes such as response to metal ion, response to oxidative stress and response to radiation. A total of 149 were identified by KEGG pathway analysis. It mainly involves Human cytomegalovirus infection, Kaposi sarcoma-associated herpesvirus infection, and Human T-cell leukemia virus 1 infection, Lipid and atherosclerosis, Chemical carcinogenesis – reactive oxygen species, etc.

Conclusion: Gancao Xiexin decoction has the characteristics of multi-component, multi-target and multi--pathway in the treatment of Behçet's disease. It plays a role in regulating the body's immune and inflammatory response by acting on the targets of MAPK1, MAPK3, AKT1, CHUK and other targets, which lays the foundation for deeper exploration of the therapeutic mechanism.

Keywords: GEO; Network Pharmacology, Gancao Xiexin Decoction, Behçet's Disease, Mechanism of Action

Abbreviations: BD = Behçet's disease, OB= bioavailability, DL= drug likeness, GO =Gene Ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes, PPI=protein protein interaction, BP=biological processes, CC=cellular components, MF =molecular functions, IL-17= interleukin-17, MCP-1= monocyte chemoattractant protein-1, MMP9 =matrix metalloproteinase -9,NTRK1= neurotrophic tyrosine kinase receptor type 1, EGFR = epidermal growth factor receptor, APP = amyloid precursor protein, TP53= tumor protein p53, MCM2 = minichromosome maintenances 2, XPO1= exportin 1, ESR1= estrogen receptors 1, FN1= fibronectin-1, HSP90= heat-shock protein 90.

Introduction

Behçet' disease (BD) is a variable vessel vasculitis with multi--system and multi-organ involvement [1] characterized by uveitis, oral ulcers, pleomorphic skin lesions, and genital ulcers. There is no effective cure for this disease. Management of Behçet's disease relies on corticosteroid therapy, conventional immunosuppressive drugs and biological agents [2]. However, BD often presents in the form of relaping-remitting. The long-term use of hormones and immunosuppressants will invitably have an impact on the body. Therefore, traditional Chinese medicine methods are sought to further understand the characteristics and curative effects of the disease. There is a discussion on "Fox confusion disease" in Treatise on febrile diseases, which holds that BD is similar. When the heat evil is multiplied, the throat swells and aches, and when wet evil is injected, the two Yin will fester. This can also be seen in Article 10 of the synopsis of the golden chamber. Treatment of Yin-Yang toxin disease and pulse syndrome of lily and fox: "Fox confusion is a disease. Gancao Xiexin decoction is the main". This disease is characterized by the accumulation of dampness and heat. Dampness blocks heat depression and damages blood collaterals. This disease is based on the accumulation of dampness and heat. Dampness blocks heat depression and heat hurts blood collaterals [3]. The "Gancao Xiexin decoction" is based on the theory of the Qi mechanism mediated by Zhongjiao [4], pay attention to the regulation of the lifting hub of the coke gas machine in the spleen and stomach. In modern clinical application, Gancao Xiexin decoction has significant curative effects on BD [5], recurrent oral ulcer [6], ankylosing spondylitis [7], rheumatoid diseases and other diseases have significant curative effects.

In order to explore the molecular mechanism of Gancaoxixin decoction in the treatment of Behcet's disease more deeply and comprehensively, this study used network pharmacology combined with GEO database mining to explore the possible pathways and targets of its action.

Materials and Methods

The Corresponding Targets of BD were Screened

The data source file GSE61399 and the Gene annotation file GPL570-55999 were obtained from the GEO database of National Center for Biotechnology Information (NCBI) using the keyword "Uveitis". Twenty-nine samples were included in the microarray data, including 17 samples from BD patients (experimental group) and 12 samples from normal people (control group). Using R software (http://www.r-project.org/, version4.1.1) for data processing, to $|\log FC| > 0.5$, $P < 0.05$ for the filter conditions for differences in gene, that is, BD re-

lated genes. The ggplot2 package was used to draw the volcano map of differentially expressed genes. The pheatmap package generated a heatmap of the top 20 differentially expressed genes.

Screening of Active Ingredients and Corresponding Targets of Gancao Xiexin Decoction

Through the pharmacological database and analysis of traditional Chinese medicine (TCM) system platform (TCMSP, http://tcmspw.com/tcmsp.php) for the soup of licorice, licorice xie heart pinellia, radix scutellariae, rhizoma coptidis, dried ginger, jujube, the active components of ginseng and targets screening, using bioavailability (OB)>30% and drug-like (DL)>0.18 as screening conditions, the active components and their corresponding targets of 7 traditional Chinese medicines were obtained.

The Traditional Chinese Medicine Compound Regulation Network and PPI Network Were Constructed

The obtained BD target genes were compared with the effective target into Gancaoxiexin decoction, and the effective targets of traditional Chinese medicine on BD were predicted. Cytoscape3.8.0 software was used for visualization. The protein-protein interaction (PPI) network was constructed by Bisogenet plug-in in Cytoscape, and the network topology was analyzed by CytoNCA plug-in. The PPI network was filtered by degree centrality (DC)>61 and betweenness centrality (BC)>600.

GO and KEGG Analysis

The gene ontology (GO) [Biological process (BP), molecular function (MF), cell component (CC)] of the intersection genes of Gyaxiexin decoction and BD were analyzed by Cluster Profiler in R software, and the Kyoto Encyclopedia of Genes and Genomes (KEGG) was analyzed. The relationship network between KEGG and core genes was constructed through Cytoscape software, and the size of each node was set according to the number of connected nodes. Visualization processing was performed.

Results

Differential Gene Analysis by GEO

The gene expression data of CD4+T lymphocytes in peripheral blood of GSE61399 were downloaded from the GEO database. The platform used was GPL570-55999, and there were 12 normal people samples and 17BD patients samples. To | logFC | > 0.5, P < 0.05 for conditions were screened 3769 differentially expressed genes, which raised cut genetic gene, 1629, 2140. Volcano map of differentially expressed genes and heat map of top 20 differentially expressed genes (Figure1-2).

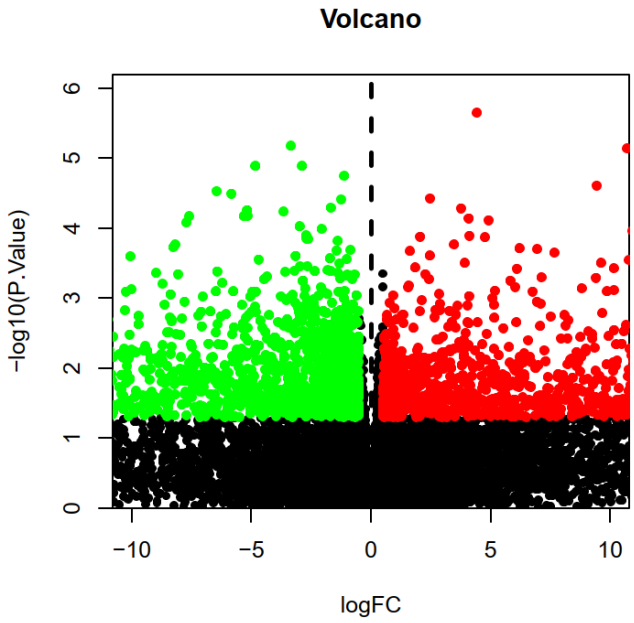

Figure1: Volcano plot of up-regulated and down-regulated genes in BD patients

Note: Red represents up-regulated genes. Green represents down-regulated genes; Black represents no difference genes

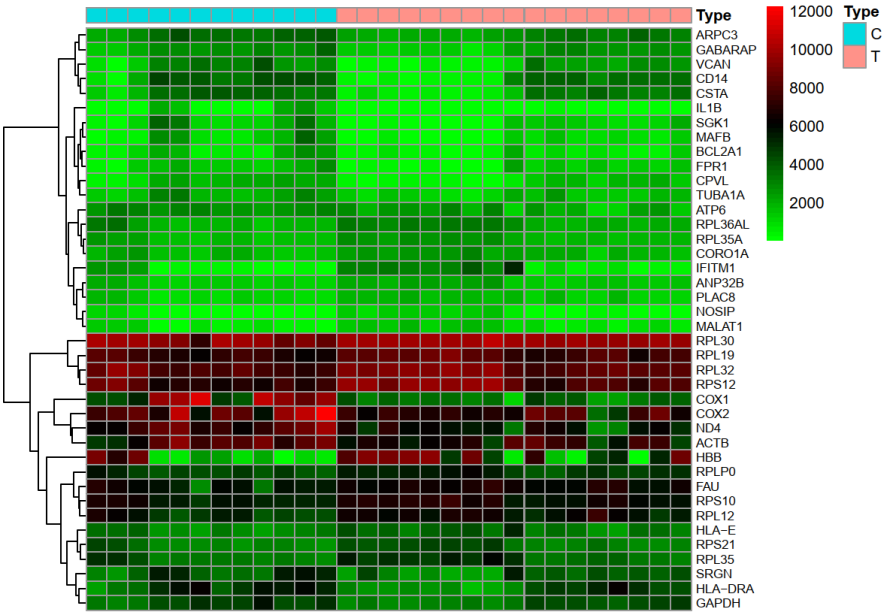

Figure 2: Heat map of differentially expressed genes between BD patients and healthy individuals

Note: The left side is the control group, and the right side is the experimental group. Red indicates high gene expression, black intermediate expression, and green low expression

Active Compounds and Targets of Gancao Xiexin Decoction

Seven traditional Chinese medicines were searched in the TCMSP database successively, and 280 chemical components of glycyrrhiza licorice, 143 chemical components of scutellaria scutellaria, 190 chemical components of ginseng, 148 chemical components of dried ginger, 48 chemical components of Chinese rhizome, 133 chemical components of jujube and 116 chemical components of pinellia pinellia were retrieved. With OB≥30.00% and medicinoid DL≥0.18 as the limit, 92 active ingredients meeting the conditions were screened out, including glycyrrhiza licorice, 36 scutellaria scutellaria, 22 ginseng, 5 dried ginger, 14 Rhizoma Phellodis, 29 jujube and 13 pinellia pinellia. The top 5 compounds with OB values were selected for data analysis (Table 1).

Construction of Drug-Compound-Target Regulatory Network

The obtained BD-related targets were matched with the targets of Gancao Xiexin decoction, and 46 potential genes of Gancao Xiexin decoction in the treatment of BD were obtained. The BD network of Gancao Xiexin decoction regulation was constructed by Cytoscape3.8.0 software, as shown in Figure 3.

Network Construction and Topological Analysis

The PPI network was constructed by Bisogenet plug-in of Cytoscape3.8.0 software. The CytoNCA plug-in was used to analyze the topology structure, and 3286 nodes were obtained after screening with degree value >61 as the standard. The core network was finally obtained by further screening with BC>600 as the standard, which contained a total of 800 nodes. The top 10 nodes with degree value were neurotrophic tyrosine kinase receptor type 1(NTRK1), epidermal growth factor receptor (EGFR), cullin3 (CUL3), Amyloid Precursor Protein (APP), tumor protein p53 (TP53), minichromosome maintenances 2(MCM2), Exportin 1(XPO1), Estrogen receptors 1(ESR1), fibronectin-1(FN1), and heat-shock protein 90(HSP90)AA1.These may be the key targets for the pharmacological effects of Gancaoxiexin decoction in the treatment of BD (Figure 4).

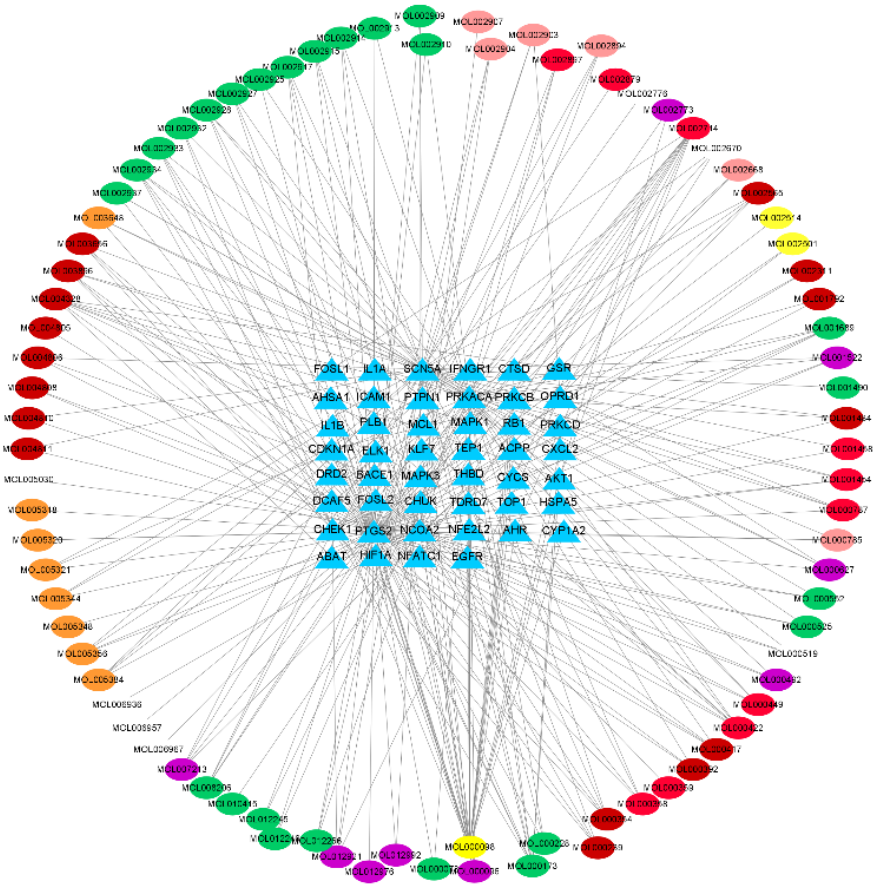

Figure 3: Drug-compound -target network of Gancao Xiexin decoction for the treatment of BD

Note: Blue triangle represents differential genes, oval represents active ingredients: dark red represents the active ingredient of licorice, pink represents the active ingredient of Rhizoma coptidis, white represents the active ingredient of pinellia, purple represents the active ingredient of jujube, green represents the active ingredient of scutellaria, yellow represents the active ingredient of dried ginger, orange represents the active ingredient of ginseng, light red represents the active ingredient of various drugs.

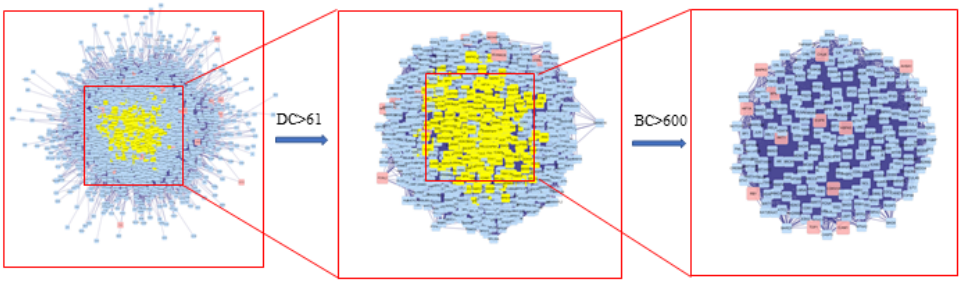

Figure 4: Process of PPI core network acquisition in the treatment of BD with Gancao Xiexin decoction

Target Pathway Analysis

GO enrichment analysis and KEGG pathway analysis of 46 intersection genes were performed by R software, and a total of 885 GO entries (P<0.05) and 149 KEGG pathways (P<0.05) were screened. The top 20 items of GO enrichment analysis were selected to draw a bubble plot (Figure 5). The targets were significantly enriched in biological processes such as response to metal ion, response to oxidative stress and response to radiation. Cellular components such as membrane raft and membrane microdomain, and molecular functions such as protein serine/threonine kinase activity.

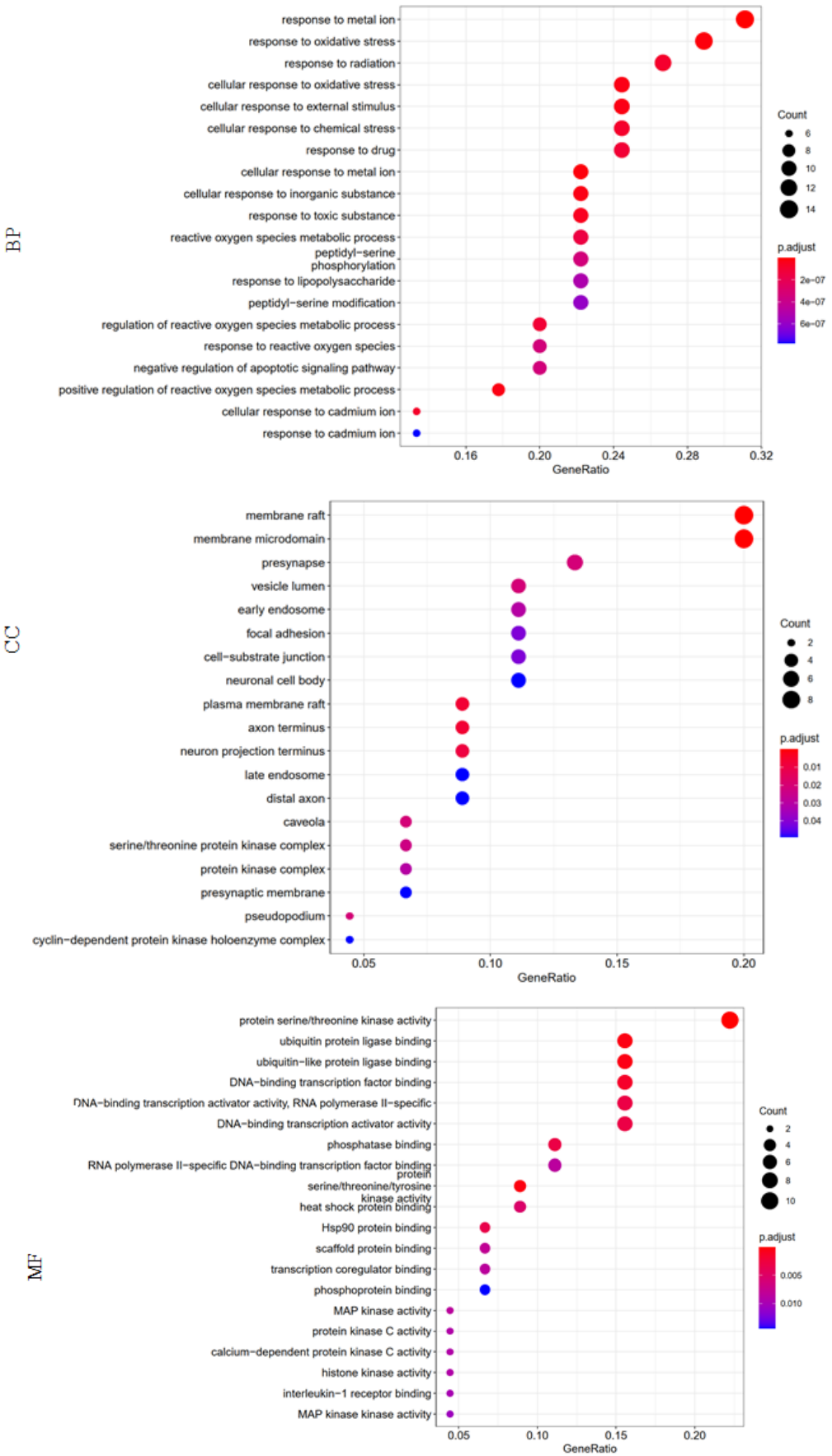

Figure 5: GO enrichment analysis of the BD targets treated with Gancao Xiexin decoction

The top 20 of KEGG pathway analysis were drawn into a bar chart (Figure 6), which is visible

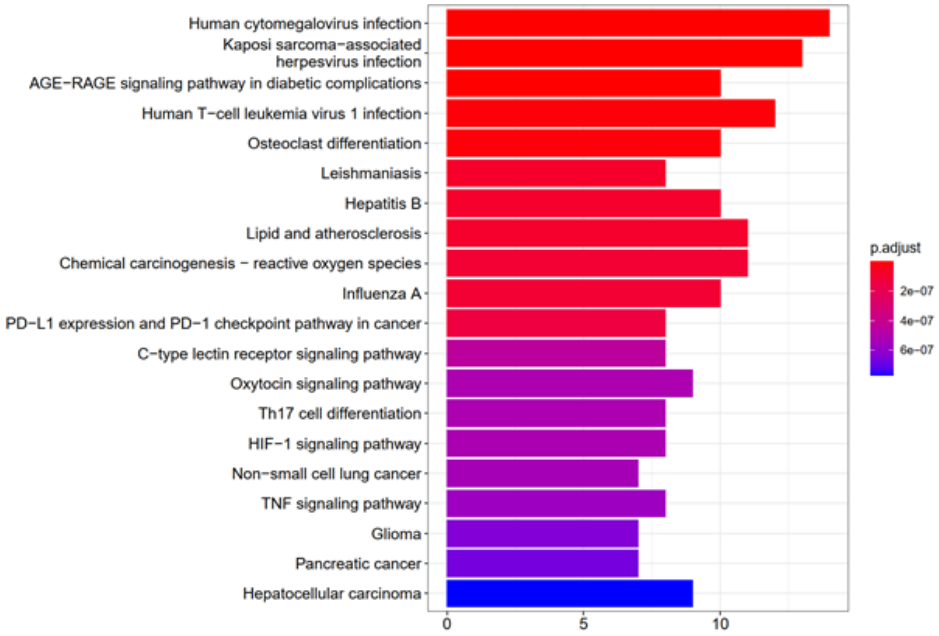

Figure 6: KEGG pathway analysis of BD targets treated with Gancao Xiexin decoction

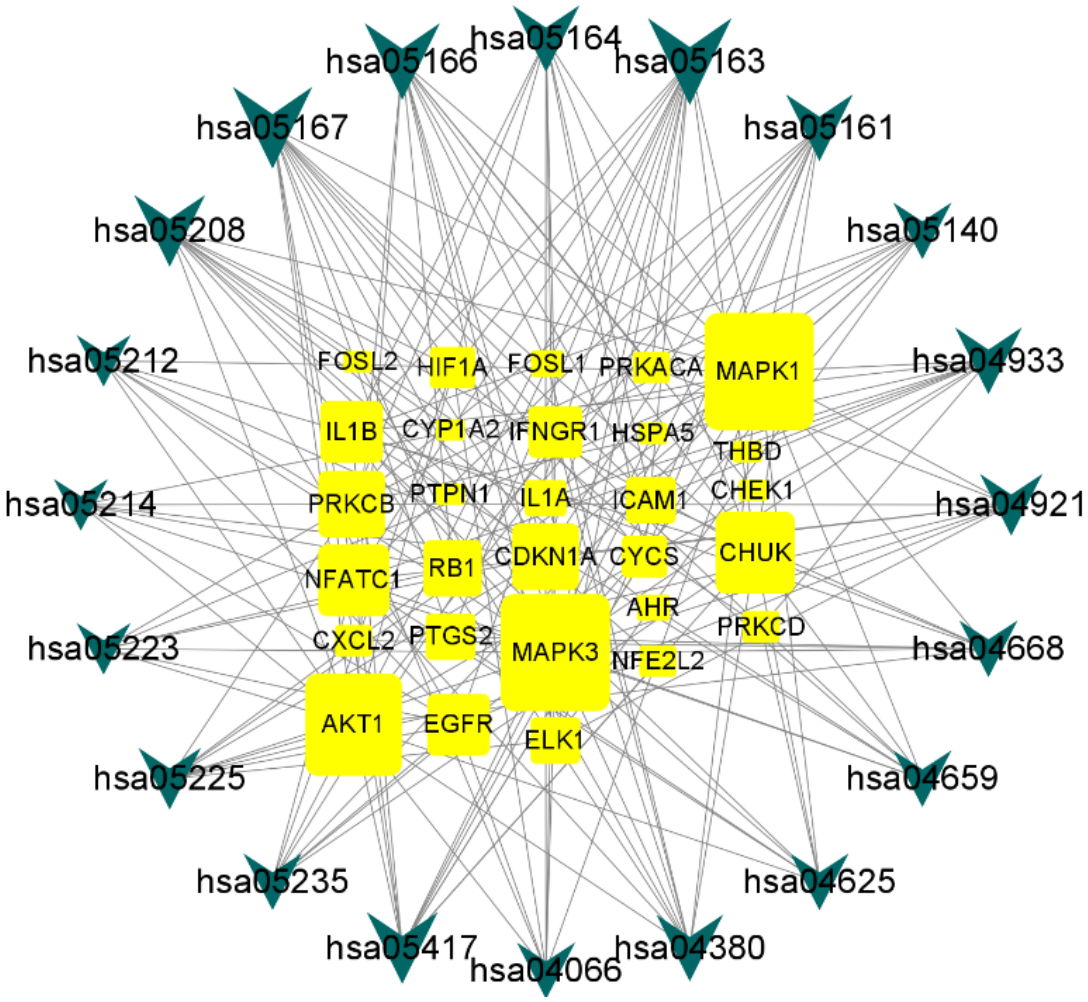

Figure 7: Network diagram of KEGG and core genes

Note: Green nodes are KEGG pathway names, yellow nodes are core genes. The size of the node graph is positively correlated with the number of connected nodes in the network.

Discussion

BD is a clinically refractory disease, and its etiology and pathogenesis are still unclear. BD belongs to the category of fox confusion in traditional Chinese medicine. The pathogenesis is the accumulation of dampness and heat, and gas corrods qi and blood. Because of the fire of the heart and stomach, liver and gallbladder damp heat accumulation knot. The evil of fire, poison, dampness and heat invades the eyes, invades the oral cavity, and radiates down into the vulva. Therefore, the triad of ulcers is seen in the eyes, mouth, and pudendum. Gancao Xiexin decoction is a classic prescription for treating fox confusion in the Jingui Yaolue (Synopsis of the Golden Chamber) by Zhang Zhongjing. With liquorice Ganping fire detoxification for the king, with scutellaria, Rhizoma coptidis cold pathogenic heat, detoxification and dehumidification, Pinellia, dried ginger, heat dry dampness open Yin coagulation and dampness, Codonopsis ginseng, licorice, jujube health in the coke to dampness. This prescription of Xinkaikujiang treats fox confusion from the spleen and stomach, because the spleen and stomach are weak, and the transportation is not good, which is the root cause of damp-heat.

In this study, 201 active ingredients of Gancao Xiexin Decoction were screened out, and the active ingredient-target network showed that quercetin, wogonin, baicalein, and ginsenoside were the main active ingredients of Gancao Xiexin decoction. Quercetin is a flavonoid, which has chemopreventive and therapeutic effects on a variety of diseases, including inhibition of oxidative stress, cytokine and COX2 production [8]. Previous studies have shown that quercetin can reduce pain and inflammation related to arthritis, inhibit mechanical hyperalgesia, edema and leukocyte aggregation in mouse knee joints in a dose-dependent manner [9]. Its mechanism includes inhibiting the proliferation and gene level of pro-inflammatory cytokine (TNF-α) in human peripheral blood monocytes, reducing the expression of inflammatory factors such as IL-1β, IL-6, and matrix metalloproteinases (MMP3, MMP9), inhibiting the production of inflammatory mediators, and improving inflammatory symptoms. Animal studies have shown that quercetin can improve pain symptoms by inhibiting the activation of MMP-9 and MMP-2 in spinal microglia in mice. Levels of interleukin-17 (IL-17) and monocyte chemoattractant protein-1 (MCP-1) [10]. Baicalein is an inhibitor, which has the functions of antibacterial xanthine oxidase, anti-oxidation, immune regulation, and so on. It can effectively inhibit itching free radicals produced in the process of xanthine oxidation. Its hydroxyl structure also gives it free radical scavenging activity, and by regulating the arachidonic acid metabolism pathway, It can inhibit the production of cyclooxygenase, dehydrogenase, nuclear factor and cytokines and play a powerful antipyretic and analgesic effect. Animal experiments have shown that baicalein can inhibit macrophage inflammatory response induced by lipopolysaccharide and adenosine triphosphate [11]. Ginsenoside Rh2 isa tetracyclic triterpenoid saponin monomer [12]. In addition to promoting the apoptosis of tumor cells, ginsenoside Rh2 has a significant anti-inflammatory effect and can inhibit the inflammatory response induced by bacterial Lipopolisaccharide (LPS). Bi [13] et al. reported that Rh2-B2 modified by increasing water-soluble groups significantly inhibited the release of TNF-α, IL-6 and IL-1β from RAW264.7 macrophages. In addition, Rh2 inhibited the production of PGE2, ROS and MMP-9 in LPS-stimulated RAW264.7 macrophages, and inhibited the TNF-α-induced MMP-9 gelatinase activity in keratinocytes.

According to the PPI network, NTRK1, EGFR, CUL3, APP, TP53, MCM2, XPO1, ESR1, FN1, and HSP90AA1 may be the key targets of Gancao Xiexin decoction in the treatment of BD. NTRK1 is a specific high-affinity receptor for NGF, which is closely related to inflammatory response [14]. As a key signaling pathway in human body, EGFR mediates cell apoptosis and proliferation [15]. CUL3 is a ubiquitinated ligase E3 protein, belonging to the Cullin protein family. Fibronectin FN1 plays an important role in normal processes such as animal development, organ growth, cell adhesion, cell migration and hemostasis [16], and promotes cell proliferation and inhibits apoptosis by activating PI3K [17]. HSP are highly conserved stress-inducing factors that manage heat stress activation, promote T cell adhesion and migration, and enhance immune surveillance. It is involved in the pathogenesis of a variety of autoimmune and chronic inflammatory diseases.

According to KEGG pathway analysis, the main pathways involved in the treatment of BD by Gancao Xiexin decoction were inflammatory response and immune-related pathways. Involved in Human cytomegalovirus (HCMV) infection, Kaposi sarcoma-associated herpesvirus infection, AGE-RAGE signaling pathway in diabetic complications, Human T-cell leukemia virus 1 infection, TNF signaling pathway, etc. Li-

u[18] et al. found that HCMV-induced anti-PP150 antibody is a common autoantibody in autoimmune diseases, which provided a clear internal link between HCMV and autoimmune diseases. Studies have shown that the AGE-RAGE pathway can be involved in the resolution of inflammation, the maintenance of cellular homeostasis, and the repair and regeneration after injury. TNF-α can mediate cell-mediated necrosis and apoptosis through a variety of ways, which plays an important role in the occurrence and development of BD [3].

In summary, this study took the active components of Gancao Xiexin decoction as the research object, and explored the mechanism of Gancao Xiexin decoction in the treatment of BD by combining GEO differential analysis and network pharmacology, which provided a new reference for the treatment of BD. However, there are still some limitations in this study and further research is still needed to improve.

Author Contributions

Conception and design of study: ZX; Data collection: ZX; Drafting the manuscript: ZX. The author reviewed the results and approved the final version of the manuscript.

Funding Statement

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Ethical Compliance

This study was approved by the First Affiliated Hospital of Zhejiang University of Traditional Chinese Medicine Ethics Committee and was conducted following the Declaration of Helsinki in its currently applicable version and applicable Chinese laws.

Data Access Statement

All relevant data are within the paper and its Supporting Information files.

Conflict of Interest Declaration

The authors declare that they have no affiliations with or involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this manuscript.

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