Discussion on the potential target and mechanism of Dachaihu Decoction in treating hyperlipidemia based on network pharmacology

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Abstract: To explore the potential targets and related signaling pathways of Dachaihu Decoction in treating hyperlipidemia, we obtained the active ingredients of the decoction by searching the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) for eight Chinese herbal ingredients, namely Radix Bupleuri, Radix Scutellariae, Radix Paeoniae Alba, Rhizoma Pinelliae, Fructus Aurantii Immaturus, Ginger, Jujube, and Rhubarb. To obtain the disease target for hyperlipidemia, we searched the English keyword "hyperlipidemia" and compared and analyzed the drug target of Dachaihu Decoction with the target of hyperlipidemia disease through an online website. We then identified the intersection between the two as the target of Dachaihu Decoction for treating hyperlipidemia. We conducted a topological analysis to screen the key effective components of Dachaihu Decoction for treating hyperlipidemia based on their degree value. Then, the gene targets of Dachaihu Decoction for hyperlipidemia were imported into the String platform to identify the key interaction modules in the PPI network. We also used the cytohubba plug-in to screen the key genes of these modules. The online database platform DAVID was utilized to perform enrichment and analysis of the key module genes with the highest number of genes and scores. The study yielded 116 active ingredients and 294 targets as the drug targets of Dachaihu Decoction. Furthermore, a total of 1349 disease-related gene targets were obtained from the CTD database, OMIM database, and TCMIP platform. After comparing the drug targets of Dachaihu Decoction with the targets related to hyperlipidemia, a total of 168 targets were found to be involved in the treatment of hyperlipidemia by Dachaihu Decoction. Using the MCODE plug-in in Cytoscape software, eight key protein modules were identified in the PPI network. Further analysis of the first key module revealed the top 15 hub genes which may play important roles in the pharmacological effects of Dachaihu Decoction for treating hyperlipidemia. To further understand the underlying mechanism, GO function annotation and KEGG pathway enrichment analysis were performed for the genes included in the key modules using the DAVID database. Based on the GO function annotation, the biological process (BP) of the key module genes was mainly related to the positive regulation of transcription from the RNA polymerase II promoter, the negative regulation of apoptosis, and the response to drugs. The cellular component (CC) was mainly located in the extracellular space, extracellular area, and pits. The molecular function (MF) was mainly focused on enzyme binding, cytokine activity, and protein binding. The KEGG pathway enrichment analysis showed that the key module genes were involved in several signaling pathways, including the hepatitis B signaling pathway, TNF signaling pathway, tumor-related signaling pathway, and PI3K-AKT signaling pathway. These pathways are known to be associated with inflammation, apoptosis, and cell growth, which are all critical processes in the development of hyperlipidemia. In conclusion, it appeared that Dachaihu Decoction could potentially be effective in the treatment of hyperlipidemia by regulating the expression of key genes, such as MMP9 and MAPK2, as well as the PI3K-AKT and TNF signaling pathways.

Keywords: Dachaihu Decoction; Network pharmacology; Hyperlipidemia

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1. Introduction

Hyperlipidemia, along with other cardiovascular risk factors, can interact with each other and easily lead to atherosclerosis, coronary heart disease, cerebral infarction, and many other heart and blood vessel diseases, resulting in increased cardiovascular morbidity and mortality[1]. When it comes to treatment, statins are the primary drugs used in the clinical management of hyperlipidemia. However, the use of statins may lead to muscle pain and other symptoms in some patients, and around 20% of the patients are resistant or intolerant to statin therapy. Additionally, patient compliance is often low, making it necessary to explore alternative treatment options[2]. Guided by the principles of traditional Chinese medicine (TCM), Chinese medicine has been widely utilized in the management of hyperlipidemia, employing the principles of holistic and dialectical treatment. As a result, Chinese medicine holds great potential for further development and application[3]. With the advancements in medicine, the integration of TCM and Western medicine has become a growing trend. The two can complement each other, providing additional options and references for clinical treatment strategies for hyperlipidemia[4]. Major Bupleurum Decoction is derived from Zhang Zhongjing's "Typhoid Miscellaneous Disease Theory". Studies have shown that Major Bupleurum Decoction can improve lipid metabolism in the body. However, the internal targets for treating hyperlipidemia have not been fully elucidated. Since hyperlipidemia is a complex disease that may involve multiple systems and pathways, relying solely on a single component or a single target drug is not a realistic approach for treating this condition. A multi-target approach is necessary for effective treatment. Modern network pharmacology, using network analysis methods, can identify and establish the relationships between drugs and diseases, providing a better understanding of the pharmacological mechanisms of TCM compounds and facilitating drug discovery. This approach offers a valuable tool for investigating the multiple roles played by targets in drug action[5].

The TCM Systems Pharmacology Database and Analysis Platform (TCMSP) contains information on 499 Chinese medicines registered in *the Chinese Pharmacopoeia*, which includes 29 384 ingredients, 3311 targets, and 837 related diseases[6]. The database uses the HIT prediction algorithm to establish relationships between drug targets, while disease information is obtained from the TTD and PharmGKB databases. This platform provides a valuable resource for researchers seeking to explore the pharmacological properties of TCM. The TCMSP database offers pharmacokinetic information for each compound, including drug-like properties (DL), bioavailability (OB), blood-brain barrier (BBB), intestinal epithelial permeability (Caco-2), and other indicators[7]. Using this database, the active ingredients of Dachaihu Decoction were screened, and the target points of these active ingredients were predicted. This database provides a valuable resource for researchers seeking to explore the pharmacokinetics of TCM and its active compounds. To obtain the drug target points of Dachaihu Decoction, gene name conversion was performed. Disease-related genes for hyperlipidemia were queried and obtained from the Comparative Toxicology Database (CTD), Online Mendelian Inheritance in Man (OMIM), and Integrated Pharmacology-based Research Platform of Traditional Chinese Medicine (TCMIP). The intersection of these databases was taken as the possible core target of Dachaihu Decoction for treating hyperlipidemia. A protein-protein interaction (PPI) network diagram was built using the String website, and the PPI network data was imported into Cytoscape 3.7.1 software to identify the key protein

modules and hub genes of the PPI network diagram. Genes included in the key modules were subjected to GO function annotation and KEGG pathway enrichment analysis using the DAVID database. The aim of this study was to investigate the potential mechanism and targets of Dachaihu Decoction for treating hyperlipidemia using network pharmacological methods. The results of this study will provide a more robust theoretical foundation for the clinical application of Dachaihu Decoction in the treatment of hyperlipidemia.

2. Materials and methods

2.1. Prediction of active components and targets of Dachaihu Decoction

To obtain the active ingredients of Dachaihu Decoction, we searched the TCMSP database and identified the corresponding protein targets for each ingredient. We then used the Uniprot website (https://www.uniprot.org/) to convert these targets into gene names, supplementing any targets that could not be matched by searching the literature. Finally, we obtained the drug targets of Dachaihu Decoction by removing any duplicates.

2.2. Target prediction of hyperlipidemia

We searched for the English keyword “hyperlipidemia” on three databases, CTD, OMIM, and TCMIP, merged and removed any duplicates among the disease-related genes retrieved from these databases, and obtained the disease targets associated with hyperlipidemia.

2.3. Target prediction of Dachaihu Decoction in treating hyperlipidemia

We compared the drug targets of Dachaihu Decoction and the targets associated with hyperlipidemia disease using the online tool (http://bioinformatics.psb.ugent.be/webtools/Venn/) for analysis. We identified the intersection of these targets as the potential targets of Dachaihu Decoction for treating hyperlipidemia.

2.4. Drug component target disease network construction of Dachaihu Decoction for hyperlipidemia

We imported the component-target and disease-target relationship networks of Dachaihu Decoction into Cytoscape 3.7.1 software and used its Merge function to construct a drug-component-target-disease network for treating hyperlipidemia. The network was analyzed topologically using the Network Analysis function of Cytoscape 3.7.1 software, and the key effective components of Dachaihu Decoction for treating hyperlipidemia were identified based on their degree. The effective ingredients of various Chinese medicines were presented in the form of acronyms, and any ingredient names that were repeated among different Chinese medicines were listed in Table 1.

2.5. Construction of protein interaction network

We imported the gene targets of Dachaihu Decoction for hyperlipidemia obtained from the previous analysis into the String platform (http://www.string-db.org/), setting the species as Homo sapiens and the Confidence Score as 0.40. We downloaded the PPI and TSV files and imported them into Cytoscape 3.7.1 software for topological analysis.

2.6. Key modules and genes screening

We utilized the MCODE plug-in in Cytoscape 3.7.1 software to identify the key modules that interact in the PPI network. We also used the cytohubba plug-in to screen the key genes of the identified key modules.

Table 1. The naming of repeated TCM effective components.

Active ingredient	TCM containing this active ingredient						Name
MOL000358	Radix Scutellariae	Radix Paeoniae Alba	Rhizoma Pinelliae	Ginger	Jujubem	Rhubar	A1
MOL000449	Bupleurum	Radix Scutellariae	Rhizoma Pinelliae	Ginger	Jujubem		B1
MOL000098	Bupleurum	Jujubem					C1
MOL000096	Jujubem	Rhubar					C2
MOL000422	Bupleurum	Radix Paeoniae Alba					C3
MOL002776	Bupleurum	Rhizoma Pinelliae					C4
MOL000359	Radix Scutellariae	Radix Paeoniae Alba					C5
MOL002714	Radix Scutellariae	Rhizoma Pinelliae					C6
MOL002914	Radix Scutellariae	Trifoliate orange					C7
MOL000492	Radix Paeoniae Alba	Jujubem					C8

2.7. GO enrichment analysis

We performed GO analysis for the key module genes with the most genes and the highest scores using the online database platform DAVID (https://david.abcc.ncifcrf.gov/). The analysis included three items: molecular function (MF), biological process (BP), and cellular component (CC). We set the parameter species to Homo sapiens, downloaded the analysis result data for each item, and conducted screening under the condition of FDR < 0.05.

2.8. KEGG pathway enrichment analysis

We conducted an enrichment analysis of KEGG pathways using the DAVID online database platform, which is a knowledge base for systematic analysis of gene functions and linking genomic information with high-level functional information. The data was downloaded and pathways with FDR < 0.05 were screened.

3. Results

3.1. Screening of active ingredients and target prediction results of Dachaihu Decoction

After conducting a search in the TCMSP database, the active ingredients of eight Chinese herbal ingredients (Radix Bupleuri, Radix Scutellariae, Radix Paeoniae Alba, Pinellia, Fructus Aurantii Immaturus, Ginger, Chinese Date, and Rhubarb) were obtained. The active ingredients were then screened based on the criteria of DL ≥ 0.18 and OB ≥ 30, and the corresponding protein targets were matched. In cases where no targets were detected, the active ingredients were removed. Ultimately, a total of 116 effective ingredients of Dachaihu Decoction were identified and are listed in Table 2.

By matching the active ingredients to protein targets, 116 active ingredients were associated with 2018 targets. After removing duplicate targets, a total of 294 targets were identified as the drug target of Dachaihu Decoction (Table 2).

3.2. Screening results of target related to hyperlipidemia

After a thorough screening of the CTD, OMIM, and TCMIP databases, a total of 1349 gene targets related to hyperlipidemia disease were obtained by merging and removing duplicates. Then, the drug target of Dachaihu Decoction was compared to the hyperlipidemia disease targets using a Venn diagram, resulting in the identification of 168 action targets of Dachaihu Decoction for the treatment of hyperlipidemia.

3.3. Target prediction results of Dachaihu Decoction in treating hyperlipidemia

The targets of Dachaihu Decoction and those related to hyperlipidemia disease were compared using a Venn

Table 2. Effective components of Dachaihu Decoction.

TCM ingredient	Number of active ingredients	Number of target gene matching ingredients	Active ingredient ID
Bupleurum	17	14	MOL001645 (CH1), MOL002776 (C4), MOL000449 (B1), MOL000354 (CH2), MOL000422 (C3), MOL004598 (CH3), MOL004609 (CH4), MOL013187 (CH5), MOL004624 (CH6), MOL004653 (CH7), MOL004718 (CH8), MOL000490 (CH9), MOL000098 (C1)
Radix Scutellariae	36	32	MOL001689 (HQ1), MOL000173 (HQ2), MOL000228 (HQ3), MOL002714 (C6), MOL002909 (HQ4), MOL002910 (HQ5), MOL002913 (HQ6), MOL002914 (C7), MOL002915 (HQ7), MOL002917 (HQ8), MOL002925 (HQ9), MOL002927 (HQ10), MOL002928 (HQ11), MOL002932 (HQ12), MOL002933 (HQ13), MOL002934 (HQ14), MOL002937 (HQ15), MOL000358 (A1), MOL000359 (C5), MOL000525 (HQ16), MOL000552 (HQ1), MOL000073 (HQ17), MOL000449 (B1), MOL001458 (HQ18), MOL001490 (HQ19), MOL002879 (HQ20), MOL002897 (HQ21), MOL008206 (HQ22), MOL010415 (HQ23), MOL012245 (HQ24), MOL012246 (HQ25), MOL012266 (HQ26)
Radix Paeoniae Alba	13	7	MOL001918 (BS1), MOL001919 (BS2), MOL000358 (A1), MOL000359 (C5), MOL000422 (C3), MOL000492 (C8)
Rhizoma Pinelliae	13	12	MOL001755 (BX1), MOL002670 (BX2), MOL002714 (C6), MOL002776 (C4), MOL000358 (A1), MOL000449 (B1), MOL005030 (BX3), MOL000519 (BX4), MOL006936 (BX5), MOL006957 (BX6), MOL003578 (BX7), MOL006967 (BX8)
Trifoliate orange	22	19	MOL013277 (ZS1), MOL013279 (ZS2), MOL013428 (ZS3), MOL013430 (ZS4), MOL013435 (ZS5), MOL013436 (ZS6), MOL013437 (ZS7), MOL013440 (ZS8), MOL001798 (ZS9), MOL001803 (ZS10), MOL001941 (ZS11), MOL002914 (C7), MOL004328 (ZS12), MOL005100 (ZS13), MOL005828 (ZS14), MOL005849 (ZS15), MOL000006 (ZS16), MOL007879 (ZS17), MOL009053 (ZS18)
Ginger	5	4	MOL000358 (A1), MOL006129 (SJ1), MOL000449 (B1), MOL001771 (SJ2)
Jujubem	29	18	MOL012921 (DZ1), MOL012946 (DZ2), MOL012976 (DZ3), MOL012981 (DZ4), MOL012986 (DZ5), MOL012992 (DZ6), MOL001454 (DZ7), MOL000211 (DZ8), MOL000449 (B1), MOL000358 (A1), MOL004350 (DZ9), MOL000492 (C8), MOL000627 (DZ10), MOL007213 (DZ11), MOL000787 (DZ12), MOL002773 (DZ13), MOL000096 (C2), MOL000098 (C1)
Rhubar	16	10	MOL002288 (DH1), MOL000358 (A1), MOL002280 (DH2), MOL002297 (DH3), MOL002259 (DH4), MOL002235 (DH5), MOL002268 (DH6), MOL000096 (C2), MOL000471 (DH7), MOL002281 (DH8)

diagram, resulting in the identification of 168 targets that are acted upon by Dachaihu Decoction for the treatment of hyperlipidemia.

3.4. Drug component target disease network of Dachaihu Decoction in treating hyperlipidemia

The drug-component-target-disease network of Dachaihu Decoction for treating hyperlipidemia was constructed using Cytoscape 3.7.1 software, which included 261 nodes consisting of 83 components, 168 targets, 8 TCM components, one disease, and one drug, as depicted in Figure 1. Topological analysis revealed that the average Degree value of TCM components in the network was 15.05, with 19 components exceeding the average Degree value, including quercetin (Degree = 214), β-glutosterol (Degree = 107), and kaempferol (Degree = 66), as illustrated in Table 3.

3.5. Construction of target PPI network of Dachaihu Decoction for PS

The PPI network was obtained through the online analysis website String, resulting in 167 nodes and 3647 edges, with an average Degree value of 43.7. The PPI enrichment *P*-value was found to be < 1.0e-16, as depicted in Figure 2.

Table 3. Effective components and molecular names of Dachaihu Decoction in treating PS (Degree score above the average).

Serial number	Name	Active ingredient	Molecular name	Degree	DL	OB (%)
1	C1	MOL000098	Quercetin	214	0.28	46.43
2	A1	MOL000358	Beta-sitosterol	107	0.75	36.91
3	C3	MOL000422	Kaempferol	66	0.24	41.88
4	B1	MOL000449	Stigmasterol	56	0.76	43.83
5	C5	MOL000359	Sitosterol	46	0.75	36.91
6	ZS15	MOL005849	Didymin	45	0.24	38.55
7	ZS11	MOL001941	Ammidin	30	0.40	49.89
8	HQ2	MOL007100	Dihydrotanshinlactone	29	0.22	34.55
9	CH2	MOL000354	Isorhamnetin	23	0.31	49.60
10	ZS13	MOL005100	5,7-Dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one	22	0.27	47.74
11	DZ10	MOL000627	Stepholidine	22	0.54	33.11
12	HQ1	MOL001689	Acacetin	18	0.24	34.97
13	ZS16	MOL000006	Luteolin	18	0.25	36.16
14	C7	MOL002914	Eriodyctiol (Flavanone)	18	0.24	41.35
15	BX2	MOL002670	Cavidine	15	0.81	35.64
16	DH4	MOL002259	Physciondiglucoside	15	0.63	41.65
17	HQ11	MOL002928	Oroxylin a	14	0.26	45.15
18	ZS1	MOL013277	Isosinensetin	14	0.44	51.15
19	C2	MOL000096	(-)-Catechin	14	0.24	49.68

3.6. PPI network key protein module and hub gene screening results

The Cytoscape software's MCODE plug-in was used to identify eight key protein modules in the PPI network. The top two key modules had MCODE scores of 49 000 and 8000, with 61 and 27 nodes and 1470 and 104 edges, respectively. The first key module played a critical role in the PPI network, as depicted in Figures 3A and 3B. Using the cytohubba plug-in, the top 15 hub genes were screened from the first key module, including MMP9, MAPK2, MAPK8, AKT1, TNF, CXCL8, HES-1, IL6, JUN, TP53, PTGS2, VEGFA, CCL2, IL10, and IL1B, as illustrated in Figure 3C.

3.7. Gene function enrichment analysis results

The gene annotation for key module A showed that the biological processes mainly involved positive regulation of transcription from RNA polymerase II promoter, negative regulation of apoptosis, response to drugs, aging, lipopolysaccharide-mediated signal pathway, cell response to lipopolysaccharide, angiogenesis, positive regulation of DNA transcription, positive regulation of nitric oxide biosynthesis, and more. The cellular component was mainly concentrated in the extracellular space, extracellular area, alveolus, outside of the plasma membrane, platelet α-granular lumen, cytoplasmic sol, protein extracellular matrix, perinuclear area of the cytoplasm, and more. The molecular function mainly included enzyme binding, cytokine activity, protein binding, transcription factor binding, tumor necrosis factor receptor binding, heme binding, MAP kinase activity, growth factor activity, and more, as shown in Figure 4.

KEGG pathway enrichment analysis mainly included the hepatitis B signaling pathway, TNF signaling pathway, tumor-related signaling pathway, PI3K-AKT signaling pathway, HIF-1 signaling pathway, Toll-like receptor signaling pathway, NOD-like receptor signaling pathway, T-cell receptor signaling pathway, and more, as shown in Figure 5. Typical signaling pathways are shown in Figures 6 and 7.

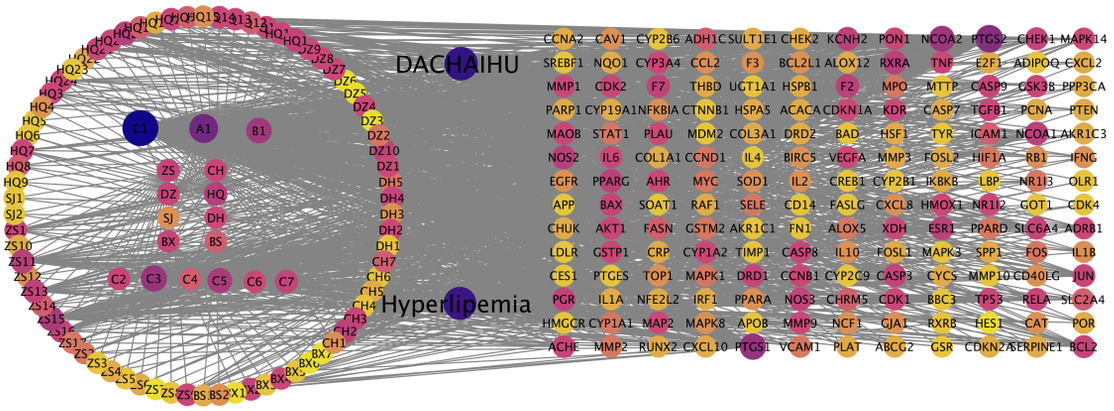

Figure 1. Drug-component-target-disease network of Dachaihu Decoction in treating hyperlipidemia.

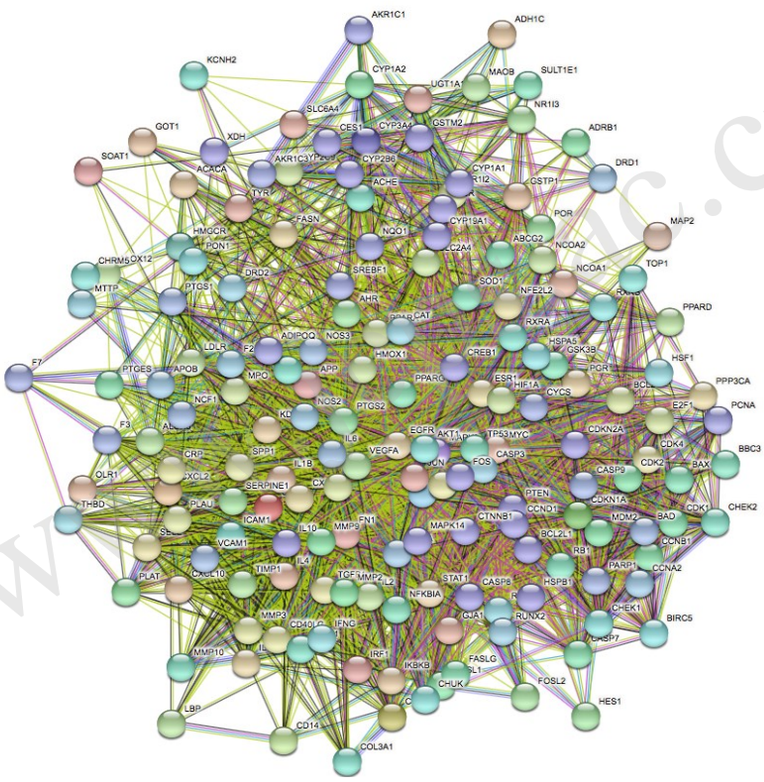

Figure 2. PPI network of Dachaihu Decoction for PS target.

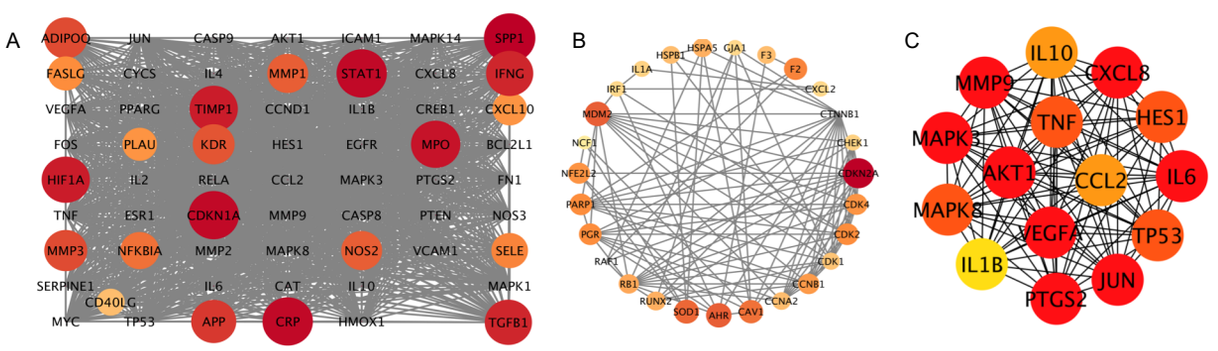

Figure 3. Key modules and hub gene of PPI network diagram of target points of Dachaihu Decoction in treating hyperlipidemia. A and B: Key modules in the top two protein scores of the PPI network; C: Hub gene of key module A.

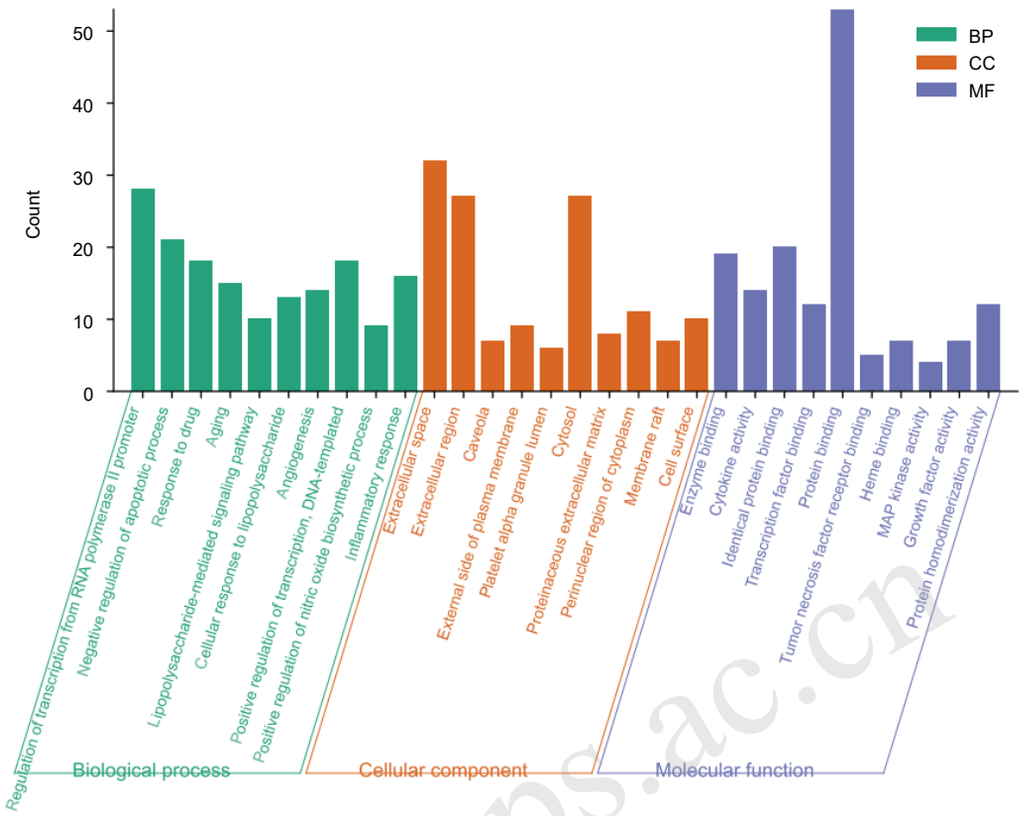

Figure 4. GO analysis of key module genes.

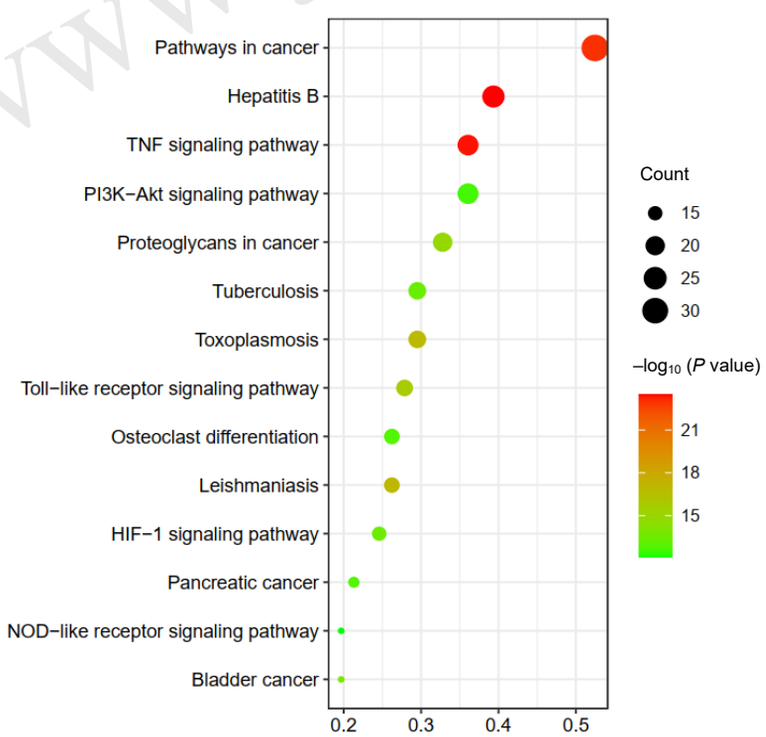

Figure 5. KEGG enrichment bubble diagram of key module A gene.

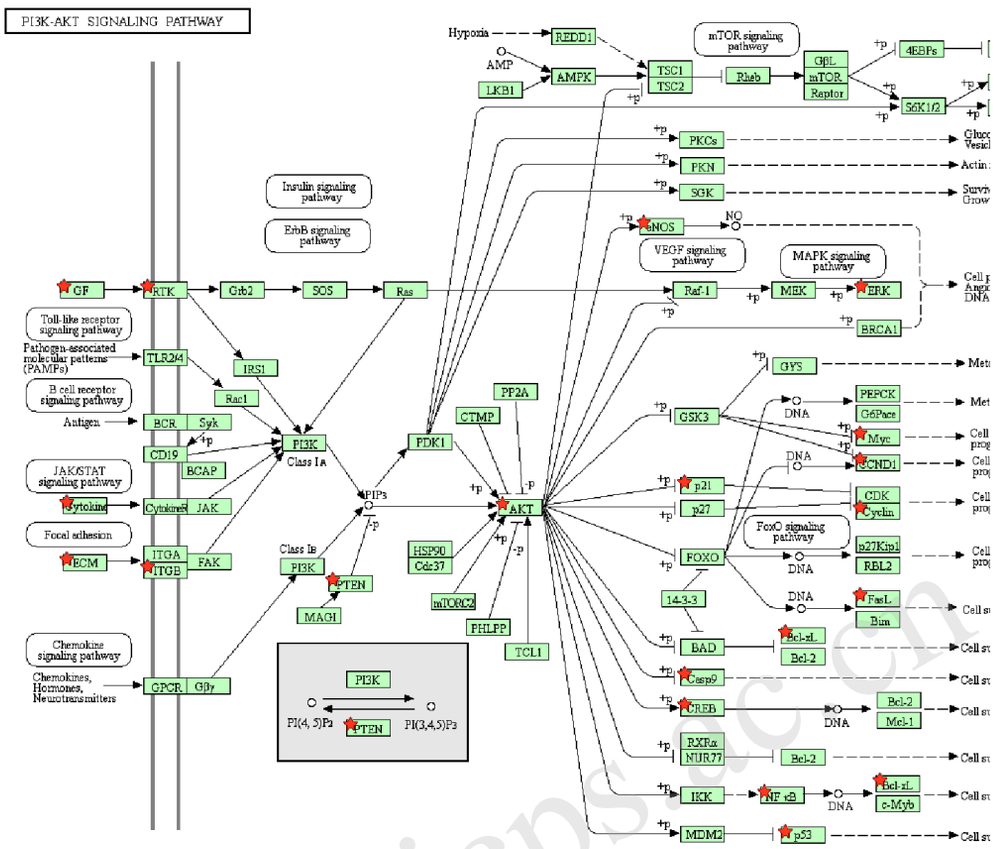

Figure 6. PI3K-AKT signaling pathway.

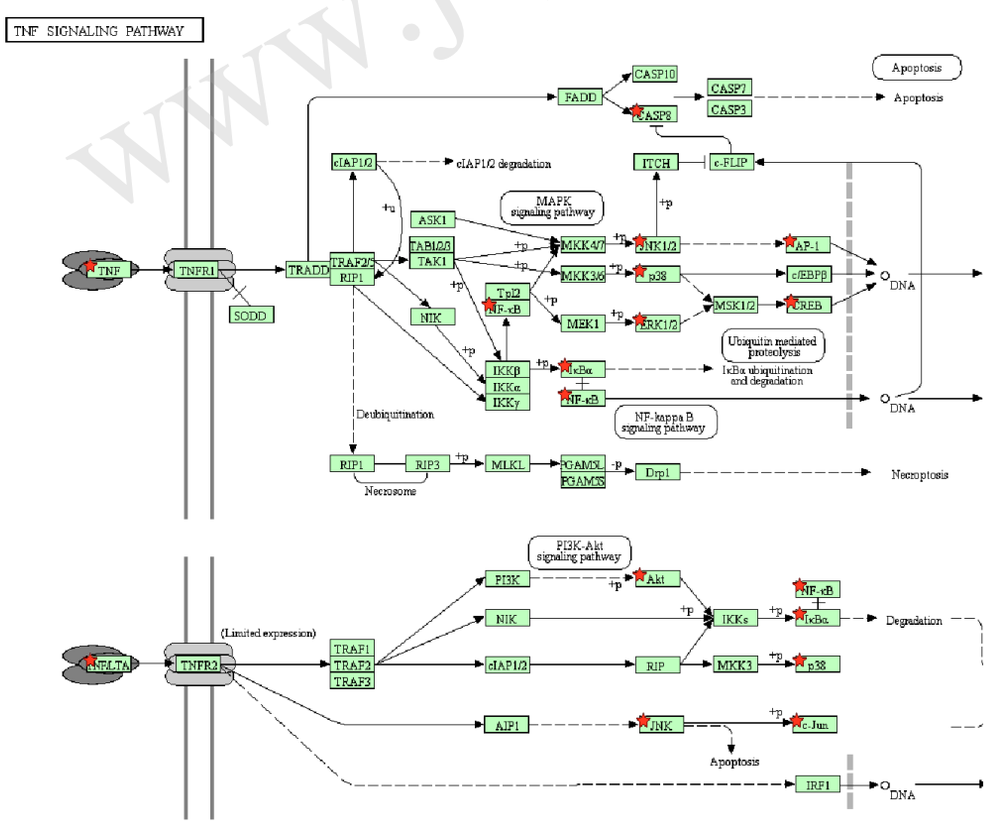

Figure 7. TNF signaling pathway.

4. Discussion

As people's living standards improve, so does the pressure on their daily lives and work. Poor lifestyle choices, such as prolonged sedentary behavior, lack of exercise, unhealthy diets, and excessive intake of fatty foods and alcohol, have led to an increasing prevalence of hyperlipidemia[8]. According to the 2016 revision of the Chinese Adult Dyslipidemia Prevention Guide, high blood triglyceride levels are found in 13.1% of Chinese adults, while the prevalence of hypercholesterolemia is 4.9%. As hyperlipidemia is a significant risk factor for cardiovascular disease, active prevention and treatment of the condition are crucial in improving the quality of life for high-risk individuals[9]. Commonly used lipid-lowering drugs in clinical practice, such as statins, are the primary medications used to treat hyperlipidemia. However, in some cases, statins may cause muscle pain and other side effects. Additionally, up to 20% of patients may exhibit statin drug resistance or intolerance, and patient compliance can be low. Therefore, it is necessary to explore alternative treatment methods.

While dyslipidemia and hyperlipidemia are not specifically named in TCM, descriptions of conditions such as sorghum can be traced back to ancient texts. Doctors in previous dynasties believed that these conditions were closely related to the liver, spleen, and kidney, with the spleen and kidney being the primary organs affected. Deficiencies in these organs were thought to be the root cause of the disease, with targets for treatment including phlegm coagulation, blood stasis, and qi stagnation. Later generations of doctors have also proposed the concepts of blood pollution and turbid pulse. Guided by TCM theory, which has been widely used in treating hyperlipidemia. It has the advantages of good efficacy, less toxicity, and side effects. It can be tailored to individuals based on their conditions and syndrome differentiation, offering broad research and application prospects. With the development of medicine, the integration of TCM and western medicine has become a trend. Both can learn from each other, providing more insights for selecting clinical treatment strategies for hyperlipidemia[3,10]. Dachaihu Decoction has been used for its effects of relieving stagnation, harmonizing qi, expelling pathogenic heat, and relieving bitterness in various disease fields. Recent studies have demonstrated its potential for improving lipid metabolism *in vivo*, although the specific internal target for its treatment of hyperlipidemia remains unclear. Chinese medicinal materials often contain a diverse range of chemical components, and the effects of each material in a prescription are not merely the sum of its chemical molecules. Moreover, the components of a medicinal material that have therapeutic effects for different diseases may vary. The efficacy of Chinese medicine is not simply the sum of its chemical components but rather the result of the coordinated and comprehensive action of various chemical components. In other words, after multiple chemical components act on different targets in the biological network simultaneously, an overall effect of network cross-coordination is produced. This is also one of the challenges in researching TCM prescriptions. While the emergence of network pharmacology cannot completely solve this problem, it provides a new approach to integrate the study of TCM with that of modern medicine. In current TCM network pharmacology research, the chemical components found in the medicinal materials of the prescription are being studied[11]. This method has several advantages, including clear chemical components, well-defined structures, and readily available and universal molecular characterization tools, which are conducive to subsequent mechanism research. At the same time, a single target or multiple targets can be identified within the entire biological network.

Network pharmacology can expedite the discovery and validation of drug targets, thereby improving the efficiency of new drug research and development. While it may not pinpoint the exact molecular pathways through which TCM prescriptions exert their effects, network pharmacology can help narrow the research scope to some extent. By rigorously screening the effective ingredients and potential molecular docking targets and validating the findings through subsequent experiments, this approach can offer a novel strategy and direction for TCM-related research.

This study identified the PI3K-AKT signaling pathway and TNF signaling pathway as the major pathways that play a crucial role. The AKT gene encodes one of three members of the human serine/threonine protein kinase family. The AKT protein family consists of an *N*-terminal pleckstrin homology domain, a serine/threonine-specific kinase domain, and a *C*-terminal regulatory domain. These proteins are phosphorylated by phosphatidylinositol 3 kinase (PI3K). The PI3K-AKT pathway is a critical component of many signaling pathways, involving the binding of membrane-binding ligands, such as receptor tyrosine kinase, G protein-coupled receptor, and integrin-linked kinase. As a result, AKT proteins regulate various cellular functions, including cell proliferation, survival, metabolism, and angiogenesis. Upon stimulation by external signals, PI3K can convert phosphatidylinositol 4,5-bisphosphate (PIP2) into phosphatidylinositol 3,4,5-triphosphate (PIP3) through a cascade reaction. When the stimulus signal source is lost or weakened, PIP3 will be converted back into PIP2, and the two will reach a dynamic balance. In this process, phosphatase and tensin homolog deleted on chromosome 10 (PTEN), which is absent from human chromosome 10, acts as an antagonist of PIP3 and promotes the conversion of PIP3 into PIP2, thereby reducing the content of PIP3 in cells. PIP3 can activate AKT not only directly but also indirectly by binding with 3-phosphoinositide-dependent protein kinase 1 (PDK1). The subsequent phosphorylation of threonine residues 308 and serine residues 473 is necessary for the complete activation of the AKT1 protein encoded by this gene[12]. The TNF gene encodes a multifunctional proinflammatory cytokine, primarily secreted by macrophages, that can bind to and function through receptors TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR. The TNF family is involved in regulating a wide range of biological processes, including cell proliferation, differentiation, apoptosis, lipid metabolism, and blood coagulation. TNF is also associated with various diseases, such as autoimmune diseases, insulin resistance, psoriasis, and rheumatoid arthritis[13].

The protein interaction analysis results suggested that MMP9, MAPK2, MAPK8, CXCL8, IL6, JUN, TP53, and other genes played a crucial role. Enrichment analysis revealed that these key genes were mainly located outside the nucleus, providing a foundation for further research on cellular localization. The primary biological processes identified included positive regulation of transcription from the RNA polymerase II promoter, negative regulation of apoptosis, response to drugs, aging, lipopolysaccharide-mediated signal pathway, cell response to lipopolysaccharide, angiogenesis, positive regulation of DNA transcription, and positive regulation of nitric oxide biosynthesis, all of which are related to lipid metabolism. The primary enriched molecular functions include enzyme binding, cytokine activity, protein binding, transcription factor binding, tumor necrosis factor receptor binding, heme binding, MAP kinase activity, growth factor activity, and more. The KEGG pathway enrichment analysis mainly focused on the hepatitis B signaling pathway, TNF signaling pathway, PI3K-AKT signaling pathway, HIF-1 signaling pathway, Toll-like receptor signaling pathway, NOD-like

receptor signaling pathway, T cell receptor signaling pathway, among others. Matrix metalloproteinase 9 (MMP9) family proteins are involved in the breakdown of the extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as disease processes, such as arthritis and metastasis. Most matrix metalloproteinases are secreted in an inactive precursor form, which is activated when cleaved by extracellular proteases. The enzyme encoded by this gene specifically degrades type IV and V collagen. MMP9 plays an important role in local proteolysis of the extracellular matrix and leukocyte migration. It can cut type IV and V collagen into large *C*-terminal three-quarter segments and short *N*-terminal one-quarter segments[14].

As a convergence point of many biochemical signals, MAPK participates in several cellular processes, such as proliferation, differentiation, transcriptional regulation, and development. This kinase can be activated by various cellular stimulators, which target specific transcription factors and regulate immediate early gene expression in response to cell stimulation[15]. The CXCL-8 gene encodes a protein that belongs to the CXC chemokine family and acts as a major mediator of the inflammatory response. This protein is commonly known as interleukin-8 (IL-8). IL-8 is secreted by monocyte macrophages, neutrophils, eosinophils, T lymphocytes, epithelial cells, and fibroblasts. It functions as a chemokine by guiding neutrophils to the site of infection. The expression of IL-8 is rapidly induced by bacterial and viral products[16]. IL-8, along with other cytokines, also participates in the inflammatory signaling cascade and contributes to systemic inflammatory response syndrome (SIRS). This proinflammatory protein is also believed to play a role in coronary artery disease and endothelial dysfunction. Additionally, this chemokine acts as an effective angiogenic factor. The combination of IL-8 and one of its receptors (IL-8RB/CXCR2) can increase vascular permeability. An increase in the level of IL-8 is positively associated with the severity of various disease outcomes, such as sepsis[17]. The IL-6 gene encodes a cytokine that contributes to inflammation and B cell maturation and can cause fever in individuals with autoimmune diseases or infections. The protein is primarily produced at sites of acute and chronic inflammation, secreted into the serum, and activates an inflammatory response by binding to the IL-6 receptor α. IL-6 is associated with various disease states related to inflammation, including diabetes and systemic inflammatory states. The Jun gene is located at 1p32-p31 and encodes a protein that shares high similarity with viral proteins. This protein can directly interact with specific target DNA sequences to regulate gene expression[18]. The TP53 gene encodes a tumor suppressor protein that contains transcriptional activation, DNA binding, and oligomeric domains. The encoded proteins respond to various cell stresses to regulate the expression of target genes. This, in turn, induces cell cycle arrest, apoptosis, aging, DNA repair, or metabolic changes[19].

In summary, the network pharmacological analysis suggests that Dachaihu Decoction may treat hyperlipidemia by regulating the PI3K-AKT and TNF signaling pathways, as well as genes such as MMP9, MAPK2, MAPK8, and CXCL8. This study provides valuable insights into the clinical application and potential mechanisms of Dachaihu Decoction for treating hyperlipidemia.

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基于网络药理学探讨大柴胡汤治疗高脂血症的潜在作用靶点和机制

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摘要: 本研究对大柴胡汤治疗高脂血症时的潜在作用靶点及相关信号通路进行了探讨，首先在中药系统药理学数据库与分析平台(TCMSP)检索大柴胡汤中柴胡、黄芩、白芍、半夏、枳实、生姜、大枣、大黄8种中药成分, 获得大柴胡汤的活性成分。通过TCMSP数据库查找每味中药的活性成分对应的蛋白靶点。在比较毒物基因组数据库(CTD)、人类孟德尔遗传综合数据库(OMIM)、中医药整合药理学研究平台(TCMIP)检索“高脂血症”英文关键词“hyperlipemia”, 得到高脂血症的疾病靶点。将大柴胡汤药物靶点与高脂血症疾病靶点通过在线网站进行对比分析, 取交集作为大柴胡汤治疗高脂血症的作用靶点。通过拓扑学分析, 根据度值筛选大柴胡汤治疗高脂血症的关键有效成分。将上述分析得到的大柴胡汤治疗高脂血症的基因靶点导入String平台, 识别PPI网络中相互作用的关键模块; 使用cytohubba插件筛选关键模块的关键(Hub)基因。采用在线数据库平台DAVID对包含基因最多、分值最高的关键模块基因进行富集分析。最终得到大柴胡汤的有效成分116个, 靶点294个, 此即为大柴胡汤的药物靶点。在CTD数据库、OMIM数据库及TCMIP平台共得到1349个疾病相关基因靶点。大柴胡汤药物靶点与高脂血症疾病相关靶点进行韦恩图对比取交集后, 得到168个大柴胡汤治疗高脂血症的作用靶点。使用Cytoscape软件MCODE插件共识别出PPI网络中8个关键蛋白模块, 对最关键的第1个关键模块进行分析, 筛选出前15位的Hub基因。GO功能注释显示, BP主要集中于从RNA聚合酶II启动子转录的正调控、凋亡过程的负调控、对药物的反应; CC主要集中于细胞外空间、细胞外区域、小窝; MF主要集中于酶结合、细胞因子活性、蛋白质结合。KEGG通路富集分析主要乙型肝炎信号通路、TNF信号通路、肿瘤相关信号通路、PI3K-AKT信号通路。可以得出, 大柴胡汤可能主要通过调控PI3K-AKT和TNF信号通路以及MMP9、MAPK2等基因的表达, 在治疗高脂血症中发挥作用。

关键词: 大柴胡汤; 网络药理学; 高脂血症