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Exploration on the Active Ingredients and Mechanisms of Ganjiang Lingzhu Decoction in the Treatment of Lumbar Disc Herniation: A Network Pharmacology Approach

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Abstract: This study aims to systematically explore the active ingredients, key targets, and potential molecular mechanisms of Ganjiang Lingzhu Decoction (GJLZD) in the treatment of Lumbar Disc Herniation (LDH) using a network pharmacology approach. Active ingredients and potential targets of GJLZD were retrieved from the TCMSP database, while LDH-related targets were collected from the GeneCards database. A protein-protein interaction (PPI) network was constructed using the STRING database to identify core targets, and KEGG pathway enrichment analysis was performed via the DAVID platform. Resultly, the findings suggest that GJLZD exerts its therapeutic effects primarily through core active components such as quercetin, kaempferol, 7-Methoxy-2-methyl isoflavone, vestitol, and beta-sitosterol. These components act on key targets including AKT1, TNF, IL1B, IL6, and TP53. Significant enrichment was observed in pathways such as Lipid and Atherosclerosis, IL-17 signaling pathway, and Fluid Shear Stress and Atherosclerosis. These pathways collectively regulate biological processes including inflammatory response, apoptosis, and extracellular matrix metabolism. In conclusion, this study preliminarily reveals that GJLZD treats LDH through a synergistic mechanism involving "multi-component, multi-target, and multi-pathway" interactions, providing a theoretical foundation for further experimental validation and clinical application.

1. Introduction

Lumbar disc herniation (LDH) is a common spinal disorder characterized by low back pain and radiating pain in the lower limbs, resulting from degenerative changes in the lumbar intervertebral discs. With a lifetime prevalence of approximately 70%, LDH significantly impairs patients' quality of life and imposes a substantial socioeconomic burden [1]. In traditional Chinese medicine (TCM), LDH falls under the categories of "low back pain"and "bi syndrome". Its etiology is often attributed to kidney deficiency as the root cause, with cold-dampness and blood stasis as the secondary pathogenic factors. Patients often present with innate kidney qi deficiency, leading to malnourishment

of the lumbar region. Combined with factors such as strain, trauma, or invasion of cold-dampness, these conditions disrupt local qi and blood circulation, resulting in meridian obstruction and pain due to blockage [2].

Ganjiang Lingzhu Decoction (GJLZD), originating from Jin Gui Yao Lue (Synopsis of the Golden Chamber), has the effects of warming the middle energizer, dispelling cold, strengthening the spleen, and eliminating dampness. The formula consists of dried ginger (Ganjiang), poria (Fuling), bighead atractylodes rhizome (Baizhu), and licorice (Gancao). It primarily works to revitalize middle energizer yang and warm-transform cold-dampness, aligning with the pathogenesis of cold-dampness internal obstruction and yang qi failure leading to heavy, cold, and painful lower back. GJLZD has demonstrated favorable clinical efficacy in treating LDH with cold-dampness syndrome [3]. However, its pharmacodynamic material basis and molecular mechanisms of action have not yet been systematically elucidated. Therefore, this study employs a network pharmacology approach to uncover the multi-component, multi-target mechanism of GJLZD in the treatment of lumbar disc herniation.

2. Materials and Methods

2.1. Collection of Active Ingredients and Target Prediction of GJLZD

The active ingredients and corresponding targets of the four herbal components of GJLZD (Dried Ginger, Poria, Bighead Atractylodes Rhizome, and Licorice) were predicted using the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP: http://tcmwsp.com/tcmsp.php) . Screening criteria were set as oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18 to identify the effective active ingredients and their targets. All obtained compound targets were integrated and deduplicated. The gene names of these targets were then standardized using the Uniprot database (https://www.uniprot.org/).

2.2. Acquisition of LDH-Related Targets

The GeneCards database (https://www.genecards.org/) was comprehensively searched using the keywords "lumbar disc herniation" and "Prolapsed Lumbar Intervertebral Disc" to retrieve disease-related targets . The targets identified from both keyword searches were consolidated in an Excel spreadsheet and subjected to deduplication processing to obtain the final set of disease target genes.

2.3. Identification of Common Targets between the Herbal Formula and the Disease

The obtained targets of GJLZD and the disease targets were mapped to each other. The intersecting target genes were identified using the Jvenn online platform (https://www.bioinformatics.com.cn/static/others/jvenn_en/example.html).

2.4. Construction of the Herb-Active Ingredient-Target Network and Screening of Key Active Ingredients

Files named "network.xlsx" and "type.xlsx" were created based on the herbal ingredients and the identified common targets. These files were imported into Cytoscape 3.10.1 to construct a herb-active ingredient-target interaction network. Network topology analysis was performed, and key active ingredients of GJLZD for treating LDH were screened based on their degree values.

2.5. Construction of the Protein-Protein Interaction (PPI) Network and Screening of Core Targets

To further investigate the protein-protein interactions underlying the therapeutic effect of GJLZD on LDH, the intersecting genes between the core components of the formula and the disease were uploaded to the STRING database (https://string-db.org/) to construct a PPI network. The species parameter was set to "Homo sapiens," the minimum required interaction score was set to 0.4, and disconnected nodes were hidden in the network. All other parameters retained their default settings. The results were saved in TSV format. The TSV file was then imported into Cytoscape 3.10.1 for network topology analysis. Based on the results of this analysis, key targets were screened using degree values.

2.6. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment Analyses

To gain deeper insights into the potential mechanism of action of GJLZD in treating LDH, GO and KEGG pathway enrichment analyses were performed on the intersecting targets between the drug action targets and the disease-related targets. The intersecting targets were uploaded to the DAVID database (https://david.ncifcrf.gov/summary.jsp) for visualization. The gene identifier was set to OFFICIAL_GENE_SYMBOL, and the species was specified as Homo sapiens. GO functional enrichment annotated the roles of the target proteins in gene function from three aspects: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). The top 10 terms for BP, CC, and MF were selected based on the enrichment score. Similarly, the top 10 KEGG pathway entries most relevant to the disease were selected as the key signaling pathways through which the formula might exert its therapeutic effects, predicting its mechanism of action. Finally, visualization analysis was conducted using the bioinformatics online platform (http://www.bioinformatics.com.cn/).

3. Results

3.1. Collection of Active Ingredients from GJLZD and Prediction of Drug-Disease Targets

Through the TCMSP database, a total of 103 active compounds were screened. After standardizing target gene names using the Uniprot database, 208 potential drug targets were obtained. A search of the GeneCards database identified 1,508 LDH-related targets. Intersection analysis between drug and disease targets revealed 88 common targets (Figure 1), suggesting their potential role as therapeutic targets of GJLZD in the treatment of LDH.

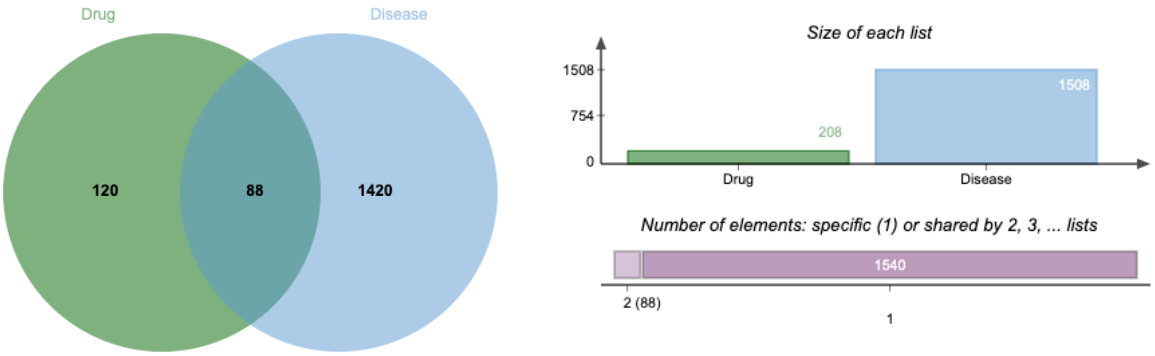

Figure 1: Venn diagram of overlapping targets between the drug (GJLZD) and disease (LDH)

3.2. Construction of the "Herb-Active Ingredient-Target" Network and Identification of Key Active Ingredients

Based on the correspondence between active ingredients and intersecting targets, an interaction network comprising 315 nodes and 1,443 edges was constructed using Cytoscape 3.10.1 (Figure 2). Network topology analysis revealed that key components with a degree ≥ 27 included: quercetin, kaempferol, 7-Methoxy-2-methyl isoflavone, vestitol, beta-sitosterol, formononetin, isorhamnetin, medicarpin, licochalcone A, and shinpterocarpin. These components are likely to play essential roles in the therapeutic mechanism of GJLZD against LDH.

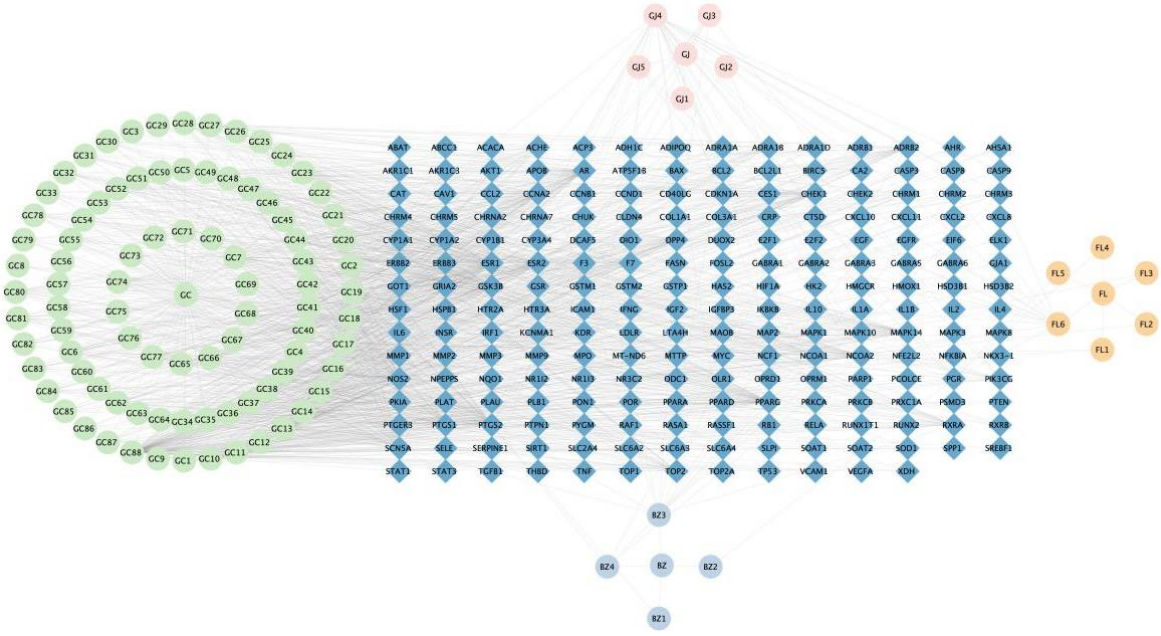

Figure 2: Herb-active ingredient-disease-target interaction network

Note: "Circles" in the figure represent traditional Chinese medicines and their constituents, while "diamonds" denote the targets corresponding to these constituents.

3.3. Protein-Protein Interaction (PPI) Network Construction and Core Target Screening

A preliminary PPI network was constructed from the 88 overlapping targets using the STRING database (Figure 3). Subsequent visualization with Cytoscape yielded an interaction network comprising 87 nodes and 1,763 edges (Figure 4). Topological analysis identified 10 core targets using a degree threshold ≥ 68: AKT1, TNF, IL1B, IL6, TP53, EGFR, BCL2, MAPK3, MMP9, and HIF1A (Figure 5, Table 1). These targets are considered hub nodes within the regulatory network.

3.4. Biofunctional Enrichment Analysis

3.4.1. GO Functional Enrichment Analysis

GO analysis of the drug-disease intersecting genes identified a total of 2,680 significant entries ($P < 0.05$). These included 2,446 biological process (BP) terms, primarily involving response to oxygen levels, regulation of apoptotic signaling pathway, cellular response to chemical stress, epithelial cell proliferation, and response to hypoxia; 80 cellular component (CC) terms, significantly enriched in structures such as membrane raft, membrane microdomain, vesicle lumen, caveola, and secretory granule lumen; and 154 molecular function (MF) terms, mainly related to DNA-binding transcription

factor binding, RNA polymerase II-specific DNA-binding transcription factor binding, protease binding, cytokine activity, and cytokine receptor binding. Figure 6 displays the top 10 most significant entries ranked by P-value from each of the three categories.

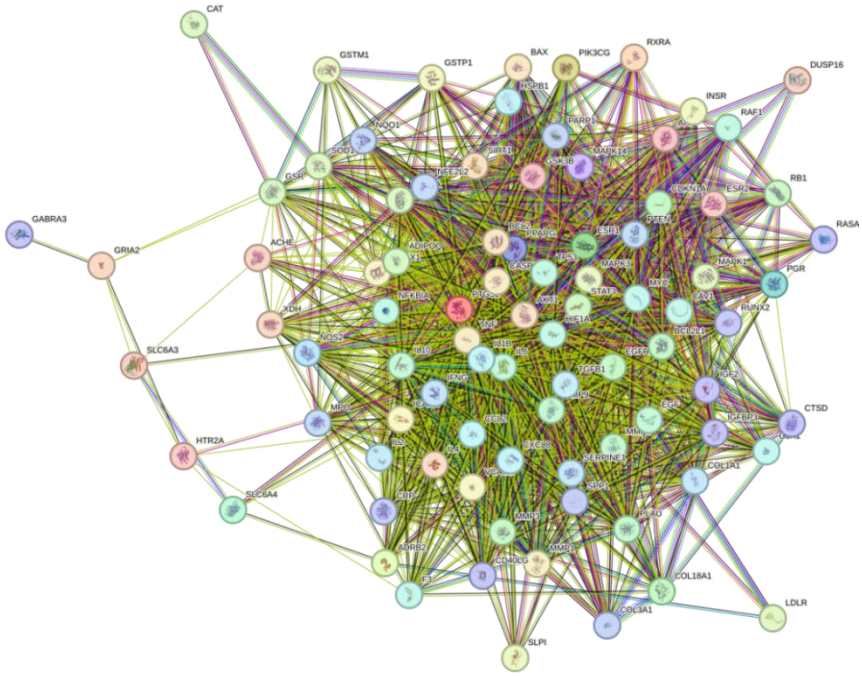

Figure 3: Preliminary PPI network from the STRING database

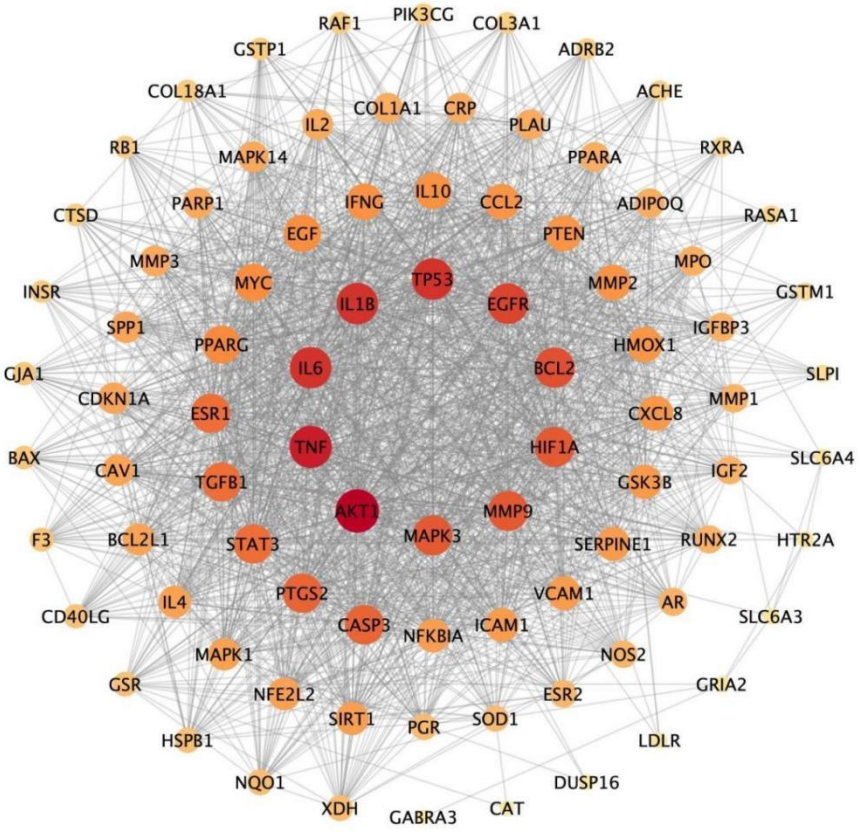

Figure 4: Protein-protein interaction (PPI) network diagram

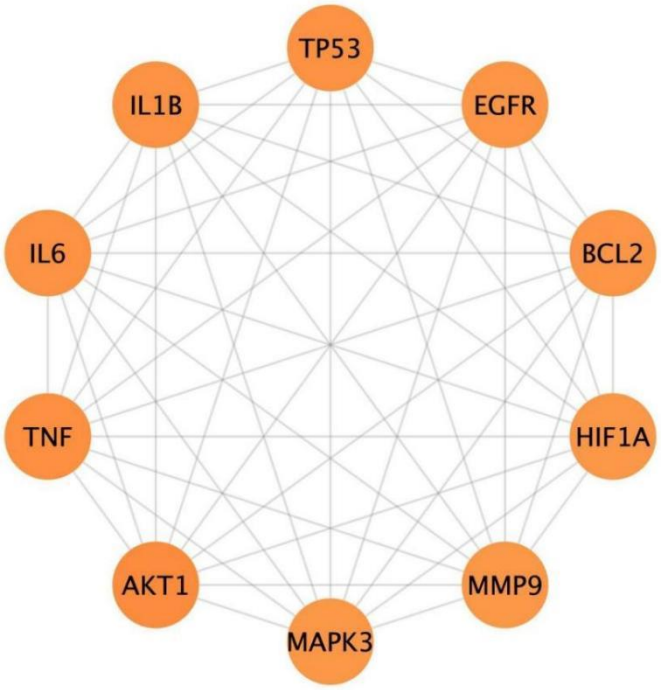

Figure 5: Protein-protein interaction (PPI) network of core targets

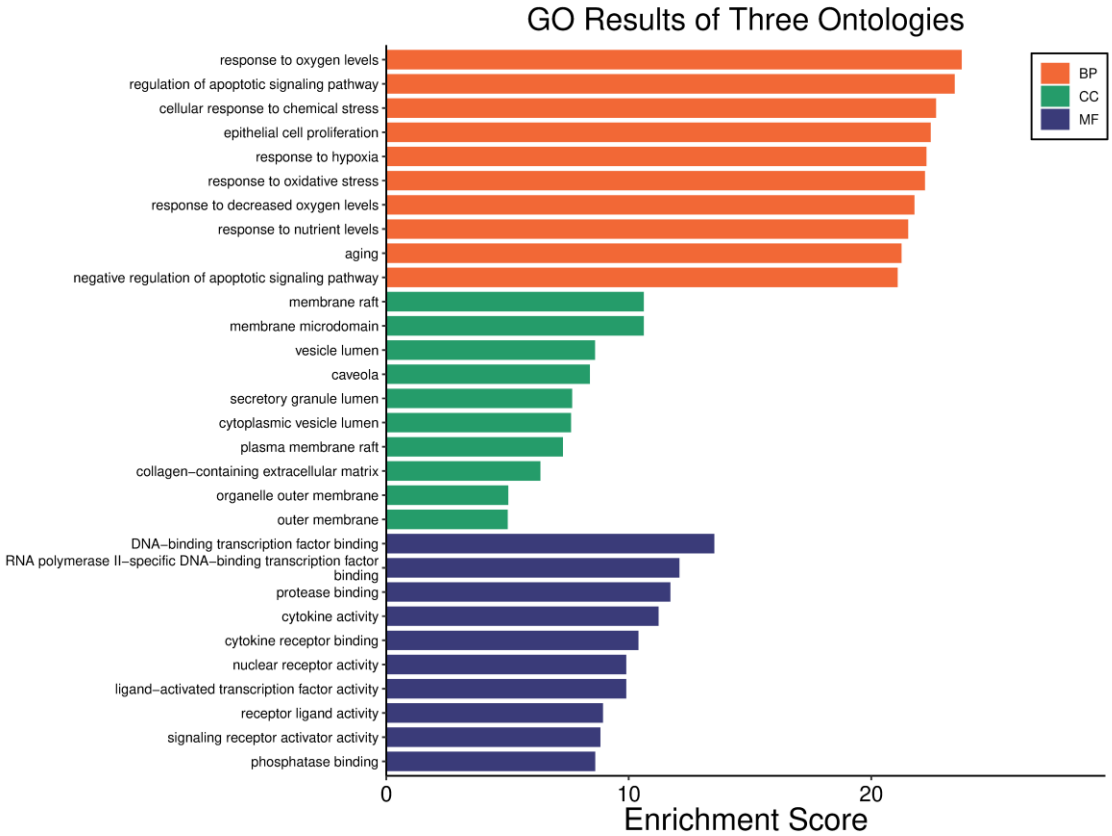

Figure 6: Top 10 entries of GO functional enrichment analysis

Table 1: Topological analysis parameters of the PPI network

Target	Degree	BC	CC
AKT1	77	0.043831845	0.895833333
TNF	74	0.027690229	0.868686869
IL1B	72	0.023483475	0.851485149
IL6	72	0.022521893	0.851485149
TP53	72	0.020483006	0.851485149
EGFR	70	0.026125725	0.811320755
BCL2	69	0.014623256	0.826923077
MAPK3	68	0.026316544	0.819047619
MMP9	68	0.013989713	0.819047619
HIF1A	68	0.013233346	0.819047619

3.4.2. KEGG Pathway Enrichment Analysis

KEGG pathway analysis of the intersecting targets identified 175 significantly enriched pathways ($P < 0.05$). Figure 7 displays the top 10 core pathways ranked by P-value, including Lipid and atherosclerosis, IL-17 signaling pathway, and Fluid shear stress and atherosclerosis. A chord diagram further illustrates the regulatory network between key targets and these pathways (Figure 8).

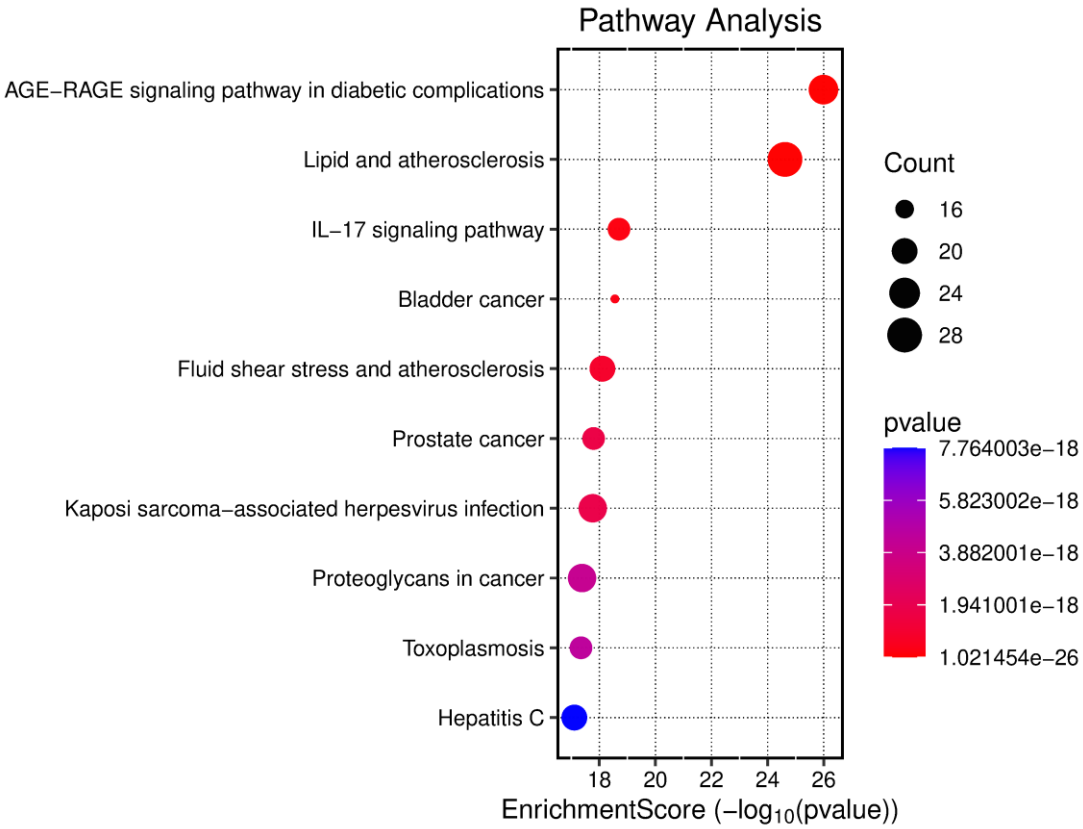

Figure 7: Top 20 entries of KEGG pathway enrichment analysis.

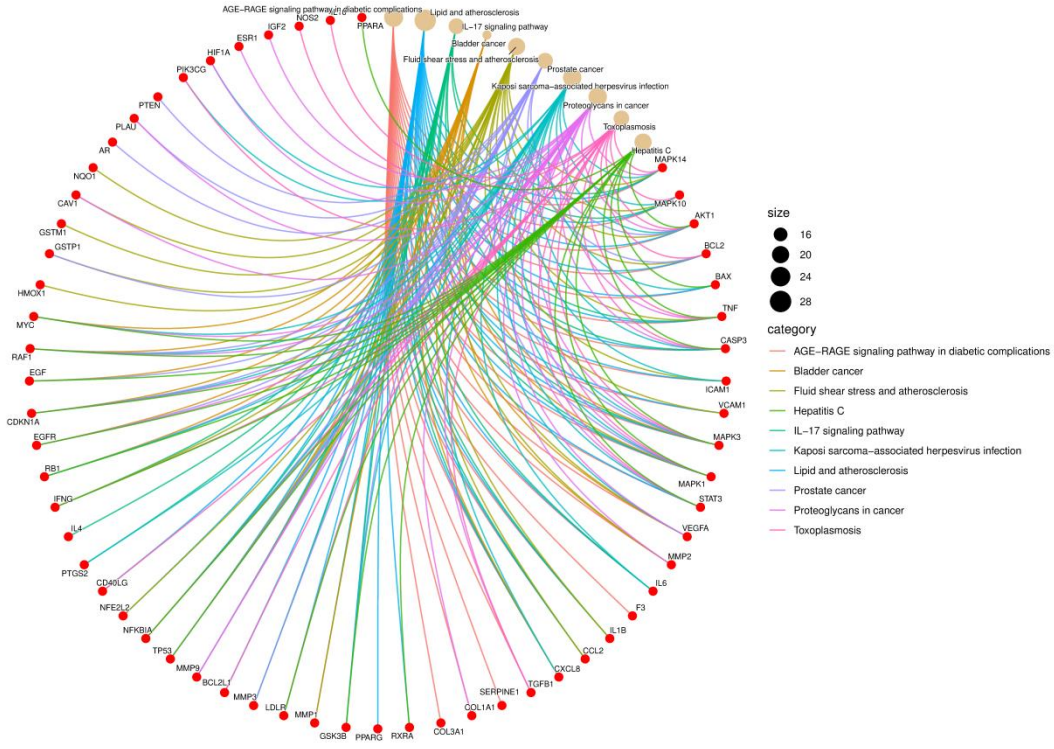

Figure 8: Inter-association map between key targets and the top 10 KEGG pathways

4. Discussion

LDH is categorized in Traditional Chinese Medicine as "low back pain", among other classifications. Chronic strain coupled with exposure to cold and dampness can easily lead to meridian obstruction and disrupted qi and blood circulation, causing pain due to blockage; or deficiency of qi and blood may fail to nourish the lumbar region, resulting in pain due to malnourishment. Cold-dampness can cause vasoconstriction of small vessels and muscle spasms, impairing local blood circulation in the intervertebral discs and leading to nutritional deficiency. Against a background of degeneration, these factors may contribute to annular fibrosis rupture, subsequently causing the nucleus pulposus to herniate posteriorly or laterally and compress nerve roots [4]. GJLZD, with its heavy use of dried ginger combined with bighead atractylodes rhizome, poria, and licorice, not only warms yang, dispels cold, and relieves pain but also promotes water diffusion and drains dampness to reduce swelling.

Traditional herbal formulas, by virtue of their multi-component, multi-target synergistic mechanisms, can effectively intervene in key pathological processes—such as anti-inflammation, anti-oxidation, tissue metabolism, and apoptosis—through the regulation of multiple signaling pathways, thereby achieving therapeutic effects against LDH [5]. Based on network pharmacology analysis, this study preliminarily identified components including quercetin, kaempferol, 7-Methoxy-2-methyl isoflavone, vestitol, and beta-sitosterol as potential core active substances in GJLZD for treating LDH.

The core clinical manifestation of LDH is lumbocrural pain, and its pathogenesis involves multiple factors, including nerve compression, inflammatory stimulation, autoimmune responses, nerve root adhesion, and electrophysiological abnormalities [6]. Essentially, LDH is a syndrome triggered by intervertebral disc degeneration (IDD), in which apoptosis of nucleus pulposus cells and

dysregulation of extracellular matrix metabolism are recognized as critical pathological bases for the initiation and progression of IDD. Studies have shown that quercetin can effectively suppress nucleus pulposus cell apoptosis and restore matrix homeostasis by activating the SIRT1-autophagy pathway, thereby delaying IDD progression [7]. Quercetin also exhibits protective effects across multiple tissues and can significantly alleviate intervertebral disc degeneration by downregulating the expression of senescence markers in nucleus pulposus tissue [8]. Furthermore, quercetin possesses anti-inflammatory, antioxidant, and joint-protective properties, effectively reducing spinal cord injury induced by nerve compression. Its mechanisms include decreasing myeloperoxidase expression in spinal cord tissue and scavenging reactive oxygen species to mitigate local inflammation [9-11]. Kaempferol, a natural flavonoid, demonstrates notable antioxidant, anti-inflammatory, and neuroprotective activities, while also promoting osteoblast proliferation and differentiation and inhibiting bone resorption [12]. Its mechanism in IDD treatment primarily involves suppressing lipopolysaccharide-induced apoptosis and regulating the expression of chondrogenic markers, thereby retarding disc degeneration [13]. Beta-sitosterol, a phytosterol, exhibits anti-inflammatory, antioxidant, and immunomodulatory functions. It exerts anti-inflammatory effects by inhibiting the expression of IL-2 and IL-10 and suppressing T-cell proliferation, effectively alleviating LDH-related pain symptoms [14-15].

PPI network analysis revealed that the key targets of GJLZD in treating LDH primarily include AKT1, TNF, IL1B, IL6, and TP53. AKT1 (protein kinase B), a core component of the PI3K/AKT signaling pathway, plays a pivotal role in cell survival, proliferation, metabolism, and inflammation regulation. Both AKT1 and AKT3 function synergistically in LDH pathogenesis [16]. TNF-α is a key pro-inflammatory factor implicated in LDH-related pain and is closely associated with multiple pathological processes in IDD, indicating its significant role in radicular pain in LDH [17-18]. TP53 can protect disc structure by delaying nucleus pulposus cell degeneration through mechanisms involving inflammation, apoptosis, and senescence [19].

Further KEGG pathway enrichment analysis demonstrated that the key mechanisms of GJLZD in treating LDH may involve multiple signaling pathways, including Lipid and atherosclerosis, IL-17 signaling pathway, and Fluid shear stress and atherosclerosis. Dyslipidemia can promote the development of atherosclerosis, leading to stenosis or even occlusion of lumbar arteries, thereby compromising blood supply and nutrient delivery to lumbar intervertebral discs and accelerating disc degeneration [20]. Additionally, fluid shear stress significantly downregulates the expression of type II collagen and aggrecan in nucleus pulposus tissue while upregulating various pro-inflammatory factors such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and nitric oxide (NO), thereby inducing or exacerbating local inflammatory responses and promoting the onset and progression of LDH [21]. Studies have shown that degenerated disc cells secrete abundant inflammatory cytokines, including TNF-α, IL-1α, IL-1β, IL-6, and IL-17, which not only drive IDD but also directly contribute to radicular pain [22]. Among these, IL-17 plays a critical role in nerve injury-associated pain, suggesting its potential as an important therapeutic target for pain modulation in LDH.

5. Conclusion

In summary, this study, utilizing network pharmacology, demonstrates that the active ingredients of GJLZD exert therapeutic effects on LDH through multiple targets and pathways, providing a theoretical foundation for its clinical application. However, certain limitations remain in the present research, and further experimental validation is required to investigate the relevant core targets and elucidate the specific mechanisms.

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