Investigation of the mechanism of *Astragalus membranaceus* in the treatment of lumbar disc herniation using network pharmacology

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Abstract

**Purpose:** To determine the underlying mechanisms of action of *Astragalus membranaceus* in lumbar disc herniation (LDH) treatment.

**Methods:** This study utilized network pharmacology analysis and STRING database to identify compound targets and visualize PPI network. Furthermore, an LDH model was induced in human nucleus pulposus cells using lipopolysaccharide (LPS), and then the vital target genes were evaluated in this model treated with active components of *Astragalus membranaceus*.

**Results:** Network pharmacology analysis indicates that several key proteins, including vascular endothelial growth factor A (VEGF A), AKT1, JUN, prostaglandin-endoperoxide synthase 2 (PTGS2), interleukin-6 (IL-6), matrix metalloproteinase 9 (MMP9), interleukin-1β (IL-1β), C-X-C motif chemokine ligand 8 (CXCL8), epidermal growth factor (EGF) and matrix metallopeptidase 2 (MMP2) may play essential roles in LDH treated with *Astragalus membranaceus*. The active components in *Astragalus membranaceus* suppressed the production of IL-1β and IL-6, and increased the expressions of VEGF A, MMP9 and MMP2 in LPS-induced LDH model.

**Conclusion:** The active components of *Astragalus membranaceus* effectively inhibits inflammation in LPS-induced LDH model, indicating that *Astragalus membranaceus* is a potential therapeutic candidate for LDH treatment.

**Keywords:** *Astragalus membranaceus*, Inflammation, Lumbar disc herniation, Network pharmacology

INTRODUCTION

Lumbar Disc Herniation (LDH) is a clinical syndrome arising from the partial or complete rupture of the annulus fibrosus in the lumbar intervertebral disc. This rupture can occur due to diverse factors, leading to the posterior protrusion of the nucleus pulposus protrudes. Consequently, this protrusion results in irritation or compression of the nerve roots and cauda equina [1]. Patients with LDH exhibit diverse clinical manifestations such as low back pain, numbness, and weakness in the lower limbs. And cauda equina nerve damage may occur, leading to defecation disorders and even paralysis. The available approaches for...
addressing LDH encompass conservative management and surgical intervention. The conservative treatment is the first choice for LDH. Therefore, it is crucial to develop complementary and alternative therapies for LDH [2].

*Astragalus membranaceus* (AM) boasts an extensive historical legacy, and is employed for clinical purpose in China. Astragalus is effective in invigorating *qi* and raising *yang*, benefiting health and strengthening appearance. It is widely reported in ancient literature that *Astragalus* directly invigorates *kidney qi*. In the Rihuazi Materia Medica, it is documented that *Astragalus* has the ability to “enhance vital energy and fortify bones and muscles”. In clinical application, *Astragalus* has been utilized to enhance lumbar bone mineral density, inhibit bone resorption, and maintain favorable balance through regulating bone metabolism and preventing bone loss among individuals with primary osteoporosis. Additionally, it holds potential to ameliorate symptoms of postmenopausal osteoporosis, promote bone formation and curtail bone resorption [3-5].

It is known that traditional Chinese medicine is characterized by multi-component, multi-target and multi-way for diseases treatment. Network pharmacology is a drug-target-gene-disease-based interaction network, including chemoinformatics, bioinformatics, network biology and pharmacology [6]. It has become a widely used tool to systematically unravel the complex network relationship between bioactive components and underlying mechanisms of traditional Chinese medicine from a system perspective [7].

To determine the mechanism of *Astragalus membranaceus* in LDH disease treatment, network pharmacology was conducted in this study, to unravel the vital target genes of *Astragalus membranaceus*, and demonstrate how *Astragalus membranaceus* inhibits inflammation in LPS-induced LDH model.

**EXPERIMENTAL**

**Screening of active components and target sites of lumbar disc herniation**

The TCMSP database (https://old.tcmsp-e.com/tcmsp.php) was used to screen the bioactive components based on the criteria of oral bioavailability ≥ 30 % and drug-likeness ≥ 0.18. The targets of these components were searched through TCMSP and Swiss target prediction websites (http://www.swisstargetprediction.ch/).

**Collection of *Astragalus membranaceus*-related targets**

The disease targets of *Astragalus membranaceus* were surveyed using two public databases: Online Mendelian Inheritance in Man (OMIM, https://omim.org/) database and GeneCards (https://www.genecards.org/) database, with the keywords “*Astragalus membranaceus*”.

**Analysis of drug regulatory network and protein interaction network**

The potential targets of components in lumbar disc herniation and *Astragalus membranaceus*-related targets were collected and imported into Cytoscape 3.8.2 software (http://www.cytoscape.org) to establish a network of lumbar disc herniation-ingredients-*Astragalus membranaceus*-target. The overlapping targets of drugs and diseases in the intersection were inputted into STRING database (https://string-db.org/) to construct protein interaction network. Cytoscape 3.8.2 was used to perform visualization and also to construct a protein-protein interaction (PPI) network.

**Functional enrichment analysis**

To investigate the biological function and signaling pathways associated with *Astragalus membranaceus*, the gene ontology (GO) analysis and the Kyoto encyclopedia of genes and genomes (KEGG) analysis were employed. KEGG and GO analysis were screened for q values < 0.05.

**Molecular docking**

For molecular docking of active components of *Astragalus membranaceus* and key genes, the 3D molecular structures of kaempferol, quercetin and formononetin were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/), and the protein structures of some key genes were downloaded through the Protein Data Bank (PDB, https://www.rcsb.org/). The molecular docking was performed using the CB-dock2 online tools (https://cadd.labshare.cn/cb-dock2/php/index.php).

*Astragalus* inhibits the inflammation of nucleus pulposus cells

Human nucleus pulposus (NP) cells isolated from lumbar disc herniation tissues were treated with LPS to establish an *in vitro* LDH model. Then, NP cells were exposed to kaempferol, quercetin, formononetin and Huangqi decoction,
respectively. The inflammatory factors, IL-1β, IL-6, VEGFA, MMP-9 and MMP2, were assessed [8].

**Enzyme-linked immunosorbent assay (ELISA)**

The concentrations of IL-1β and IL-6 in the cell medium were evaluated using ELISA kits of IL-1β (ab214025, Abcam) and IL-6 (ab178013, Abcam). A 100 μL sample was introduced into the ELISA well and incubated for 2 h. Then 100 μL detected antibody was added, and the mixture underwent an additional 1 h incubation. Afterward, the wells were subjected to treatment with enzyme working reagent and TMB reagent. The reaction was ultimately terminated by addition of stop solution. The absorbance value was read at 450 nm.

**Western blot assay**

The total protein were extracted using lysis buffer (25 mM Tris-HCl pH 7.4, 250 mM NaCl, 50 mM KCl, 10 % glycerol, 0.5 % NP-40). The lysates were subjected to SDS-PAGE gel running and transferred to nitrocellulose membrane followed by incubation with the primary antibodies against VEGF A (19003-1-AP, ProteinTech, IL, USA), MMP9 (27306-2-AP, ProteinTech), MMP2 (10373-2, ProteinTech) and β-actin (20536-1-AP, ProteinTech). After incubation with horseradish peroxidase labeled with anti-rabbit IgG (B900210, ProteinTech). The target bands were visualized with ECL reagents (Solarbio, Beijing, China). The relative intensity of each band was measured using ImageJ software and normalized to β-actin [9].

**Statistical analysis**

The data are expressed as mean ± standard deviation (SD). Statistical evaluation was performed by one-way analysis of variance (ANOVA) to compare the means among the groups, using SPSS 25.0 version [10]. P<0.05 was taken as indicating statistical significance.

**RESULTS**

**Potential targets of LDH and Astragalus membranaceus**

Based on oral bioavailability ≥ 30 % and drug-likeness ≥ 0.18, 20 bioactive components of Astragalus membranaceus were screened using TCMSP database. The targets of these components were searched through TCMSP and Swiss target prediction websites, and a total of 525 targets were acquired (Figure 1 A). The targets of LDH were screened using GeneCards and OMIM databases, resulting in the acquisition of 698 targets. Statistical computing, conducted using the R project, was employed for data screening. Through the intersection of 525 Astragalus membranaceus-related targets and the 698 LDH-related targets, 84 shared recognitions were identified. These overlapping targets were designated as the core targets for subsequent investigations (Figure 1 B).

**Enrichment analysis of GO and KEGG**

To investigate the potential signaling pathway linked to Astragalus membranaceus in the treatment of LDH, GO and KEGG enrichment analysis were applied based on the potential targets. As suggested in the results, the response to endopeptidase activity was identified as the most enriched biological progress by GO functional enrichment analysis (Figures 2 A and B). As revealed by the analysis of overlapping genes via KEGG pathway enrichment analysis, the predominant enriched signaling pathways associated with the treatment of LDH using Astragalus membranaceus were the TNF, IL-17

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*Figure 1: Potential targets of LDH and Astragalus membranaceus. (A) 20 bioactive ingredients of Astragalus membranaceus were screened using TCMSP database; (B) 84 overlapping recognitions were obtained by intersection targets between 525 Astragalus membranaceus-related targets and 698 LDH-related targets. LDH: Lumbar disc herniation.*
and HIF-1 signaling pathways (Figure 2 C and D).

**PPI network of LDH and Astragalus membranaceus**

A PPI network was created using the STRING database, which was then visually analyzed using Cytoscape 3.8.2. The edges represent protein-protein and nodes represent proteins. To identify the core proteins of *Astragalus membranaceus* intervention for LDH, Cytoscape software was applied to mine important targets (Figure 3 A). Based on the results from the analysis, a new PPI network was acquired to uncover more critical proteins. Eventually, the final PPI network concentrated on the top 10 targets of *Astragalus membranaceus* in treating LDH, sorted by degree values. The most prominently valued target nodes include JUN (c-Jun), vascular endothelial growth factor A (VEGF A), AKT1, prostaglandin-endoperoxide synthase 2 (PTGS2), interleukin-6 (IL6), matrix metalloproteinase 9 (MMP9), MMP2, interleukin-1beta (IL-1β), C-X-C motif chemokine ligand 8 (CXCL8) and epidermal growth factor (EGF). These high-value target nodes are likely to play crucial roles in the treatment of LDH using *Astragalus membranaceus* (Figure 3 B).

**Molecular docking of Astragalus membranaceus with its key target genes**

The active components of *Astragalus membranaceus* quercetin, kaempferol and formononetin were used for the molecular docking. Their 3D molecular structures were downloaded from PubChem. The key target genes contain VEGFA, IL-6, IL-1β, MMP9 and MMP2, and their protein structures were downloaded from the Protein Data Bank. The molecular docking was performed using CBlowdock2. VEGFA, IL-6, IL-1β, MMP9 and MMP2 were successfully performed for molecular docking with each active component (Figures 4 A - E), and the vina scores were added to the table (Figure 4 F). The data suggested that *Astragalus membranaceus* binds with the predicted key target genes.

**Astragalus inhibited inflammation in LDH model**

Given that *Astragalus membranaceus* bound to VEGFA, IL-6, IL-1β, MMP9 and MMP2 which are typical molecules involved in inflammation, the *in vitro* LDH model was established to study the effect of the inhibition by *Astragalus on inflammation*. The data from ELISA revealed that IL-1β and IL-6 levels were significantly decreased in groups treated with quercetin, kaempferol, formononetin and huangqi decoction (Figure 5 A). Moreover, western blots data showed that the expression of VEGFA, MMP-9 and MMP2 were significantly increased when the cells were treated with quercetin, kaempferol, formononetin and huangqi decoction (Figure 5 B). Therefore, *Astragalus* suppressed the inflammatory activity in LPS-induced nucleus pulposus cells.

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**Figure 2:** Enrichment analysis of GO and KEGG. (A) Bar plot diagram of GO analysis for mainly biological pathways; (B) Dot plot diagram of GO analysis for mainly biological pathways; (C) Bar plot diagram of KEGG enrichment analysis of the mainly enriched genes; (D) Dot plot diagram of KEGG enrichment analysis of the mainly enriched genes
Figure 3: PPI network of LDH and Astragalus membranaceus. (A) PPI network among the core proteins of Astragalus membranaceus intervention for LDH. The edges represent protein-protein and nodes represent proteins; (B) The top 10 targets of Astragalus membranaceus in treating LDH were sorted by degree values.

Figure 4: Molecular docking of Astragalus membranaceus with its key target genes. (A - E) The molecular docking of target genes VEGFA, IL-6, IL-1β, MMP9 and MMP2 with Astragalus membranaceus active components containing quercetin, kaempferol and formononetin. (F) The vina score of molecular docking.

DISCUSSION
Lumbar disc herniation affects the lumbar spine and presses on nearby nerves, causing pain, numbness, and weakness in the lower back, buttocks, legs, and feet. In severe cases, cauda equina nerve damage may occur, leading to defecation disorders and even paralysis. Developing complementary and alternative therapies are necessary for LDH treatment [2]. Astragalus membranaceus inhibits bone resorption, regulates bone metabolism, and prevents bone loss in patients with primary osteoporosis [3-5]. However, the mechanism of action of Astragalus membranaceus in LDH treatment remains unclear.
In this work, a network pharmacology approach was conducted to analyze *Astragalus membranaceus*. A total of 20 bioactive ingredients of *Astragalus membranaceus* were screened, and 525 targets of these bioactive ingredients were acquired. There were 698 targets of LDH acquired from online databases, and 84 overlapping recognitions between *Astragalus membranaceus*-related targets and LDH-related targets were designated as core targets for further study.

Network pharmacology analysis combines high-throughput histology, bioinformatics, and systems biology [11,12], and which was applied in this study to the therapeutic effects of *Astragalus membranaceus* in LDH treatment [2,13]. The active components of *Astragalus membranaceus* include kaempferol, quercetin and formononetin, which have been widely used clinically for a long time. It is well known that traditional Chinese prescriptions are characterized by multi-components and multi-targets, and cannot be compared to Western drugs which have single chemical components [14]. Direct at the 84 overlapping genes, a new subject of PPI network was conducted for a comprehensive analysis on the illness mechanism of actions and ingredients of *Astragalus membranaceus*.

Molecular docking is used to predict the binding affinity of *Astragalus membranaceus* to its target proteins[7]. This program uses different algorithms and scoring functions to evaluate the binding energy and determine the best conformation of the ligand within the binding site of the target protein. The results of the docking simulations provide insights into the interaction between the ligand and target genes, including the binding site, binding mode, and binding affinity [15]. This information may be instructive for designing new ligands with improved potency and selectivity. Hence, molecular docking is a useful tool for predicting the interaction between *Astragalus membranaceus* and its key target genes.

*Astragalus membranaceus* has been shown to have anti-inflammatory effects in *in vitro* and *in vivo* models [16]. *Astragalus membranaceus* has also been reported to stimulate the production of anti-inflammatory cytokines and inhibit the production of pro-inflammatory cytokines such as IL-1β and IL-6 [17,18]. This may help to reduce the activation of immune cells and the subsequent release of inflammatory mediators. Additional research is required to comprehensively grasp the mechanisms of action and establish the ideal dosage and treatment duration.

**CONCLUSION**

This study employed network pharmacology analysis to acquire the active components of *Astragalus membranaceus* and their target genes. GO, KEGG and PPI network analysis demonstrates that VEGFA, AKT1, JUN, PTGS2, IL6, MMP9, IL1β, CXCL8, EGF and MMP2 exert vital activities in LDH treated with *Astragalus membranaceus*.
membranaceus. Molecular docking results suggest that these vital proteins bind active components of Astragalus membranaceus, and the active components inhibit inflammation in the LDH model. Thus, the data obtained have shed more light on the prevention and treatment of LDH with the traditional Chinese prescription, Astragalus membranaceus.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. WenJie Ke, Chengwei Yu designed the study and carried them out; WenJie Ke, Chengwei Yu, Wei Liu, Haifeng Liu supervised the data collection, analyzed and interpreted the data; WenJie Ke, Chengwei Yu prepared the manuscript for publication and reviewed the draft of the manuscript. All authors read and approved the manuscript for publication.

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