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Study on the mechanism of action of Lycii Fructus in treating spinal cord injury based on network pharmacology

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Abstract: The purpose of this study was to to explore the effective active ingredients and specific mechanisms of *Lycii Fructus* in treating spinal cord injury (SCI). The method used in this study was to obtain the main active ingredients of *Lycii Fructus* and their corresponding targets through the Chinese Medicine System Pharmacology Database and Analysis Platform (TCMSP), Perl, UniProt and other databases. The targets of SCI were obtained through the four databases of OMIM, GeneCards, DrugBank and PharmGkb. The potential therapeutic targets of *Lycii Fructus* against SCI were determined by the Venn diagram online tool. The protein interaction network was established with the help of the String database, and the PPI network diagram was topologically analyzed by Cytoscape software to select core targets. DAVID database was used to perform GO analysis and KEGG pathway enrichment analysis on potential therapeutic targets, and the visualization

was performed using the R language. The results of Venn diagram showed that there were 156 cross targets between the active components of *Lycii Fructus* and SCI. The core targets were TP53, MAPK1, AKT1, HSP90AA1, ESR1, etc. GO analysis showed that the biological processes involved in *Lycii Fructus* were mainly DNA binding transcription factors, activation of nuclear receptors and transcription factor activity, activation of G protein-coupled amine receptor activity, etc. KEGG pathway enrichment analysis found that *Lycii Fructus* played a role in the treatment of SCI involving PI3K-Akt signaling pathway, IL-17 signaling pathway, TNF signaling pathway, HIF-1 signaling pathway and p53 signaling pathway. This study preliminarily reveals *Lycii Fructus* can treat SCI through multiple pathways, multiple components and multiple targets. This experiment lays the foundation for further research on the therapeutic effect of *Lycii Fructus.*

1. Introduction

Spinal cord injury (SCI) is a kind of injury that the spinal cord function changes temporarily or permanently caused by severe spinal injury or related diseases[1]. It has the characteristics of high incidence, high treatment cost, high disability rate and low onset age[2]. SCI includes primary injury and secondary injury, but its specific mechanism is not clear, but most scholars believe that it includes blood circulation disorder, changes in biochemical active substances and energy metabolism disorder[3]. Traditional Chinese medicine also believes that SCI belongs to the category of “body laziness” and “paralysis”. As stated in “Lingshu·Cold and Heat Diseases”: “If the body is injured, there is a lot of blood loss, and it is like a stroke and cold, if there is a fall, the limbs are laziness and cannot be retracted, which is called body laziness.” It can be seen that SCI is mostly caused by trauma, and the Governor Vessel is damaged, resulting in dysfunction of Qi, blood, meridians, and internal organs, which causes the body to become paralyzed over time. The treatment principle is to replenish Qi and blood, promote blood circulation and remove blood stasis, strengthen the spleen and kidney, nourish blood and soften the liver, and replenish the kidney and replenish essence[4].

Lycii Fructus is the dried mature fruit of the Ningxia *Lycii Fructus* plant of the Solanaceae family. It has the effects of nourishing the liver and kidneys, improving blood and essence, and improving eyesight[5]. Studies have shown that *Lycii Fructus* contains a variety of active ingredients such as polysaccharides, polyphenols, and carotenoids, which can play a variety of pharmacological effects such as antioxidant, anti-inflammatory, anti-tumor, neuroprotective, and immune enhancement[6,7]. So, whether it has the effect of treating SCI needs further in-depth research. This study used the method of network pharmacology to systematically analyze the active ingredients, targets, and

signal pathways of *Lycii Fructus*, explore the molecular mechanism of *Lycii Fructus* in treating SCI, and provide a scientific basis for the further development and utilization of *Lycii Fructus*.

2. Materials and Methods

2.1. Screening of active Components and targets of *Lycii Fructus*

All active components and targets of *Lycii Fructus* were retrieved fromthe Traditional Chinese Medicine system pharmacology database analysis platform (TCMSP, *http://lsp.nwu.edu.cn/temspsearch.php*) with the key word of "*Lycii Fructus*"[8]. Based on the oral bioavailability (OB)≥30% and drug-like properties (DL)≥0.18, the active components and corresponding targets of *Lycii Fructus* were screened, and the target names were standardized by UniProt database (*https://www.uniprot.org/*) based on the principle of species being human[9].

2.2. Target screening of SCI

SCI-related genes were searched through the GeneCards database(*http://www.genecards.org*)[10], the Online Mendelian Inheritance in Man database(*https://omim.org/*)[11], the TDD database (*http://db.idrblab.net/ttd/*) [12]and the DrugBank database (*https://go.drugbank.com/*)[13], and duplicate genes were deleted after summary.

2.3. Screening of relevant targets for the treatment of SCI by Lycii Fructus

Upload the obtained drug targets and SCI-related targets to Venny platform (*http://bioinformatics.psb.ugent.be/webtools/Venn/*), and take the intersection of the two to initially obtain the target of the wolfberry in treating SCI.

2.4. Construction of protein-protein interaction networks and acquisition of core targets

Input the targets of wolfberry fruit in treating SCI into the "STRING" database (https://string-db.org/)[14], select the species "Homo sapiens", set the lowest interaction score to "highest confidence" (0.900). The topology analysis was carried out through the CytoNCA plug-in in Cytoscape software to select the core targets for the treatment of SCI.

2.5. Functional enrichment analysis

Input the action targets of Lycium barbarum into the DAVID database (*https://david.ncifcrf.gov/*)[15] for Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Species selection "Homo sapiens", screening with $P<0.01$ as the condition, and sorting according to P value, select the top 10 items of biological process (BP), cytological component (CC), molecular biological function (MF) in the GO

functional annotation and the top 30 pathways in the KEGG pathway enrichment, and visualize the results using R language.

3. Results

3.1. Screening of effective active components and targets in Lycii Fructus

45 known pharmaceutical components of *Lycii Fructus* were retrieved from the TCMSP database. 34 active components were screened out according to the conditions of OB≥30% and DL≥18%, corresponding to 178 action targets. Active components with high connectivity were selected and used Cytoscape software to visualize the protein network to build a "*Lycii Fructus*-action targets" network diagram, in which the outer sky blue nodes represent the active components of *Lycii Fructus* and the inner pink nodes represent the action targets. See Figure 1. Among them, the active components with high degree of connectivity include quercetin, β-sitosterol and stigmasterol.

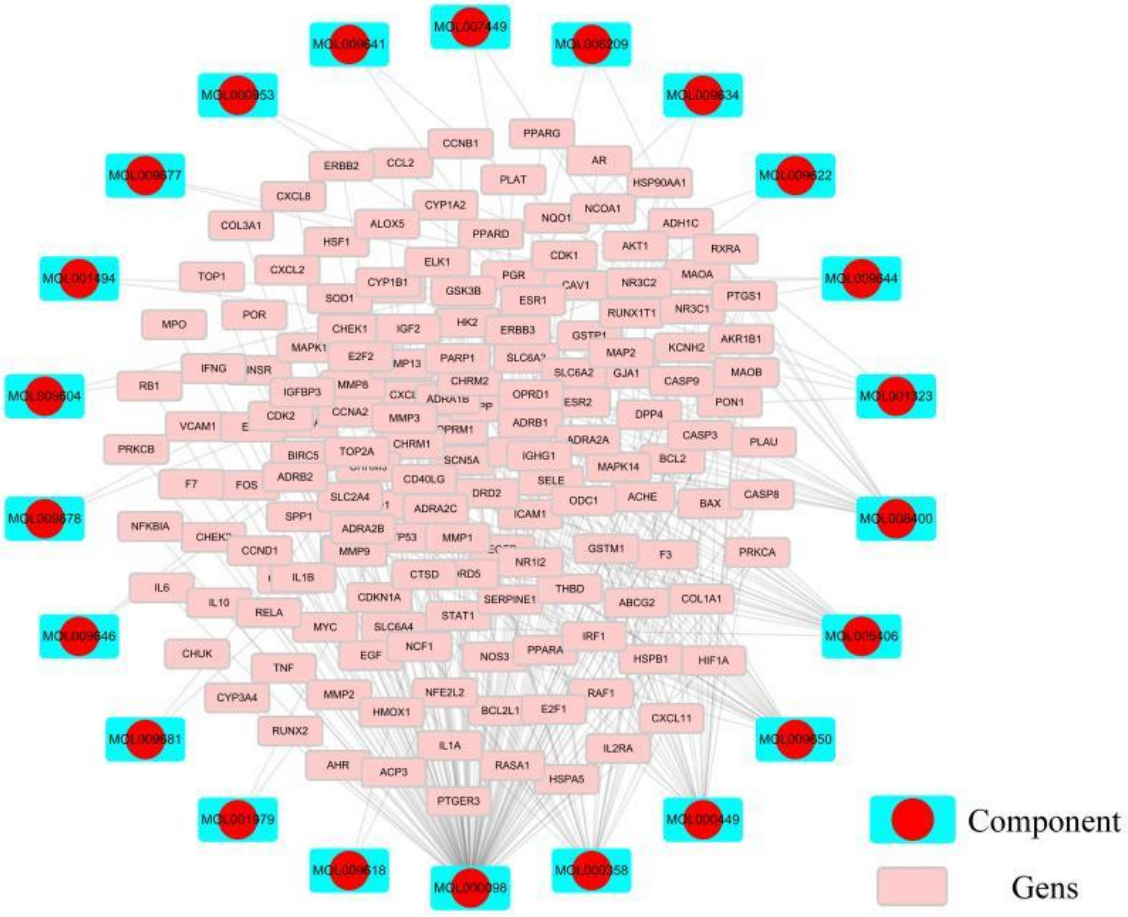

Figure 1: Active Component-target genes network diagram.

3.2. Target screening of SCI

6738, 60, 16, and 52 SCI disease targets were screened from GeneCards, OMIM, TDD, and DrugBank databases respectively, and a total of 6753 SCI disease targets were obtained after removing duplicates. See Figure 2A.

3.3. Screening of relevant targets for the treatment of SCI by Lycii Fructus

The Venny platform was used to intersect the 178 targets corresponding to the 34 active components in the screened *Lycii Fructus* with the 6753 SCI disease targets, and 156 common targets were screened as the target of the *Lycii Fructus* in treating SCI. See Figure 2B.

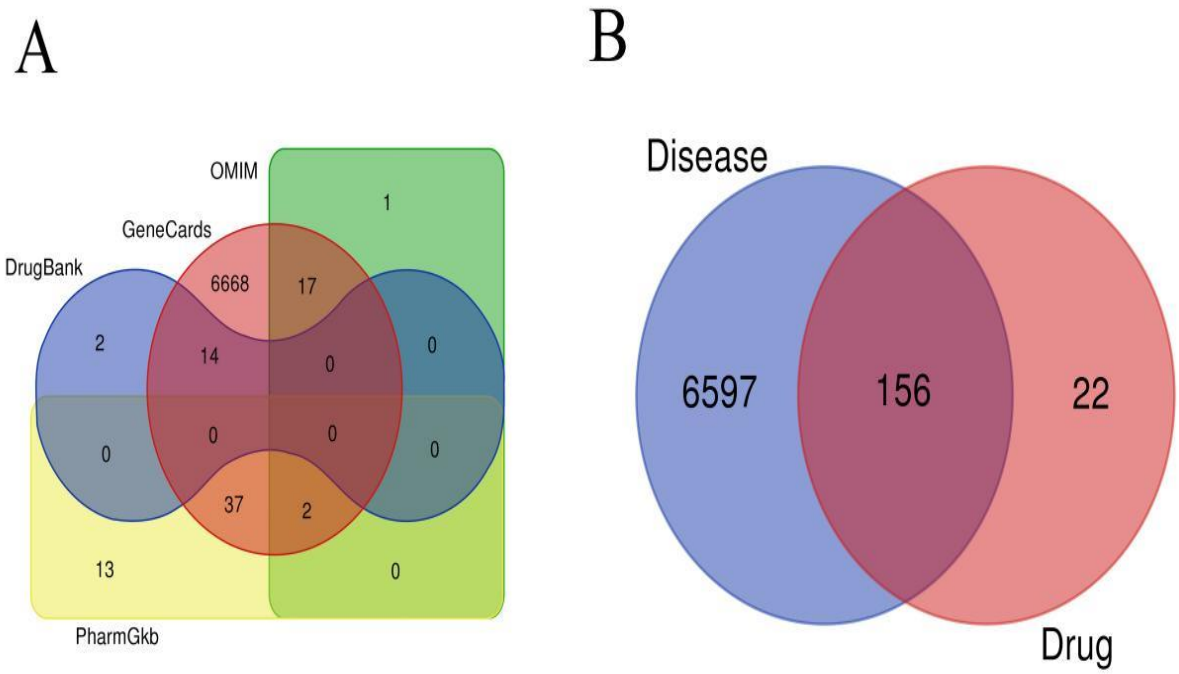

Figure 2: *Lycii Fructus*-SCI target map.

3.4. Construction of protein-protein interaction (PPI) network and acquisition of core targets

In order to further understand the functions and mechanisms of the targets of *Lycii Fructus* in treating SCI, the 156 targets of *Lycii Fructus* in treating SCI selected above were imported into the String database to build a PPI network, as shown in Figure 3. The CytoNCA plug-in was used to conduct topological analysis of the PPI network, and genes with BC>10.94, CC>0.50, DC>8, EC>0.13, LAC>3.83, and NC>5.67 were taken together to screen out 8 targets, namely TP53, MAPK1, AKT1, HSP90AA1, ESR1, TNF, BCL2, and IL6, as shown in Figure 4. The above genes may be the core target of *Lycii Fructus* in treating SCI.

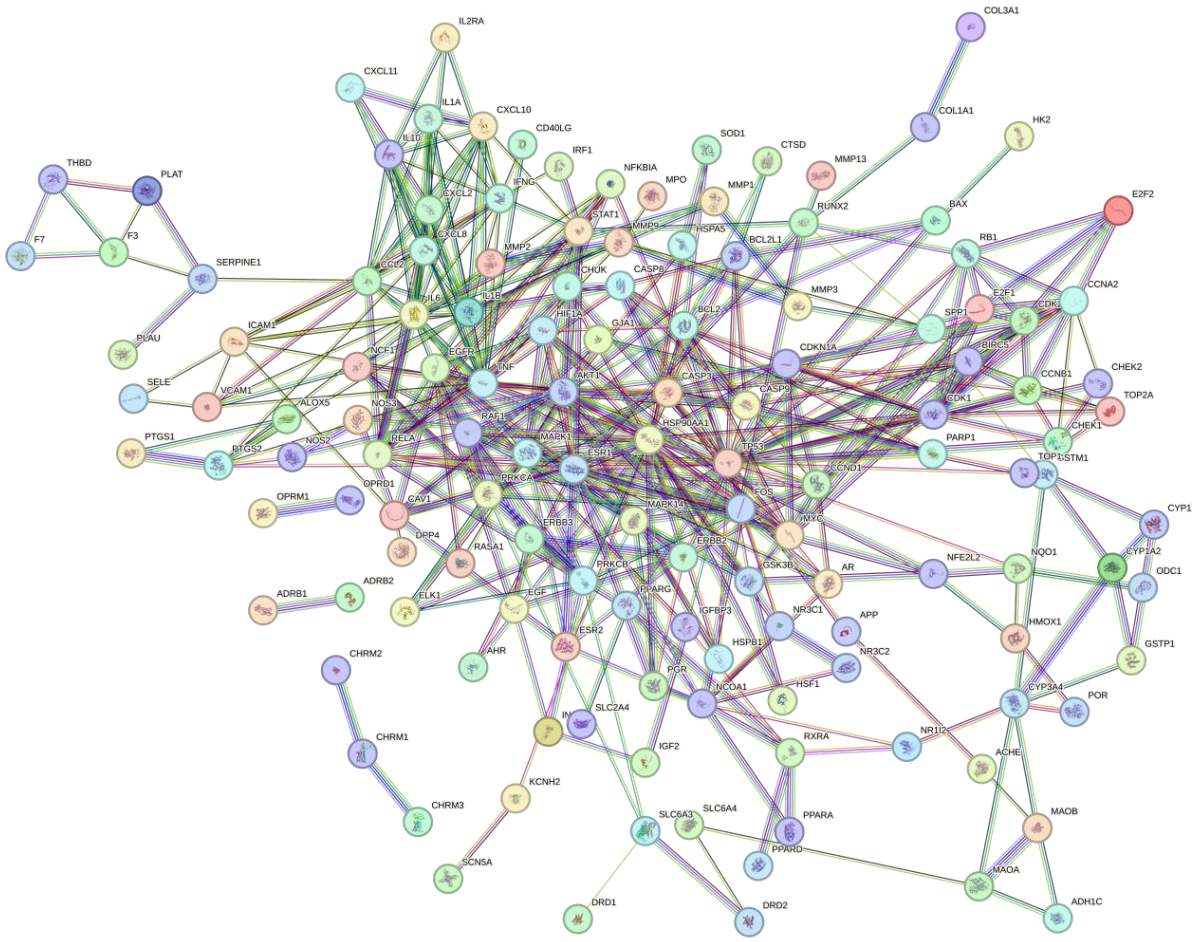

Figure 3: PPI network of *Lycii Fructus* target for SCI

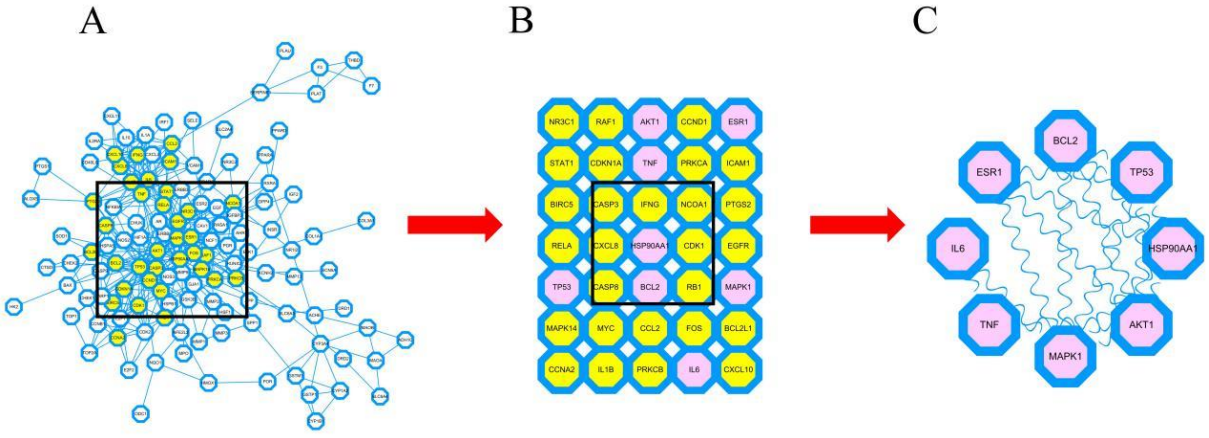

Figure 4: The PPI network construction

3.5. GO analysis

The 178 targets of active ingredients of *Lycii Fructus* in treating SCI were imported into the

DAVID online database for GO analysis. The threshold was set to P<0.01, and the 10 items with the highest P value were selected to draw a histogram through R language. GO analysis results showed 2523 items, of which 2236 were obtained for biological processes (BP), mainly involving responses to foreign body stimulation, responses to oxidative stress, responses to biological stimulation, etc. There are 91 cell compositions (CC), mainly involving membrane rafts, membrane microdomains, neuron cell bodies, synaptic membranes, vesicular cavities, etc. There are 196 molecular functions (MF), mainly involving DNA-binding transcription factors, specific RNA polymerase II binding transcription factors, regulation of kinase activities, etc. as shown in Figure 5.

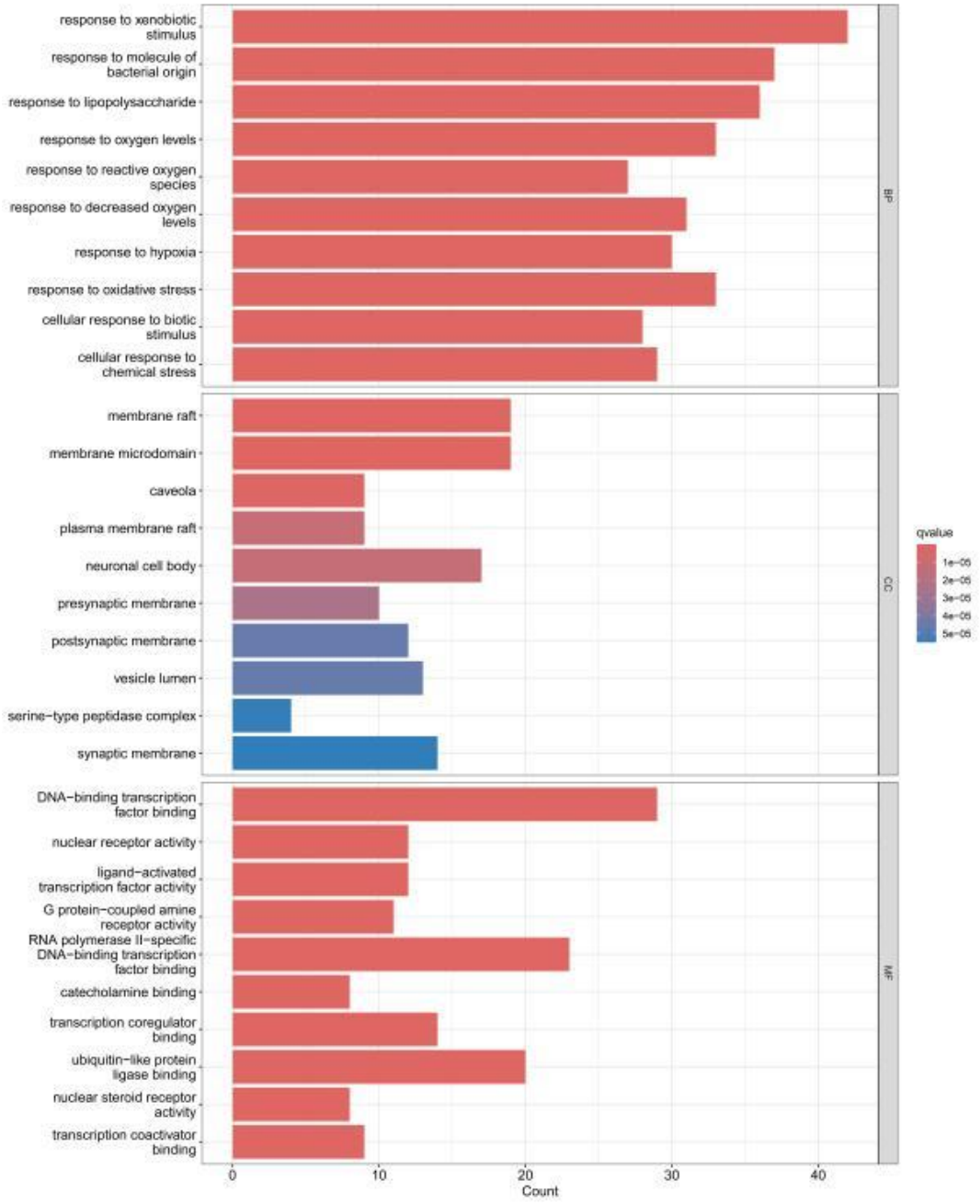

Figure 5: GO analysis of therapeutic targets of *Lycii Fructus* in the treatment of SCI

3.6. KEGG pathway enrichment analysis

KEGG signaling pathway enrichment analysis was performed on 178 targets of *Lycii Fructus* in the treatment of SCI, and the threshold was set to $P < 0.01$. The 30 signaling pathways with the highest P values were selected and a bar graph was drawn using R language. The results showed that the above 178 targets were mainly enriched in the AGE-RAGE signaling pathway, IL-17 signaling pathway, TNF signaling pathway, and HIF-1 signaling pathway. The above results suggest that *Lycii Fructus* may play a role in the treatment of SCI through the above signaling pathways, as shown in Figure 6.

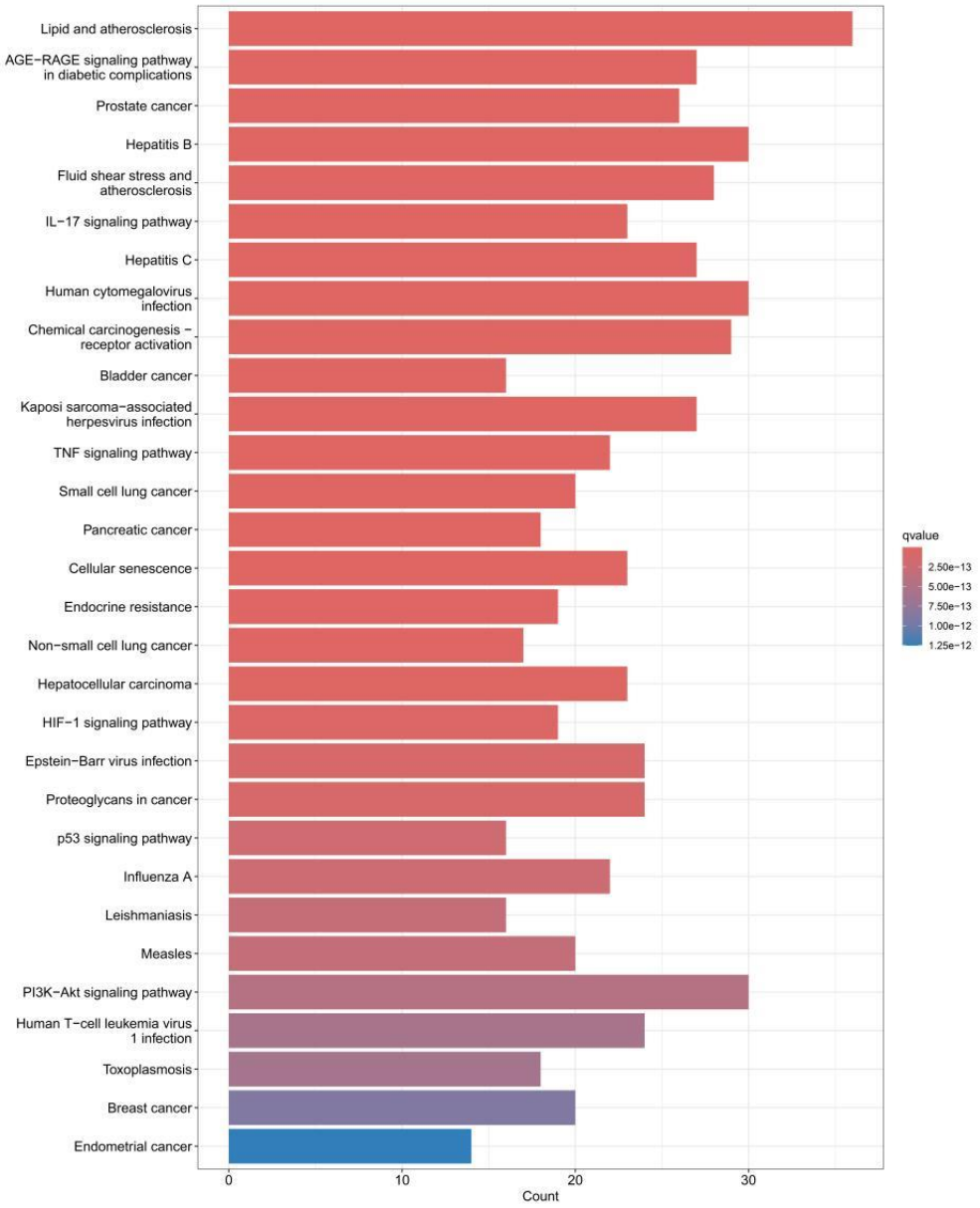

Figure 6: KEGG pathway enrichment analysis of the therapeutic targets of *Lycii Fructus* in the treatment of SCI (The top 30 signaling pathways)

4. Discussions

The incidence of spinal cord injury is increasing year by year worldwide. SCI is not only highly dangerous in itself, but also accompanied by serious secondary injuries, which leads to a poor quality of life for patients. Currently, there is still a lack of effective therapeutic drugs in clinical practice[16]. Therefore, it is crucial to find effective drugs and explore their treatment mechanisms. Based on traditional Chinese medicine theory and clinical practice, the pathogenesis of SCI can be summarized as stagnation of the Du vessel. Treatment should promote blood circulation to remove blood stasis and clear the Du vessel[4]. *Lycii Fructus* was first recorded in the Shennong Materia Medica Classic. It mainly governs five internal pathogenic factors, including heat, thirst, and arthralgia. However, it did not distinguish the medicinal parts of *Lycii Fructus*. It was not until the Compendium of Materia Medica that the *Lycii Fructus* fruit mainly nourished, while the *Lycii Fructus* root mainly consumed thirst and fever. The Chinese Pharmacopoeia also includes *Lycii Fructus*. Its main functions are: nourishing liver and kidney, replenishing essence and improving eyesight. It is used for deficiency of essence, waist and knees soreness, dizziness and tinnitus, impotence and spermatorrhea, internal heat and thirst, blood deficiency and yellowing, and unclear vision.

This study used network pharmacology methods to explore the mechanism of *Lycii Fructus* in treating spinal cord injury. The results showed that the 34 active ingredients screened by *Lycii Fructus* corresponded to 178 targets of action, and there were 156 intersection targets with 6753 stroke-related targets. By building a PPI network of 156 target proteins, 8 core targets were screened. The top three topologically ranked are TP53, MAPK1, and AKT1, which are the key targets for *Lycii Fructus* to play a role in SCI treatment. TP53 is an apoptosis regulatory factor that exerts an apoptosis-inducing effect by stimulating the expression of BAX and FAS antigens or by inhibiting the expression of Bc1-2. It activates its pro-apoptotic activity through interaction with PPP1R13B/ASPP1[17]. Mitogen-activated protein kinase 1 (MAPK1) is an important protein in MAPK signaling. It inactivates MAPK by dephosphorylating serine, threonine and tyrosine. As a negative regulator of MAPK signaling, MAPK1 plays an important role in regulating cell proliferation, growth and differentiation [18]. Related studies have shown that in the serum of SCI patients and spinal cord injury mice, miRNA-433- 5p is significantly down-regulated and MAPK1 is up-regulated. Overexpression of miRNA-433-5p may slow the inflammatory response by targeting MAPK1, thereby promoting spinal cord injury rats. Restoration of motor function. AKT1 protein, also known as protein kinase B (PKB), is a key molecule in the cellular signaling pathway and is involved in regulating multiple processes such as cell growth, survival, metabolism and differentiation. Brunet A [19] found that the AKT or PI3K/Akt pathway is closely related to neuron survival. Brain-derived neurotrophic factors and nerve growth factors can mediate or promote the survival of nerve cells by activating AKT[20]. In addition, AKT1 in the signaling pathway plays an important role in neuronal apoptosis. Up-regulating the expression of AKT1 can effectively reduce

nerve damage and inhibit axon degeneration [21].

KEGG pathway enrichment analysis found that Lycium barbarum mainly plays a therapeutic role through the AGE-RAGE signaling pathway, the PI3K-AKT signaling pathway, and the HIF-1 signaling pathway. AGE-modified peripheral nerve myelin can promote segmental demyelination. When advanced glycation end products modify major axon cytoskeletal proteins, they can cause axon degeneration and obstruction of axon transport. AGEs and AGE/RAGE interact with each other, they can induce oxidative stress and induce pro-inflammatory responses, which in turn lead to segmental demyelination and axon dysfunction. Therefore, effective regulation of the AGEs-RAGE axis plays a key role in alleviating the pathogenesis of diabetic peripheral neuropathy [22,23]. The PI3K-AKT axis is one of the important signaling pathways that regulate autophagy, apoptosis, inflammatory response, and oxidative stress response of spinal cord cells. Effectively regulating the PI3K-AKT axis can up-regulate the expression level of Bcl-2 in spinal cord tissue and down-regulate the expression level of CASP-3, IL-6, IL-1β, PI3K, and AKT, thereby playing a protective role in SCI. Hypoxia inducible factor-1 (HIF-1) is involved in physiological and pathological processes such as neovascularization, apoptosis, autophagy, and axon regeneration after SCI. Activation of the HIF-1 signaling pathway can induce autophagy in spinal cord cells, inhibit apoptosis, improve nerve survival. The microenvironment, and ultimately play a role in promoting nerve axon regeneration [24]. When the HIF-1/VEGF signaling pathway is activated, it can promote the formation of new blood vessels after SCI, improve hypoxia and ischemia, and then promote the repair of neural function after SCI in rats.

In summary, this study used network pharmacology to analyze the pharmacological connections of *Lycii Fructus* in the treatment of SCI at the molecular level, and found that effective molecular targets such as TP53, MAPK1, and AKT1 are key targets in its treatment process. These targets affect each other in series and synergize with each other at multiple molecular levels, and jointly play a role in treating SCI through the AGE-RAGE signaling pathway, IL-17 signaling pathway, TNF signaling pathway, HIF-1 signaling pathway, etc.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Acknowledgments

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