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Study on the mechanism of action of Phellodendron amurense in treating spinal cord injury based on network pharmacology

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Abstract: The purpose of this study was to explore the mechanism of action of *Phellodendron amurense* in treating spinal cord injury based on network pharmacology. The method of this study was to obtain the main active ingredients and target genes of *Phellodendron amurense* from the Traditional Chinese Medicine System Pharmacology Database and Analysis Platform (TCMSP), and standardize the gene names through the Uniport database. The four databases of OMIM, GeneCards, DrugBank and PharmGkb were used to identify the disease genes of spinal cord injury. The target genes of drug active ingredients and the disease genes of spinal cord injury were intersected by the online tool of Venn diagram to determine the potential therapeutic targets of *Phellodendron amurense* against spinal cord injury. The protein-protein interaction (PPI) network of potential therapeutic targets was obtained through the STRING database, and the PPI network diagram was topologically analyzed by the CytoNCA plug-in in the Cytoscape software to select the core key targets. The biological information annotation database DAVID was used to perform GO analysis and KEGG pathway enrichment analysis on the potential therapeutic targets, and the visualization was performed by R language. The results showed that a total of 37 effective active ingredients and 511 effective active ingredient targets of *Phellodendron amurense* were screened. A total of 6753 targets for spinal cord injury and 157 drug-disease intersection targets were screened. Quercetin (MOL000098), isoberberine (MOL000790), (S)-hydroberberine (MOL001455), β-sitosterol (MOL000358) and other compounds were the main active ingredients, and TP53, TNF, ESR1, AKT1, IL6, MAPK1, HSP90AA1, RELA, IL1B, MYC, CCND1, and BCL2 were the main action targets. GO analysis showed that the biological processes involved in *Phellodendron amurense* mainly involved responses to xenobiotic stimulation, responses to bacterial-derived molecules, responses to lipopolysaccharides, and responses to nutritional levels. KEGG pathway enrichment analysis found that *Phellodendron amurense* plays a role in the treatment of spinal cord injury involving AGE-RAGE signaling pathway, IL-17 signaling pathway, TNF signaling pathway, HIF-1 signaling pathway, PI3K-Akt signaling pathway, etc. The study concluded that *Phellodendron amurense* has the characteristics of multi-target and multi-pathway effects in

the treatment of spinal cord injury, and it may intervene in the spinal cord injury process by promoting neuronal survival, promoting axonal regeneration, inhibiting neuronal apoptosis, and anti-inflammatory processes.

1. Introduction

Spinal cord injury (SCI) is caused by a traumatic event and can lead to impaired neurological function, partial or complete loss of sensation and motor function, paralysis, loss of bowel and bladder control, and sexual dysfunction. It has profound psychological, social, and economic impacts not only on the SCI individual, but also on the entire family [1]. According to the latest research, the global prevalence of spinal cord injury in 2016 was about 368/105, the prevalence of spinal cord injury in my country was about 236/105, and about 3.8 million people are suffering from spinal cord injury. According to the latest research, the global prevalence of spinal cord injury in 2016 was about 368/105, the prevalence of spinal cord injury in my country was about 236/105, and about 3.8 million people are suffering from spinal cord injury[2]. Spinal cord injury is divided into two stages: primary and secondary. Primary injury is related to the destruction of nerve and vascular structures. Secondary injury occurs within a few minutes to a few days after the primary injury, including a series of cascade pathological and physiological changes, including vascular dysfunction, inflammatory response, oxidative stress, glutamate excitotoxicity, cell apoptosis, etc[3]. Since primary injury is irreversible, how to inhibit the pathological and physiological changes after spinal cord injury and reduce secondary injury to achieve neuroprotection and neuroregeneration is the focus of current research. is born in mountainous miscellaneous wood forests or near valley streams. It is the dry bark of the Rutaceae plant yellow bark tree. It is mainly produced in Sichuan, Shanxi, Guangxi, Yunnan, Hubei, Guizhou and other places[4]. It is bitter and cold in nature. It belongs to the kidney and bladder meridians. It has the effects of clearing heat and dampness, purging fire and steaming, and detoxifying and treating sores. It is mainly used for damp heat and diarrhea, jaundice and red urine, heat and astringent pain, bone steaming, eczema and wet sores, etc[5]. It mainly contains alkaloids such as berberine, magnoliine, and palmotedrine, and phenolic acids such as syringin and coniferous; in addition, there are also limonins such as phellodendron ketone and phellodendron lactone, terpenoids such as freedelins, phenylpropanoids such as zanthoxylum, as well as various chemical components such as volatile oils and trace elements. They have various pharmacological activities such as anti-inflammatory, antibacterial, anti-cancer, lowering blood sugar, and lowering uric acid[6]. At present, most research focuses on the medicinal properties of *Phellodendron amurense*, but the research on its molecular mechanism has not yet been in-depth.

Traditional Chinese medicine has the characteristics of multiple components, multiple targets, and diverse regulation methods, and contains a huge amount of information. It is difficult to reflect the systematicness of traditional Chinese medicine by adopting the research ideas of single target and single component in Western medicine. However, network pharmacology is a new method developed based on the concept that drugs function through "multiple targets, multiple pathways, and multiple pathways". It can integrate various drug, protein, gene and other databases, and use bioinformatics technology to analyze and build a drug-target-disease network interaction model to explore the mechanism of drug action. Its holistic and systematic characteristics are very suitable for application to the research of traditional Chinese medicine monomers and compounds. This paper uses network pharmacology methods to discuss the mechanism of in treating spinal cord injury, and provides a methodological basis and theoretical basis for future clinical application and scientific research.

2. Materials and Methods

2.1. Prediction of targets

With the help of the TCMSP database (*http://lsp.nwu.edu.cn/tcmsp.php*)[7], the potential active ingredients of were screened and their related target genes were predicted. This study uses "*Phellodendron amurense*" as the key word, which will meet both the requirements of oral bioavailability (OB) ≥30% (OB greater than or equal to 0.3 indicates good oral absorption of the drug ingredients) and Drug-likeness (DL) ≥ 0.18 (DL greater than or equal to 0.18 indicates suitable for drug development), and the targeted ingredients are stored as potential active ingredients of *Phellodendron amurense* and tabulated. TCMSP is then used to obtain the corresponding target genes of the drug active ingredients, and all target genes with the species "Homo sapiens" are genetically annotated through the UniProt database to standardize gene names, eliminate duplicate and non-human target genes, and obtain candidate target genes for drug ingredients. The screened main active ingredients and potential drug targets of *Phellodendron amurense* were introduced into Cytoscape software to build a "drug-ingredient-target" network. The degree values, mesticity centrality and tightness centrality of component nodes in the network are calculated through topological analysis, taking twice the median of the degree value, the median of mesticity centrality and the median of tightness centrality as thresholds, and determining the candidate core active ingredients corresponding to the target of the active ingredients of *Phellodendron amurense* bark based on the above parameters and conditions, and then sorting them according to the degree value. The first four ingredients are regarded as core active ingredients. The connection lines between nodes are used to represent the mutual relationship between the target and the active ingredient of the drug.

2.2. Screening of SCI-related gene targets

SCI-related genes were identified by the GeneCards database（*http：//www.genecards.org*）[8], the Online Mendelian Inheritance in Man database（*https://omim.org/*）[9], the TDD database (*http://db.idrblab.net/ttd/*)[10]and the DrugBank database (*https://go.drugbank.com/*)[11], and the repeated genes were deleted after summary.

2.3. Screening of targets related to in the treatment of SCI

The obtained drug targets and SCI-related targets were uploaded to the Venny 2.1.0 platform (*http://bioinformatics.psb.ugent.be/webtools/Venn/*), and the intersection of the two was taken to initially obtain the target of in the treatment of SCI.

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

Import the gene targets of *Phellodendron amurense* for treating SCI into the String database (*https://string-db.org/*)[12], limit the species to human beings, obtain protein interaction relationships, save the results in TSV format, and import the interaction network into Cytoscape 3.10.2 statistical software. First, the overall analysis of this protein interaction network was carried out, and then the cytohubba plug was used to determine the five most important protein interaction relationships, and the calculation method was selected to determine the Maximal Clique Centrality (MCC). In a network, nodes are used to represent components and targets, and edges are used to represent interactions between components and targets.

2.5. GO analysis and KEGG pathway analysis

The biological information annotation database DAVID[13] was used to conduct GO analysis and KEGG pathway enrichment analysis on potential treatment targets. The Gene Ontology (GO) analysis includes three parts: Cellular component (CC), Molecular function (MF) and Biological process (BP), which are used to describe the biological functions of genes. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis is used to describe the signal pathways regulated by genes. When searching for potential therapeutic targets for drugs, the species was set to "Homo sapiens", and $P<0.05$ was considered statistically significant. Non-channel entries from KEGG channel enrichment analysis was removed. The results of GO enrichment analysis and KEGG pathway enrichment analysis were visualized using R language, and the top 30 GO items (including BP, CC, and MF) were screened out based on the number of target enrichment to draw a bubble chart; and the top 30 KEGG items were drawn a bubble chart. Finally, Cytoscape software is used to build a "drug-target-pathway" network.

3. Results

3.1. Prediction of gene target

A total of 37 effective active ingredients of were retrieved, corresponding to 1346 targets. The targets of invalid components were removed, and 181 potential targets of *Phellodendron amurense* were obtained (Figure 1). The large circle on the outside is the drug active ingredients, and the square on the inside is the potential target of the drug. The degree of connection between the drug active ingredients and the target is sorted by degree value. The top 4 ingredients are regarded as core active ingredients. They are quercetin (MOL000098), isocorypamine (MOL000790), (S)-hydrogenated berberine (MOL001455), and β-sitosterol (MOL000358).

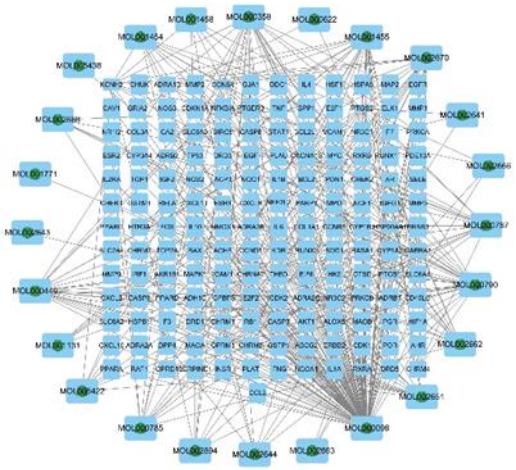

Figure 1: Active Component-target genes network diagram.

3.2. Screening of SCI-related gene targets

The genes related to them were searched through OMIM, GeneCards, DrugBank, and PharmGkb databases using "spinal cord injuries" as the keyword, and 60, 6738, 16, and 52 results were obtained respectively. After deleting duplicate genes, 6753 genes related to spinal cord injury were obtained. (Figure 2A)

3.3. Collection of targets related to myrrh in treating stroke

By mapping and comparing the targets of with the genes related to the pathogenesis of SCI, 157 common genes were found, which speculated that these genes may be related gene targets for in treating SCI. (Figure 2B)

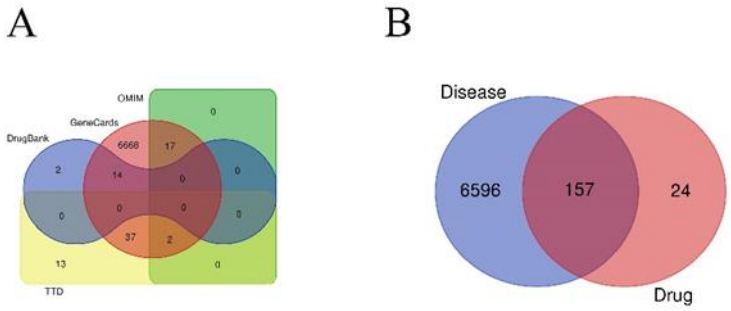

Figure 2: -SCI target map

3.4. PPI network construction and core target analysis

In order to further explore the function and mechanism of in treating potential targets for SCI, the 157 potential targets screened above were imported into the String database, and a protein interaction network diagram with a total of 134 nodes and 413 edges was obtained. See Figure 3 for the PPI network. The CytoNCA plug-in was used to conduct topological analysis of the protein interaction network. BC value>15.20, CC value>0.47, DD value>8.00, EC value>0.12, LAC value>3.83, and NC value>5.40 were screened out. The core targets were sorted out. It was initially concluded that the top 12 targets that play an important role in the treatment of spinal cord injury by *Phellodendron amurense* were TP53, TNF, ESR1, AKT1, IL6, MAPK1, HSP90AA1, RELA, IL1B, MYC, CCND1, BCL2. The protein interactions of 12 core targets were plotted. (Figure 4).

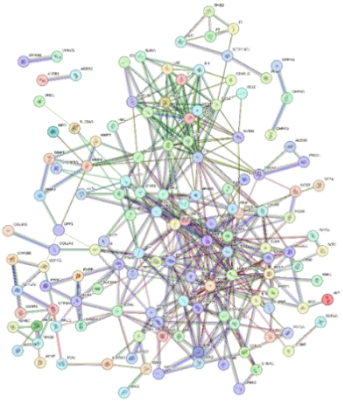

Figure 3: PPI network of target for SCI

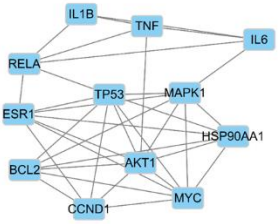

Figure 4: The PPI network construction

3.5. PPI network construction and core target analysis

The 157 effective targets of the active ingredient of in anti-SCI were imported into the DAVID online database for GO functional analysis and KEGG pathway enrichment analysis, and the set value was P<0.05. GO analysis results showed that a total of 2496 items were obtained. Top 10 items in BP, CC, and MF for visual analysis was selected. (Figure 5). Among them, there are 2207 entries for BP, mainly involving responses to alien stimuli, responses to molecules derived from bacteria, responses to lipopolysaccharides, responses to nutritional levels, responses to oxidative stress, wound healing, and responses to oxygen levels, responses to reduced oxygen levels, responses to chemical stress, responses to reactive oxygen species, etc. There are 83 items in CC, mainly involving membrane rafts, membrane microdomains, neuron cell bodies, outer plasma membrane, synaptic membrane, postsynaptic membrane, RNA polymerase reverse transcription regulatory complex, recess, plasma membrane rafts, serine-type peptidase complex, etc. There are 206 items in MF, mainly involving DNA-binding transcription factor binding, ribonucleic acid polymerase II-specific DNA-binding transcription factor binding, transcription co-regulatory factor binding, nuclear receptor activity, ligand-activating transcription factor activity, postsynaptic neurotransmitter receptor activity, neurotransmitter receptor activity, G protein-coupled amine receptor activity, nuclear steroid receptor activity, catecholamine binding, etc.

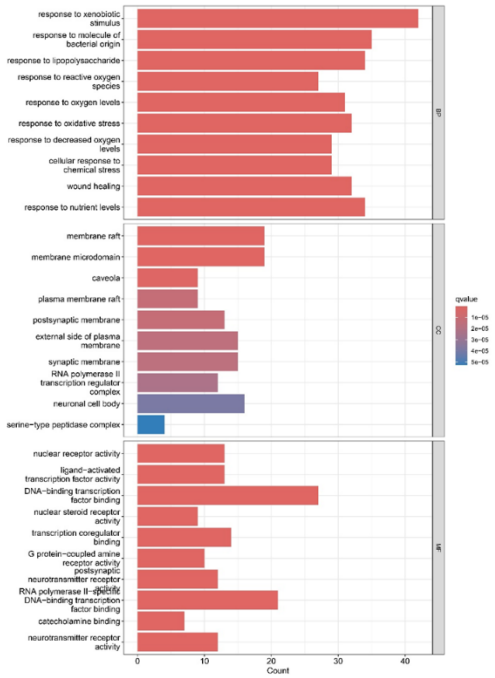

Figure 5: GO analysis of therapeutic targets of in the treatment of SCI

The results of KEGG pathway enrichment analysis showed that a total of 175 pathways were involved. In this study, the top 30 signal pathways were selected to make bubble plots (Figure 6). The AGE-RAGE signaling pathway enriches 26 hub genes, the IL-17 signaling pathway enriches 21 hub genes, the TNF signaling pathway enriches 21 hub genes, the HIF-1 signaling pathway enriches 19 hub genes, the PI3K-Akt signaling pathway enriches 31 hub genes, and the p53 signaling pathway enriches 16 hub genes.

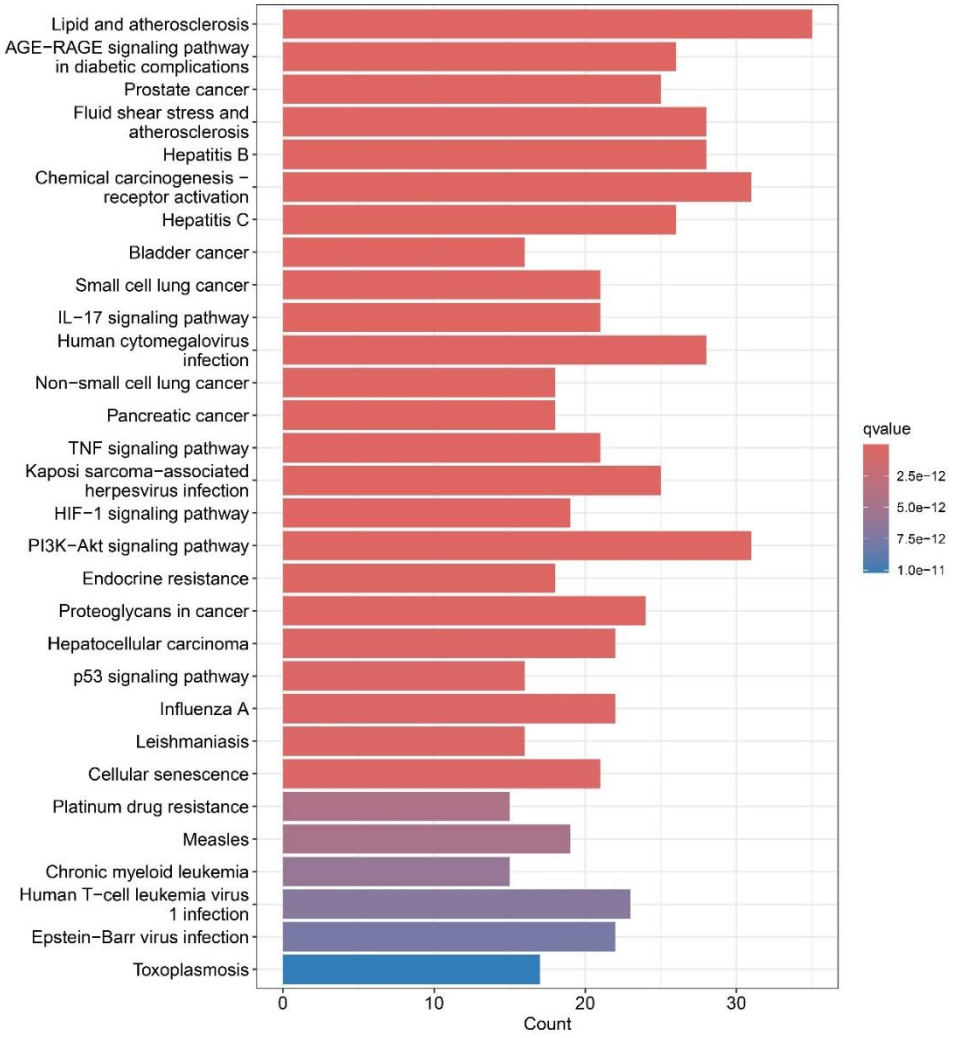

Figure 6: KEGG pathway enrichment analysis of the therapeutic targets of in the treatment of SCI (The top 30 signaling pathways)

4. Discussions

SCI can be divided into two stages: primary injury and secondary injury according to the progression of the disease and its mechanism. The degree of secondary injury plays a key role in the prognosis of SCI. Secondary injuries mainly include changes in local microcirculation after injury, apoptosis of nerve cells, accumulation of oxygen free radicals, etc., and are also important factors affecting the development and prognosis of SCI[14].

Traditional Chinese medicine believes that spinal cord injury belongs to the categories of “body inertia” and “weakness syndrome”. As in “Ling shu Cold and Heat Disease”: “If there is an injury to the body, there is a lot of blood, and if there is a stroke and a cold, if there is something falling, the limbs will be slack and lazy, which is called physical laziness.” It can be seen that spinal cord injury is mostly caused by trauma. Damage to the governor vessel leads to dysfunction of qi and blood, meridians and viscera, causing the body to become flaccid for a long time. The treatment 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 nourish the kidney and replenish essence[15].

This study used online pharmacology methods to explore the mechanism of Cortex Phellodendri

in treating SCI. The results showed that the 24 active ingredients screened by *Phellodendron amurense* corresponded to 181 action targets, and there were 157 intersection targets with 6753 SCI-related targets. By building a PPI network of 157 target proteins, 12 core targets were screened. The top three topologically ranked are TP53, TNF, and ESR1, which are the key targets for *Phellodendron amurense* 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[16]. Studies have shown that after spinal cord injury, activated glial cells produce a variety of inflammatory factors, such as tumor necrosis factor (TNF-α). It has been reported that levels of inflammatory factors increase significantly within 24 hours after spinal cord injury and play a key role in secondary injury after spinal cord injury, leading to spinal cord demyelination and neuron death[17].

KEGG pathway enrichment analysis found that *Phellodendron amurense* mainly plays a role in treating SCI through the AGE-RAGE signaling pathway, TNF signaling pathway, and HIF-1 signaling pathway. Studies have found that 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[18, 19]. TNF, as an important cytokine, can induce multiple intracellular signaling pathways, including apoptosis, cell survival, inflammatory response and immunity. Studies by Karova K and others have shown that transplantation of neural precursor cells can significantly reduce the production of TNF-α in the injured spinal cord, and reduce the formation of glial scars, enhance gray matter preservation, and reduce cavities in the transplanted rats[20]. HIF-1 is a transcription factor that regulates oxygen homeostasis. It consists of two subunits: HIF-1α and HIF-1β. HIF-1 is the main regulator of many hypoxia-induced genes under hypoxia conditions. The target gene of HIF-1 encodes a protein that increases the transfer of oxygen molecules and mediates an adaptive response to oxygen deprivation. Studies have shown that selective inhibition of the hypoxia-inducible factor prolyl hydroxylase 1(HIF-PH1) mediates neuroprotection through HIF-and CREB-against normoxic oxidative death[21].

In summary, this study used network pharmacology to analyze the pharmacological relationship of *Phellodendron amurense* in the treatment of spinal cord injury at the molecular level, and found that effective molecular targets such as TP53, TNF, and ESR1 are key targets in its treatment process. These targets affect each other in series and synergize with each other at multiple molecular levels to jointly play a role in treating spinal cord injury through the AGE-RAGE 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.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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