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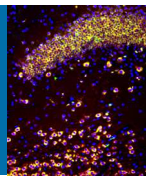

Original Article

Construction of the gene network in the spinal cord injury treated by coptidis rhizoma based on network pharmacological and molecular docking

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Abstract

Objective: To explore the potential molecular network mechanism of coptidis rhizoma in the treatment of spinal cord injury (SCI) based on network pharmacology and molecular docking technology.

Methods: The compounds of coptidis rhizoma and therapeutic targets for SCI were predicted and screened from TCMSP. Genes related to SCI were searched in GeneCards. Protein-protein interaction (PPI) network was constructed and the key targets were screened. The drug-component-target-pathway network was constructed. AutoDock software was used for molecular docking verification.

Results: Fourteen compounds were screened from coptidis rhizoma, consisting of 148 potential targets, 133 of which were related to SCI. Interleukin-6 (IL-6), cysteine aspartate protease 3 (Casp3), proto-oncogene protein c-Jun (Jun), cyclooxygenase 2 (Ptgs2), and proto-oncogene protein c-Myc (Myc) were constructed as an interaction network. The biological processes involved in the treatment of SCI by coptidis rhizoma mainly include adenylate cyclase-inhibiting G protein-coupled acetylcholine receptor signaling pathway, etc. The results of molecular docking showed that the main active components in Coptis chinensis had a stable binding activity with the core target.

Conclusion: The treatment of SCI by coptidis rhizoma involves multiple targets, to promote neuronal survival, inhibit nerve cells apoptosis, in the process of SCI, which is useful to understand the mechanism of coptidis rhizoma for the treatment of SCI in future clinical applications.

Key words: *Coptidis rhizoma; Spinal cord injury; Network pharmacology; Bioinformatics; Therapeutics*

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Introduction

Spinal cord injury (SCI) is a serious neurological injury common in clinical neurosurgery and orthopedics. The disruption of ascending and/or descending neuronal axons through the injured area leads to sensory, motor, and autonomic nerve dysfunction and spinal cord function changes. This usually results in irreversible primary damage to the SCI center immediately after injury (Cardozo MJ, et al., 2017). The body goes through a continuous process of primary injury and secondary injury after SCI. Secondary injury is the main component of the pathological cascade reaction after SCI, which is reversible. Therefore, current studies mostly focus on secondary injury. The glial scar formed after SCI hinders the growth of axons. The lack of local inflammatory reaction, neurotrophic factors, and growth factors leads to a large number of axonal degeneration and diffuse demyelination, as well as the production of a large number of inhibitory factors in the microenvironment of the injured area after SCI, which constitute the main factors of poor repair of SCI. SCI affects millions of people around the world every year, and it can cause great harm to patients' physical and mental health. With the

acceleration of the pace of life in various countries, the incidence of SCI has gradually increased in recent years, there is one SCI patient in about 273,000 people (about 80% of whom are male) (Grégoire CA, et al., 2015) and the rate is increasing at 10,000/year in the United States, and it is 2,000/year in Germany (Jain NB, et al., 2015). The number of cases is about 43 per million in China (Qiu J, 2009). The main causes of SCI are vehicle traffic accidents, wrestling, violence, sports, and work-related injuries, while traffic accidents and sports injuries are the main causes of teenagers' SCI (Yang R, et al., 2014). Wrestling is the most common cause of traumatic SCI in people over 65 years old (Sabapathy V, et al., 2015). SCI usually damages motor and sensory functions and causes serious complications, such as neuropathic pain, spasm, fecal incontinence, pressure ulcers, etc. (Westgren N, et al., 1998), which seriously affects the quality of life of patients and brings severe pressure and economic burden to families and society. The current clinical treatment for SCI is surgery within the effective time to stabilize the spine, reduce external pressure, and control inflammation (Cofano F, et al., 2019), prevent further damage. Clinical research focuses on surgical therapy (Bohl MA, et al., 2018), stem cell therapy (Xu LQ, et al., 2016), electrophysiological stimulation therapy (Hachmann JT, et al., 2017), rehabilitation therapy (Krucoff MO, et al., 2016), traditional Chinese medicine (Zhang Q, et al., 2016), acupuncture (Fan Q, et al., 2018). However, how to effectively recover SCI patients is a great challenge for us.

Coptidis rhizoma, also known as Sichuan coptidis rhizoma, is traditional Chinese medicine. It is bitter in taste and cold in nature, and enters the heart, liver, stomach etc. It can clear away heat and expel fire, detoxify dampness and detoxify. It is a good heat-clearing, detoxifying and anti-inflammatory drug with the function of broad-spectrum antibiotics. At present, more than 100 kinds of chemical components have been isolated from Chinese traditional medicine coptidis rhizoma, including alkaloids, lignans, flavonoids, acidic components, etc., among which alkaloids are the most important pharmacodynamic components, accounting for 70%-80% of the total alkaloids of coptidis rhizoma. It is suitable for dampness and heat internal steaming, ruffian bloating, irritability of heart fire, stomach heat and vomiting, dampness and heat diarrhea, sores, swelling and pain, and other diseases. In recent years, the effects of coptidis rhizoma have been discovered continuously, especially its antibacterial and anti-inflammatory effects, making it known as a "traditional Chinese medicine antibiotic", which has attracted special attention of people (Wu J, et al., 2019). The role of coptidis rhizoma in nervous system diseases has been paid more attention to. Studies have shown that coptisine can protect motor neurons and alleviate cerebral ischemia-reperfusion injury, thus having a potential therapeutic effect on neurodegenerative diseases (Hsu YY, et al., 2012; Jiang W, et al., 2015; Chen W, et al., 2016). Studies have confirmed that berberine can resist oxidative stress and protect mitochondria. Zhang et al. proved that berberine can protect stem cells and reduce the apoptosis rate induced by hypoxia through *in vitro* experiments (Zhang W, et al., 2009). Gomes et al. found that berberine can mediate the sirT1-dependent mitochondrial pathway to reduce insulin resistance, but its molecular mechanism is still not well studied (Gomes AP, et al., 2012).

Network pharmacology is based on systems biology, integrating the database retrieval, target function analysis, target pathway enrichment analysis, and other research methods, revealing the "drug-composition-targets-disease" complex relationship network, from the angle of the whole Chinese medicine ingredient of the interaction between the target relationship, from the comprehensive understanding of the molecular basis of disease (Wu HN, et al., 2019). It is a new method developed based on the idea that drugs play a role through "multi-target and multi-pathway" (Boezio B, et al., 2017). It can integrate drug, protein, and gene databases, and use bioinformatics technology to analyze and construct drug-target-disease network models to explore the mechanism of medicine. Therefore, its characteristics of integrity and systematization are very suitable for the study of Chinese medicine (Yuan H, et al., 2017).

In this study, network pharmacology combined with molecular docking technology was used to explore the mechanism of coptidis rhizoma in the treatment of SCI, and to provide a methodological basis and theoretical basis for future clinical application and scientific research.

Materials and methods

Prediction of compound composition and target of coptidis rhizoma

All compounds related to coptidis rhizoma were retrieved from the TCMSP database (https://tcmsp-e.com/). Based on various literature reports, oral bioavailability (OB) ≥ 30% and drug-like degree (DL) ≥ 0.18 were stipulated as the screening conditions for compound components according to pharmacokinetic (ADME) parameters. The active components and related targets of coptidis rhizoma were screened.

Screening of SCI related gene targets

Screening of SCI gene in GeneCards database (https://www.genecards.org/) for treatment of SCI targets.

Collection of SCI related targets of coptidis rhizoma

Venn figure (https://bioinfogp.cnb.csic.es/tools/venny/index.html) is used to change the coptidis rhizoma target genes associated with SCI intersection, the overlapping gene is likely to be coptis function target gene for the

treatment of SCI.

Construction of protein-protein interaction (PPI) networks

String database (https://string-db.org/) is a database with a large number of protein interaction relationships, including experimental validation and bioinformatics prediction data, involving a total of 9 643 763 proteins and 1 380 838 440 interactions. The gene targets of coptidis rhizoma for the treatment SCI were imported into the String database, and the human species were restricted to obtain the protein interaction relationship. The results were exported in TSV format and imported into Cytoscape3.7.2 statistical software to draw the interaction network. Firstly, the protein interaction network is analyzed as a whole, and then the most important five protein interaction relationships are obtained by using Tool→Network Analysis→Analyze Network in Cytoscape3.7.2 statistical software. In the Network, nodes are used to represent components and targets. Edge is used to represent the interaction between component and target.

GO functional analysis and KEGG pathway enrichment analysis

Metascape database (http://metascape.org/gp/index.) can provide a system for large-scale gene or protein synthesis of biological function annotation information, to find out the most significant enrichment of biological annotation. The gene targets of coptidis rhizome acting on SCI were imported into the Metascape database. Select Enrichment Analysis from Custom Analysis, then get the data import microscopic (http://www.bioinformatics.com.cn/), respectively, on the GO Biological Processes (BP), Molecular function (MF), Cell components (CC) were used for Enrichment Analysis, and the results were preserved to draw a bar chart, while KEGG Pathway was used to draw a bubble chart.

Molecular docking verification

The 2D structure of compounds in Buyanghuanwu Decoction (BYHWT) was obtained in PubChem. The 3D structure was obtained by importing Chem3D software, Optimized 3D structure files of key target proteins were screened from the PDB database in PDB format. Water molecules and ligands were removed by PYMOL software, and protein structures were isolated. AutoDock software and Grid module were used to set the protein ligand as the center of the docking box, and the box size of the lattice point was set as 40 × 40 × 40, and the area where small ligand molecules may act was set. AutoDock Vina molecular simulation software was used for molecular docking analysis of drug small molecules and target protein receptors.

Results

The compounds and genetic target prediction of coptidis rhizoma

A total of 48 components were retrieved from coptidis rhizoma and the remaining 14 components were screened according to OB 30% and DL 0.18 including Berberine, Obacunone, berberrubine, epiberberine, etc., and have been shown in Table. Then the corresponding related targets of the components were derived, and 148 related targets corresponding to the components were obtained after screening.

Target prediction of SCI

SCI was used as keywords to retrieve related genes in GeneCards, and 6404 related genes were collected and ranked by closeness.

Table. The compounds of coptidis rhizoma

Number	Mol	Compound	OB	DL
1	MOL001454	Berberine	36.86124504	0.77665
2	MOL013352	Obacunone	43.28625365	0.76724
3	MOL002894	Berberrubine	35.73551127	0.7269
4	MOL002897	Epiberberine	43.09233228	0.7761
5	MOL002903	(R)-Canadine	55.36687348	0.77465
6	MOL002904	Berlambine	36.68090144	0.81596
7	MOL002907	Corchoroside A_qt	104.9542429	0.77599
8	MOL000622	Magnograndiolide	63.70888436	0.18833
9	MOL000762	Palmidin A	35.35818795	0.65003
10	MOL000785	Palmatine	64.60111294	0.64524
11	MOL000098	Quercetin	46.43334812	0.27525
12	MOL001458	Coptisine	30.671852	0.85647
13	MOL002668	Worenine	45.833181	0.86552
14	MOL008647	Moupinamide	86.71215907	0.26454

Collection of related targets of coptidis rhizoma

The Venn figure was used to map and compare the action targets of coptidis rhizoma with the target genes of SCI-related diseases, and 133 common genes were found, which were speculated to be the related gene targets of coptidis rhizoma in the treatment of SCI, as shown in **Figure 1.**

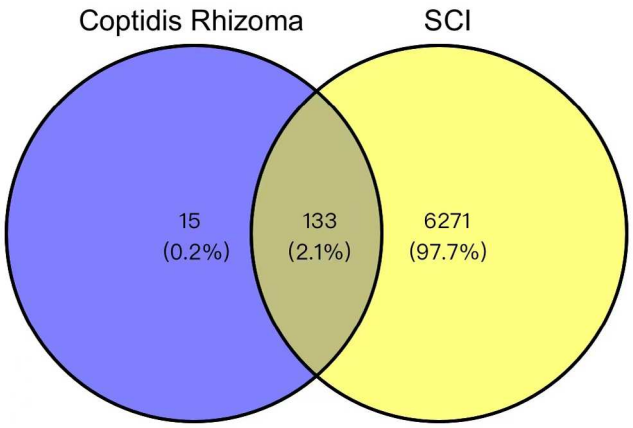

Figure 1. Related targets of coptidis rhizoma and SCI

Construction and analysis of PPI network between coptidis rhizoma and SCI

PPI network obtained by String Database was imported into Cytoscape3.7.2 and ranked by degree value **(Figure 2)**, nodes represent proteins and edges represent associations between proteins. A total of 133 nodes and 1745 edges are involved (CHRM4, EIF6, and CHRM4 were not reflected in the interaction network because they did not interact with other proteins). The average node degree was 26.2, and the average local clustering coefficient was 0.59. The 5 core-protein interactions were identified using Tool in Cytoscape3.7.2 **(Figure 3)**. The

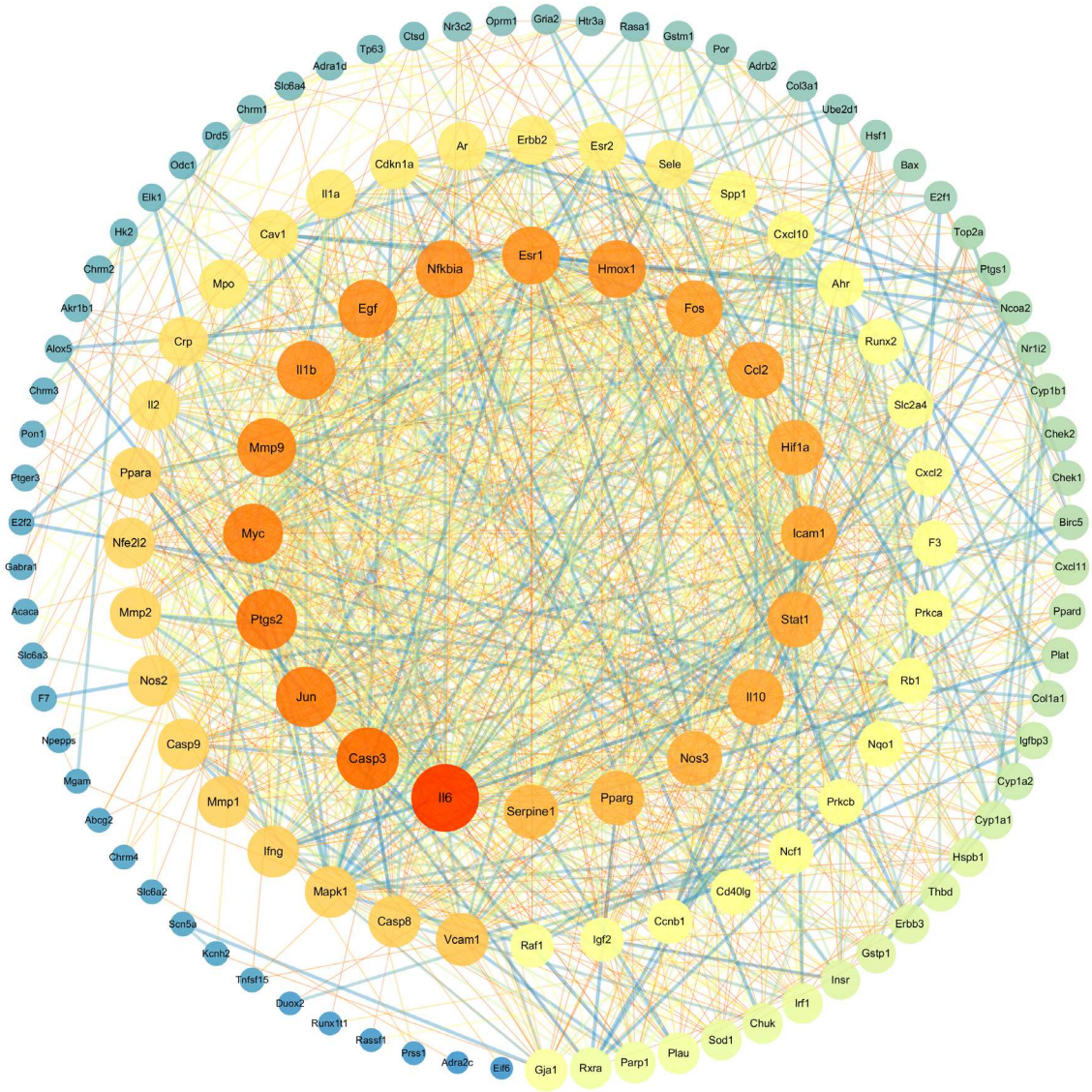

Figure 2. Protein interaction network between coptidis rhizoma and SCI

results showed that IL-6 and Casp3, Jun, Ptgs2 and Myc may be important targets in this network.

Construction of compound-target interaction network

The compound target interaction network of coptidis Rhizoma treatment SCI **(Figure 4)** constructed with Cytoscape3.7.2 has found the treatment of SCI by coptidis Rhizoma with multiple components and multiple targets.

GO analysis

Metascape database was used to perform GO analysis on related targets of coptidis Rhizoma and SCI, and the BP, MF, and CC-level data were obtained by bioinformatics (www.bioinformatics.com) and made into a visual bar chart of the top 20 items **(Figure 5)**. The results showed that the most important biological processes in BP analysis were adenylate cyclase-inhibiting G protein-coupled acetylcholine receptor signaling pathway, smooth muscle adaptation, positive regulation of apoptotic DNA fragmentation, positive regulation of DNA catabolic process, mycotoxin metabolic process, etc. Molecular functions such as estrogen 2-hydroxylase activity, G protein-coupled acetylcholine receptor activity, estrogen 16-alpha-hydroxylase activity, hydroperoxy eicosatetraenoate dehydratase activity, histone threonine kinase activity ranked the top in MF analysis. In CC analysis, neuronal cell body membrane, caveola, cell body membrane, dendrite membrane, an integral component of the presynaptic membrane were the most important components.

KEGG pathway analysis

Metascape database was used to perform KEGG pathway analysis on related targets of coptidis rhizoma and SCI. According to the P-value, the top 10 representative pathways were shown in **Figure 6**. The color of nodes in the figure from green to red reflects the *p*-value from large to small, and the node from small to large reflects the number of related genes from small to large, including Bladder cancer, African trypanosomiasis, AGE-RAGE signaling pathway in diabetic complications, non-small cell lung cancer, Malaria Prion, Prion diseases, Leishmania infection, IL-17 signaling pathway, Legionellosis, TNF signaling pathway, etc.

Results of molecular docking

IL-6, Casp3, Jun, Ptgs2, and Myc were selected according to the above compound-target interaction network by closest connection. Finally, Ptgs2 was determined to carry out molecular docking by searching literatures and screening unreported molecules. According to relevant research, binding energy less than -5.0 kcal/mol is the standard for the stable binding of ligand and receptor in molecular docking, and the lower the binding energy is, the higher the stability of the substructure. The docking results showed that the minimum binding energy of the active ingredient and the receptor protein was mostly less than -5.0 kcal/mol, indicating that the active ingredient and the receptor protein had good binding activity. The specific docking process was shown in **Figure 7**. The above docking results fully indicated that the active components of coptidis rhizoma had a good binding effect on SCI key core target genes.

Discussion

The treatment of SCI is always a difficult problem in clinics. Although a lot of efforts and attempts have been made to treat SCI with tissue engineering technology and stem cell technology, the progress has not been obvious. The regeneration of neurons in the adult central nervous system is not a one-step process. It is a process that needs to protect the survival of neurons, help them migrate to the original neuron target, re-myelinate, and form functional synapses. Therefore, it is possible for a large number of physical and chemical events to occur simultaneously to restore the function of damaged neurons. In recent years, there has been a trend towards combination therapy in this area to synergize and improve the efficacy of monotherapy. Coptidis rhizoma has a wide range of pharmacological effects and has significant therapeutic effects on a variety of diseases. Especially, pharmacological effects of coptidis rhizoma have been studied and acquired many achievements. Coptidis rhizoma not only has a broad spectrum of antibacterial and antiviral effects, but also has the effects of anti-arrhythmia, improving heart failure, protecting myocardial ischemia, improving acute cerebral ischemia, and hypoxia in the treatment of cardiovascular and cerebrovascular diseases, as well as hypoglycemia and anti-hypertension. Not only that, but coptidis rhizoma

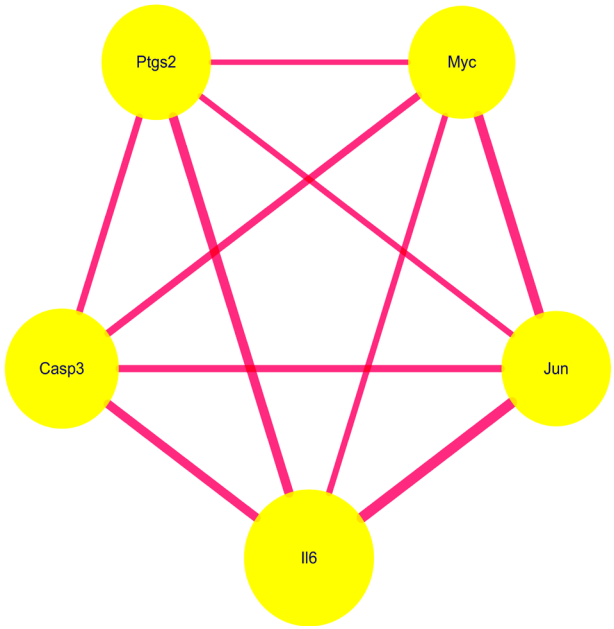

Figure 3. The 5 core-protein-interactions

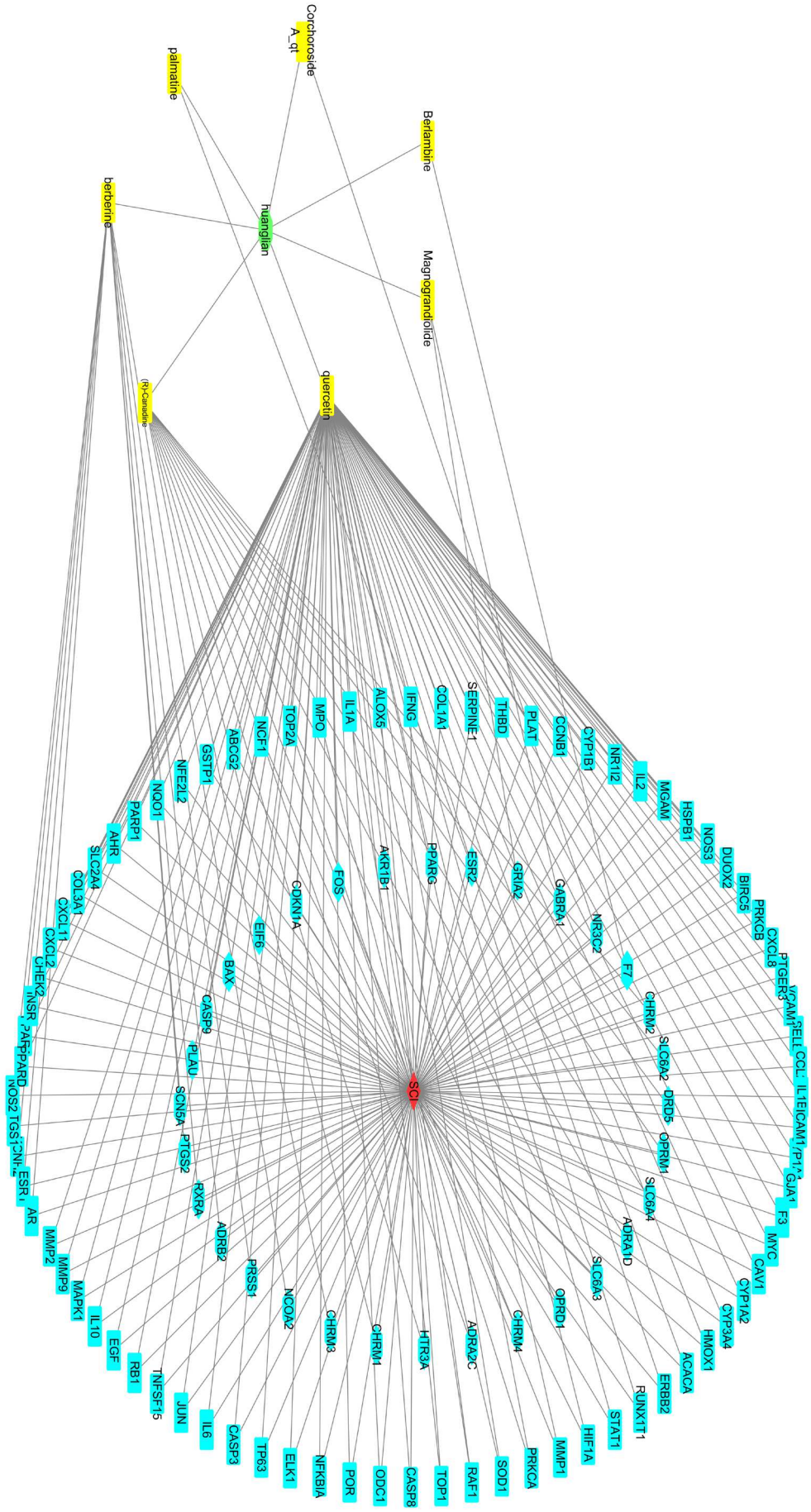

Figure 4. Compound-target interaction network

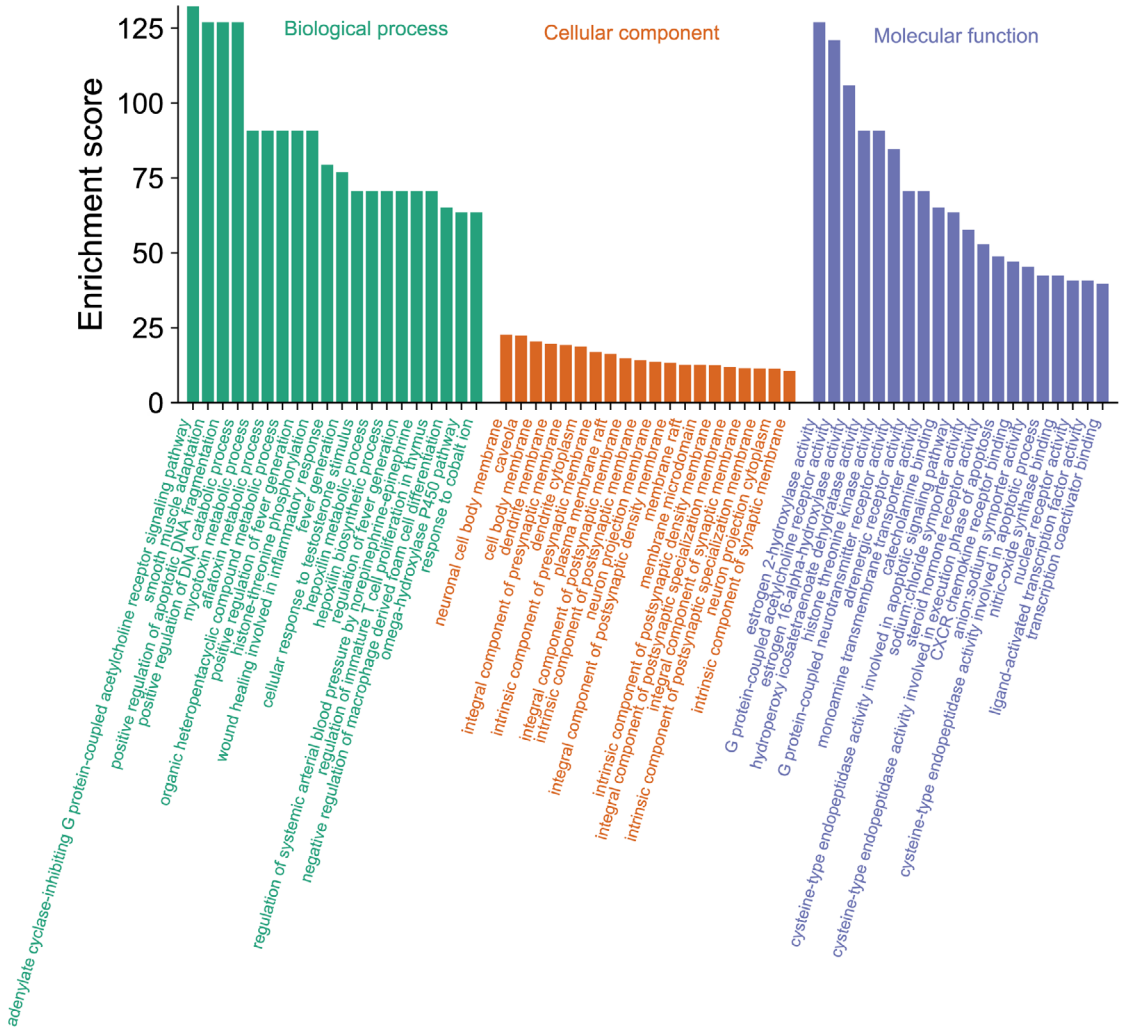

Figure 5. GO analysis of targets of coptidis rhizoma and SCI

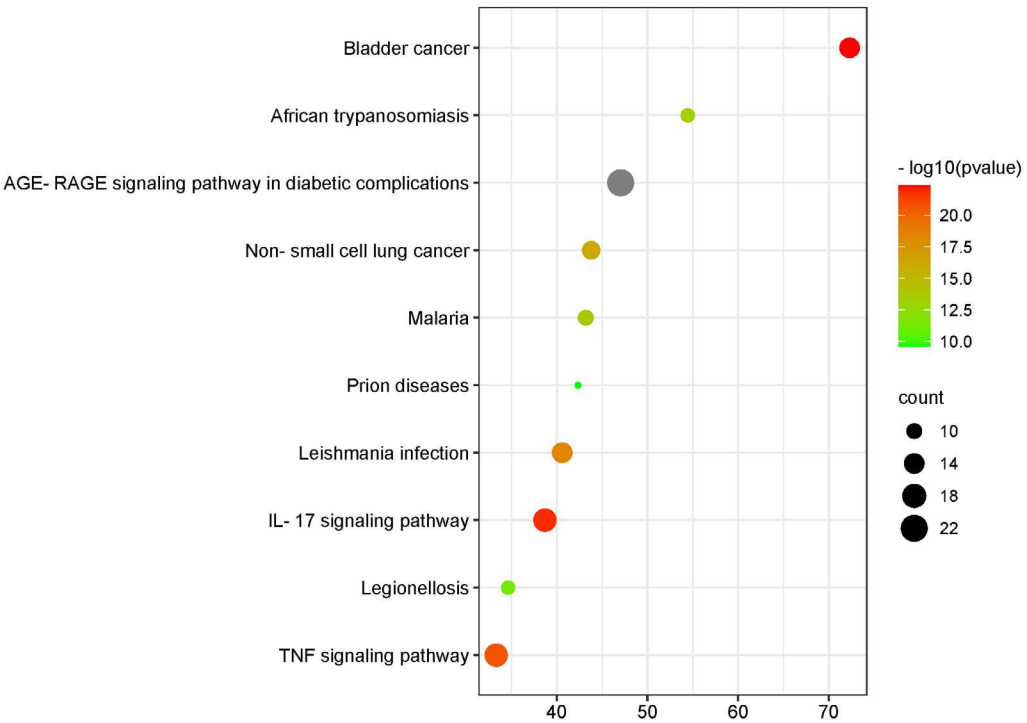

Figure 6. KEGG pathway analysis of coptidis rhizoma and SCI

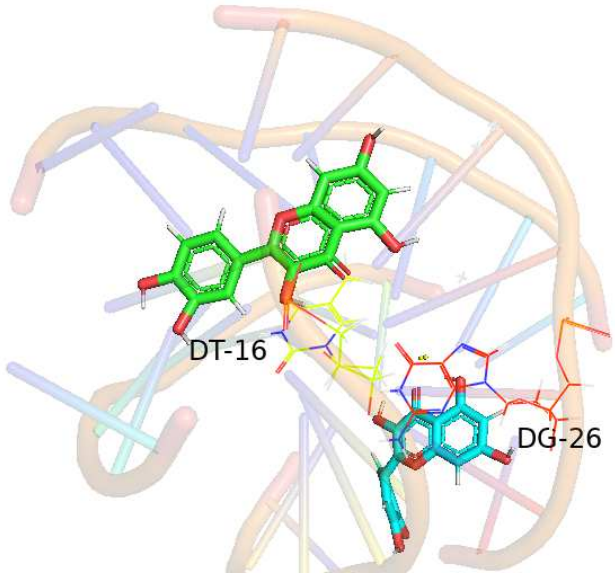

Figure 7. Molecular docking. *Molecular docking between target protein and active ingredient.*

also has the advantages of extensive drug sources and low price, and has a wide range of promotion value and application prospects.

The active components of coptidis rhizoma that may act on SCI include berberine, obacunone, epberberine, etc. SCI is a serious neurological disease that causes biochemical reactions based on the primary injury, leading to spinal cord ischemia, excitatory toxicity, ion imbalance, and free radical-mediated peroxidation (Hutson TH, et al., 2019). It is the process of tissue degeneration necrosis, mainly by the expansion of the idiopathic edema, ischemia, hypoxia injury, inflammation, free radicals and peroxide production, mitochondrial damage and energy metabolism, and microcirculation disorder. It destroys the original lesions around the complete organization, arises a series of cellular and molecular mechanisms, triggers the level of cell necrosis and apoptosis effect. The above chemical components have anti-inflammatory, antioxidant, immune regulation, coagulation function, and other functions conducive to the survival, development, and normal function maintenance of nerve cells, protect abnormal discharge of brain cells. After the treatment of coptidis rhizoma in rats with SCI the experimental results showed that berberine could up-regulate the autophagy activity of rat SCI tissue, reduce the death rate of cells at the injury site, thus promoting the recovery of nerve function, and the experimental results showed that it could reduce the apoptosis of cells by reducing the expression level of Caspase-3 (Zhao W, et al., 2019).

Network pharmacology, which can predict the targets and pathways of drug therapy at the molecular level, has been recognized and studied more and more in recent years. In this study, the possible mechanism of coptidis rhizoma in the treatment of SCI was investigated by network pharmacology. 148 relevant action targets of coptidis rhizoma were predicted based on network pharmacology concepts and techniques and literature retrieval against the background of multiple databases. 6404 genes related to SCI were retrieved from the GeneCards database. The mapping between coptidis rhizoma action target and SCI-related pathogenesis target revealed that 133 genes might be the related gene target of coptidis rhizoma treatment for SCI. BP enrichment analysis showed that the biological processes involved in the treatment of SCI of coptidis rhizoma mainly included adenylate cyclase-inhibiting G protein-coupled acetylcholine receptor signaling pathway, smooth muscle adaptation, positive regulation of apoptotic DNA fragmentation, positive regulation of DNA catabolic process, mycotoxin metabolic process, etc. The molecular functions involved mainly include estrogen 2-hydroxylase activity, G protein-coupled acetylcholine receptor activity, estrogen 16-alpha-hydroxylase activity, hydroperoxy eicosatetraenoate dehydratase activity, histone threonine kinase activity PPI network showed that there were a large number of interactions between each target.

IL-6, Casp3, Jun, Ptgs2, and Myc may be the five most important target proteins according to the degree value in this network. IL-6 is produced by T lymphocytes, B lymphocytes, monocytes, macrophages, and other cells. It is a cytokine involved in the acute response, playing an important role in the regulation of infection and injury stimulation. Body stress response, tissue damage, or inflammation can elevate the level of IL-6. Studies have shown that serum IL-6 increases after burning, amputation, and closed injury in mice (Barros AGC, et al., 2019). Apoptosis is a type of programmed cell death. Caspase-3 is the most important terminal splicing enzyme in cell apoptosis and plays an irreplaceable role in cell apoptosis. Studies have shown that caspase-3 plays an important role in SCI (Dasari VR, et al., 2007). Adjan et al. found that caspase-3 was positively expressed in neurons and glial cells within 6-7 mm from the end of the injury center at 4 hours after SCI (Adjan VV, et al., 2007). McEwen found that caspase-3 protein was significantly expressed in the damaged tissues 4 hours after SCI and lasted for about 8 days (McEwen ML, et al., 2005). In addition, Grossman studied the apoptosis regulating protein caspase-3 in neurons within 15 minutes to 24 hours after SCI and also found that caspase-3 protein was expressed in neurons at 4 hours after injury (Grossman SD, et al., 2001). Jun is an intranuclear proto-oncogene that is believed to play an important role in nerve regeneration. Danzi et al. examined the effects of dimers containing Jun on the axon growth of dorsal root ganglion, cortex, and hippocampus neurons. The results showed that dimers composed of Jun and transcriptional activator 3(Atf3) promoted the axon growth of rat central nervous system neurons and mouse dorsal root ganglion neurons (Danzi MC, et al., 2018). Myc is a gene related to cell cycle regulation. DiGiovanni S's research showed

that Myc was significantly overexpressed at 4 and 24 hours after SCI, leading to inflammatory response, oxidative stress, DNA damage, and other related reactions, ultimately leading to neuronal apoptosis (Di Giovanni S, et al., 2003).

Conclusion

This study shows that the same components of a drug could control multiple targets, the same target could participate in different signaling pathways. This shows that we are in the network as a single or multiple important control targets that can be adjusted to the whole network. It provides a scientific basis for the clinical application of coptidis rhizoma in the treatment of SCI and a direction for exploring the potential mechanism of coptidis rhizoma. In this study, the network pharmacology method was used to explore the mechanism of coptidis rhizoma in the treatment of SCI, and it was found that coptidis rhizoma may play a therapeutic role in SCI from a variety of signaling pathways and biological processes, which also provided an idea for the mechanism study of SCI. Some databases are mined in this study, these hypotheses need to be further verified in basic and clinical research. In addition, the clinical treatment of SCI is mostly composed of traditional Chinese medicine, but this paper only studied the coptidis rhizoma, which needs to be improved continuously.

Ethical statement

Not applicable.

Acknowledgements

Not applicable.

Conflict of interest

The authors declare that they have no conflicts of interest.

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None.

Transparency statement

All the authors affirm that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Authors' contribution

Hao Yuan, Zhong-Fu Zuo, Min Sai: Conceptualization, Methodology; Yi-Bo Wang, Qiu-Lin Wang: Data curation, Writing-Original draft preparation; Tin-Hua Wang, Hao Yuan, Zhong-Fu Zuo: Visualization, Investigation; Yi-Bo Wang, Qiu-Lin Wang: Writing-reviewing and editing

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