Observational Study

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Huangqi Guizhi Wuwu decoction in peripheral neurotoxicity treatment using network pharmacology and molecular docking

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Abstract

In this study, we predicted the core active compounds of Huangqi Guizhi Wuwu decoction in treatment of oxaliplatin-induced peripheral neuropathy and the related potential mechanism. Corresponding database was used to complete the interaction (PPI) network of key targets and the enrichment analysis of corresponding genmes. Molecular docking of key targets and key compounds was carried out using relevant software. The 60 chemical components corresponding to the oral absorption of Huangqi Guizhi Wuwu decoction correspond to 157 unique targets, and the 233 chemical components corresponding to percutaneous absorption in vitro correspond to 155 unique targets. There were 1074 unique targets for chemotherapy-induced peripheral neuropathy. Finally, three common key targets (SLC6A2, SLC6A3, and SLC6A4) and two key compounds (6-Gingerol and nuciferin) were screened according to the above three target datasets. The results showed that The PPI network of common key targets involved 23 associated proteins. In the related GO enrichment results, there were 33 items related to biological processes, 13 items related to cell composition, 21 items related to molecular function, and four KEGG pathway enrichments. L1000 kinase and GPCR perturbation analysis showed that the associated protein had an effect on the expression of multiple groups of kinase genes. HPA revealed that the enrichment of three common key targets was tissue-specific. The docking results showed that the 6 groups were structurally stable. The oral and topical use of Huangqi Guizhi Wuwu decoction can prevent and control peripheral neurotoxicity. The prevention and control effects may be related to its participation in the regulation of neurotransmitter transport, sympathetic activity, and transport. The histological parts of the mechanism are mainly distributed in the adrenal gland, placenta, brain, intestine, and lung, the blood is not specific. According to the prediction results of molecular docking, 6-Gingerol and nuciferin can closely bind to three common key targets.

Abbreviations: AlogP = fat water partition coefficient, Caco-2 = intestinal epithelial permeability, CIPN = chemotherapy-induced peripheral neuropathy, DL = drug similarity, GO = gene biological process, Homology modeling = starting from the amino acid sequence of the protein and taking the three-dimensional structure of the homologous protein analyzed by experiment as the template, the three-dimensional structure of the target protein was modeled. This is a highly reliable protein structure modeling method, KEGG = Kyoto Encyclopedia of Genes and Genomes, Molecular docking = molecular docking is a method of drug design based on the characteristics of receptors and the interactions between receptors and drug molecules. In recent years, molecular docking has become an important technology for computer-aided drug research, Network pharmacology = explains the occurrence and development process of diseases from the perspective of system biology and biological network balance, understands the interaction between drugs and the body from the perspective of improving or restoring the balance of biological network, and guides the discovery of new drugs In 2007, Andrew L. Hopkins, a pharmacologist at Dundee University in the UK, first put forward this concept. After that, “network Pharmacology” was rapidly applied to research in many fields, showing important theoretical and practical application value, OB = oral bioavailability, PPI = protein-protein interaction network, Prescription = a prescription is the embodiment of a therapeutic method. It is a prescription composed of several drugs, according to the principle of compatibility, summarizing clinical experience, Shanghan Lun = treatise on typhoid and miscellaneous diseases has been a special book on medical theory and practice since the Qin and Han Dynasties. It is one of the most influential classical medical works in the history of Chinese medicine and the first great work on clinical therapeutics in China, SLC6A2 = sodium-dependent noradrenaline transporter, SLC6A3 = sodium-dependent dopamine transporter, SLC6A4 = sodium-dependent serotonin transporter, Xue Bi = numbness and pain of the skin or fingertip and a series of clinical symptoms aggravated by cold symptoms, Zhang Zhongjing = at the end of the Eastern Han Dynasty, he was a famous medical scientist and was honored as “medical saint” by later generations.

Keywords: network pharmacology, molecular docking, alternative medicine, oxaliplatin-induced peripheral neurotoxicity, Huangqi Guizhi Wuwu decoction

The authors have no funding and conflicts of interest to disclose.

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

The treatise does not involve human experiments. Data from multiple public databases will not expose private information. This systematic review also does not involve endangering participant rights. Ethical approval will not be required.

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1. Introduction

Peripheral neuropathy is one of the most serious adverse reactions to platinum drugs. This clinical symptom not only affects the quality of life of patients, but also limits the dosage of chemotherapy drugs and even leads to the cancelation of chemotherapy regimens containing platinum drugs in clinical treatment.[1] Currently, there is no effective method to treat peripheral neurotoxicity (CIPN) induced by chemical drugs. Clinically, CIPN occurrence and symptom progression are often prevented by reducing the drug dose.[2–4] This clinical problem has been one of the key points of clinical research on adverse reactions to chemotherapeutic drugs in recent decades.

Huangqi Guizhi Wuwu decoction is a prescription used to treat Xue Bi (numbness and pain of skin or finger tip, aggravation of cold symptoms) in the medical book named Shanghan Lun, which compiled by Zhang Zhongjing in the Han Dynasty. It is composed of five herbs, namely Huangqi (Hedysarum Multijugum Maxim), Guizhi (Cinnamomi Ramulus), Shaoyao (Paeoniae Radix Alba), Shengjiang (Zingiber Officinale Roscoe), and Dazao (Jujubae Fructus).[5] In modern clinical practice, it has been widely used to treat peripheral neuropathy in China. The use method is not only confined to the oral administration of decoction, but is also often used topically after decoction.[6,7] Some researchers have not only used the Huangqi Guizhi Wuwu decoction to treat peripheral neuropathy and achieved clinical efficacy, but also conducted meta-analyses[8] and clinical control tests,[9] and conducted animal experiments on the corresponding efficacy observation of Huangqi Guizhi Wuwu decoction in the prevention and treatment of peripheral neuropathy,[10,11] however an in-depth and comprehensive mechanism still needs to be carried out.

In recent years, in drug mechanism research and discovery in the pharmaceutical industry, to improve the R&D (research and development) probability and save R&D resources, computer technology has been introduced into the R&D process, and a variety of calculation methods can be used for rational drug design. Using combinatorial computer technology, such as hierarchical virtual screening and molecular dynamics simulation, to complete molecular recognition of natural sources the new computational research methods for disease-resistant molecules.[12] Molecular docking combined and Molecular dynamics model is used to simulate the biological activity of molecules against diseases.[13] Molecular docking studies were conducted to test the interaction between the compound and the molecular active pocket of the anti-disease target.[14,15] Molecular dynamics research reveals the favorable binding energy mode in the whole simulation process of molecular clustering and evaluates the stability of the complex.[16,17]

As an emerging and interdisciplinary subject studying TCM at the system level, network pharmacology can quickly and systematically predict and analyze drug action mechanisms.[18] Molecular docking has important applications in virtual high-throughput screening and drug discovery. Protein ligand recognition through molecular docking analysis has become an effective tool for drug design.[19,20] Therefore, this study would analyze Huangqi Guizhi Wuwu Decoction according to the requirements of network pharmacological evaluation method guide, so as to provide new basis and ideas for systematically revealing the multi-target and multi-channel prevention and treatment of oxaliplatin induced peripheral neuropathy. Whole study design (Fig. 1).

2. Materials and Methods

2.1. Screening of active components and target prediction of Huangqi Guizhi WuWu decoction

Relying on a laboratory platform for computational systems biology (LSP) (tcmspw. Com), the corresponding chemical components of five Chinese herbal medicines, were retrieved in the LSP tcmsp module to establish a chemical composition data set, According to the meaning of pharmacokinetic information: oral bioavailability (OB) value, patent drug similarity (DL) value, lipid water partition coefficient (Alogp) value and drug intestinal absorption (Caco-2) value, combined with experience and relevant theories,[21] the compounds contained in traditional Chinese medicine were preliminarily screened for the first time. When screening the effective components of Huangqi Guizhi Wuwu Decoction for the prevention and treatment of peripheral neurotoxicity caused by oxaliplatin, OB values ≥ 30% and DL value ≥ 0.15 were set, and the corresponding data set was obtained. When screening the effective components of Huangqi Guizhi Wuwu decoction for topical use in the prevention and treatment of peripheral neurotoxicity caused by oxaliplatin, we set the value of Alogp ≥ 3 and Caco-2 ≥ 0 to obtain another data set. By combining LSP and DrugBank (https://go.drugbank.com/), the protein targets corresponding to each chemical component were searched in the database, and the target protein datasets of chemical components contained in Huangqi Guizhi Wuwu Decoction for oral and topical use were constructed.

2.2. Screening of disease corresponding targets

With "Oxaliplatin-induced peripheral neurotoxicity," "Peripheral neurotoxicity due to oxaliplatin," "Peripheral neurotoxicity induced by oxaliplatin" and "chemotherapy-induced peripheral neuropathy" as key words, access to OMIM (https://omim.org/), TTD (http://bidd.nus.edu.sg/group/cjttd/), PharmGkb (https://www.pharmgkb.org/), DiGSeE (http://210.107.182.61/geneSearch/), DisGeNET (http://www.disgenet.org/web/DisGeNET/menu), PolySearch (http://polysearch.cs.ualberta.ca/), and KEGG (http://www.kegg.jp/kegg/) databases, widely collect human gene targets of oxaliplatin induced peripheral neuropathy, and sort out the corresponding target data set of the disease.

2.3. Screening of key targets and key compounds of Huangqi Guizhi WuWu decoction for oral and topical use in the prevention and treatment of oxaliplatin induced peripheral neuropathy

The corresponding targets of the chemical components contained in Huangqi Guizhi Wuwu Decoction for oral use, the corresponding targets of the chemical components contained in Huangqi Guizhi Wuwu Decoction for topical use and the corresponding targets of peripheral neuropathy caused by oxaliplatin were used on the Bioinformatics & Evolutionary Genomics Platform (http://bioinformatics.psb.ugent.be/webtools/Venn), take the intersection and extract the target corresponding to the intersection, As a common key target of drugs acting on diseases, it is ready for further correlation analysis of the above common key targets.

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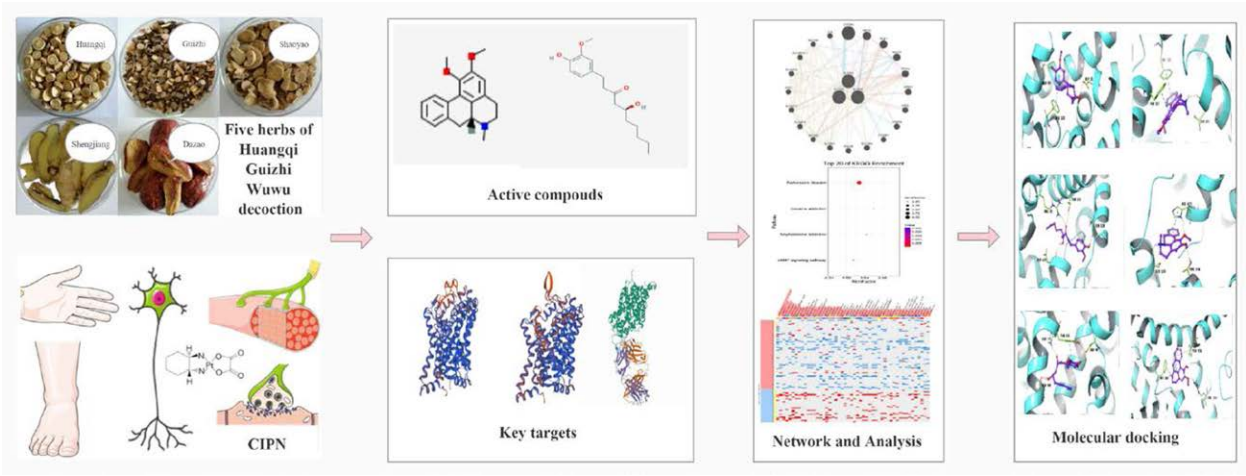

Figure 1 Shows our technology roadmap. CIPN = chemotherapy-induced peripheral neuropathy.

At the same time, the oral active components and external use components corresponded to the common key targets screened in the previous step. Similarly, bioinformatics and evolutionary genomics platforms were used to intersect the above two groups of chemical components to screen the main key compounds to prepare for molecular docking with the common key targets.

2.4. The distribution of common key targets in different human tissues was analyzed

We selected the common key targets selected in 2.3 through the Human Protein ATLS (https://www.proteinatlas.org/) database (HPA) to study the expression of common key targets in different human tissues and organs from the protein level, so as to pave the way for subsequent experimental research.

2.5. Construction of protein-protein interaction network

In order to illustrate the role of common key targets at the system level, the gene names of common key targets selected in 2.3 are passed through UniProt database (http://www.uniprot.org/) Correct and upload to genemania database (http://genemania.org/), the PPI network of protein-protein interaction information was obtained and analyzed.

2.6. Study on gene ontology (GO) function and KEGG signaling pathway

Using the Enrichr database (http://amp.pharm.mssm.edu/Enrichr/), The proteins contained in the PPI network are in the David database (https://david.ncifcrf.gov/tools.jsp), The correlation analysis of GO function enrichment and KEGG pathway enrichment was carried out to systematically predict the mechanism of internal and topical use of Huangqi Guizhi Wuwu Decoction in the prevention and treatment of peripheral neurotoxicity caused by oxaliplatin from the perspective of system biology.

2.7. LinCS l1000 characteristic matrix analysis

Find the kinase perturbations (L1000 kinase and GPCR perturbations) of the genes corresponding to the 23 proteins contained in the PPI network in the enrichment database and download the corresponding data packets on the clustergrammer platform (http://amp.pharm.mssm.edu/clustergrammer/).

2.8. Molecular docking of selected key compounds with common key targets

Gas chromatography and spectroscopy are commonly used to determine the crystal structure of separated chemical components.[22,23] In order to fully apply the database to save research costs and reflect the role of computer technology in drug research and development, the molecular structure of 6-Gingerol (PubChem CID: 442793) and nuciferin (PubChem CID: 10146) was selected from the public chemistry database (PubChem), converted into a three-dimensional structure with ChemBio3D Ultra12.0,[24] and optimized using the MMFF94 force field (Hagler, 1996). To evaluate the interaction mode of 6-Gingerol and Nuciferin with SLC6A2 (Sodium-dependent noradrenaline transporter), SLC6A3 (Sodium-dependent dopamine transporter), and SLC6A4 (Sodium-dependent serotonin transporter) proteins, AutoDock Vina 1.1.2[25] was used.

The three-dimensional structures of SLC6A4 (PDB ID: 5i74) proteins were obtained from the public protein database (https://www.rcsb.org/). The PDB did not contain the crystal structures of SLC6A2 and SLC6A3. For this study, we first studied SLC6A2 and SLC6A3 in the Swiss model (http://swissmodel.expasy.org/). Homology modeling was performed on,[26] and the three-dimensional structures of SLC6A2 and SLC6A3 proteins were obtained, as shown in Figure 2A. A Ramachandran plot[27] was used to evaluate the quality of the three-dimensional protein structure.[28] The results showed that SLC6A2 and SLC6A3 were most similar to the sequence of the protein dopamine transporter (PDB ID: 4xp4), with similarities of 59.70% and 56.18%, respectively. A relatively reasonable conformation can be obtained when the similarity of the model is greater than 30%.[29] The analysis of the pull conformations of SLC6A2 and SLC6A3 showed that 95.39% of the amino acid residues of SLC6A2 and 97.04% of the amino acid residues of SLC6A3 fell in the best region (green region), as shown in Figure 2B. It is generally considered that this value is greater than 90%, indicating that the protein model is reasonable and can be used for molecular docking in the next step.[30] Homology modeling (Fig. 2).

3. Results

3.1. Screening of compounds and prediction of corresponding targets

According to the set screening conditions, a total of 81 effective oral compounds (Table S1, Supplemental Digital Content, http://links.lww.com/MD/H708) of the Huangqi Guizhi Wuwu

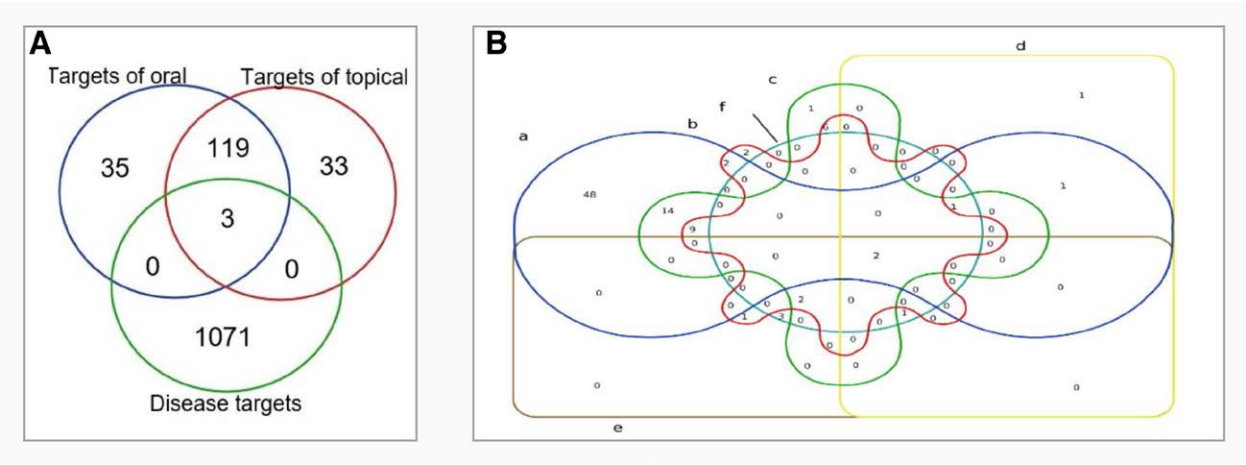

Figure 2 Homology modeling of SLC6A2 and SLC6A3. A. Three dimensional structure of SLC6A2 and SLC6A3 proteins, B. SLC6A2 and SLC6A3 pull conformations. SLC6A2 = sodium-dependent noradrenaline transporter, SLC6A3 = sodium-dependent dopamine transporter.

decoction were collected. After removal and repetition, a total of 60 compounds were identified, and a total of 1369 corresponding targets were identified, including 157 unique elements, 385 effective topical compounds (Table S2, Supplemental Digital Content, http://links.lww.com/MD/H709), 233 compounds in total after repeated removal, and 3718 corresponding targets, including 155 unique elements. The above data can form an effective oral compound corresponding to the target dataset (Table S3, Supplemental Digital Content, http://links.lww.com/MD/H710), and effective topical compounds correspond to the target dataset (Table S4, Supplemental Digital Content, http://links.lww.com/MD/H711).

3.2. Disease corresponding target

Based on the set keywords, a number of public databases were consulted, and 1156 human gene targets corresponding to the disease were collected. After removing duplicate target names, 1074 human gene targets of the unique item were obtained to form the disease-corresponding target dataset (Table S5, Supplemental Digital Content, http://links.lww.com/MD/H712).

3.3. Common key targets and major key compounds

Three target datasets (effective oral compound corresponding target data set, effective topical compound corresponding target data set, and disease corresponding target dataset) sorted in 3.1 and 3.2 are intersected on the Bioinformatics & Evolutionary Genomics Platform (Fig. 3A). Three common key targets were also identified. The human genes corrected by UniProt were SLC6A3, SLC6A4, and SLC6A2, and we identified the compounds corresponding to the three common key targets in the data set sorted in 3.1, including six oral effective compounds corresponding to SLC6A2, 77 topical effective compounds, 9 oral effective compounds corresponding to SLC6A3, 35 topical effective compounds, 9 oral effective compounds corresponding to SLC6A4, and 24 topical effective compounds, based on the above six groups of compound data (Table S6, Supplemental Digital Content, http://links.lww.com/MD/H713). The intersection of Bioinformatics & Evolutionary Genomics Platform was also used (Fig. 3B). The two main key compounds are 6-Gingerol (PubChem CID: 442793) and nucifferin (PubChem CID: 10146), and the two main compounds obtained are compounds that have preventive and therapeutic effects on peripheral neuropathy caused by chemotherapy when taking Huangqi Guizhi Wuwu decoction topical and orally. The three common key targets obtained are targets that have effects on peripheral neuropathy caused by chemotherapy when taking Huangqi Guizhi Wuwu Decoction topically or orally. Wayne diagram showing common key targets and major key compounds (Fig. 3).

3.4. Distribution analysis of common key targets

Using the HPA database, we obtained the expression of the main key targets SLC6A2, SLC6A3, and SLC6A4 in different human tissues and organs. When SLC6A2 was used as the research object, its tissue specificity was mainly manifested in the enrichment and enhancement of the adrenal gland and placenta, and the subcellular localization was mainly in the cytoplasm and mitochondria. The regional enrichment of the human brain was mainly in the pons and medulla, and no specificity was detected in blood immune cells. This gene product is not a prognostic factor for the disease, as it can terminate the action of norepinephrine by high-affinity sodium-dependent reuptake to the presynaptic terminal. The main biological processes involved were neurotransmitter transport, sympathetic activity, and transmission (Fig. 4A).

When SLC6A3 is used as the research object, its tissue specificity is mainly manifested in the increased tissue enrichment of the brain, which is mainly localized in the cytoplasmic vesicles; the human brain region is mainly enriched in the midbrain, and no specificity is detected in the blood immune cells. This gene product may be a marker for adverse prognosis of renal cell carcinoma. This protein can terminate the effect of dopamine through high-affinity sodium-dependent reuptake, and the main biological processes involved are neurotransmitter transport, sympathetic activity, and transmission (Fig. 4B).

When SLC6A4 is taken as the research object, its tissue specificity is mainly manifested in the enrichment and enhancement of intestinal and lung tissues; the subcellular localization is mainly in the Golgi apparatus and vesicles, the enrichment of human brain regions is mainly in the pons and medulla, and the enhancement of blood-specific immune cells and neutrophils. This gene product is not a prognostic factor for the disease, as the target protein can terminate the action of serotonin by recycling in a sodium-dependent manner. The main biological processes involved were neurotransmitter transport, sympathetic activity, and transmission (Fig. 4C).

In a word, SLC6A2 is enriched in adrenal gland and placenta, SLC6A3 is enriched in brain, SLC6A4 is enriched in intestine and lung, and the subcellular localization of SLC6A2, SLC6A3, and SLC6A4 is located on different subcellular organelles in cells, but the main biological processes involved in the three

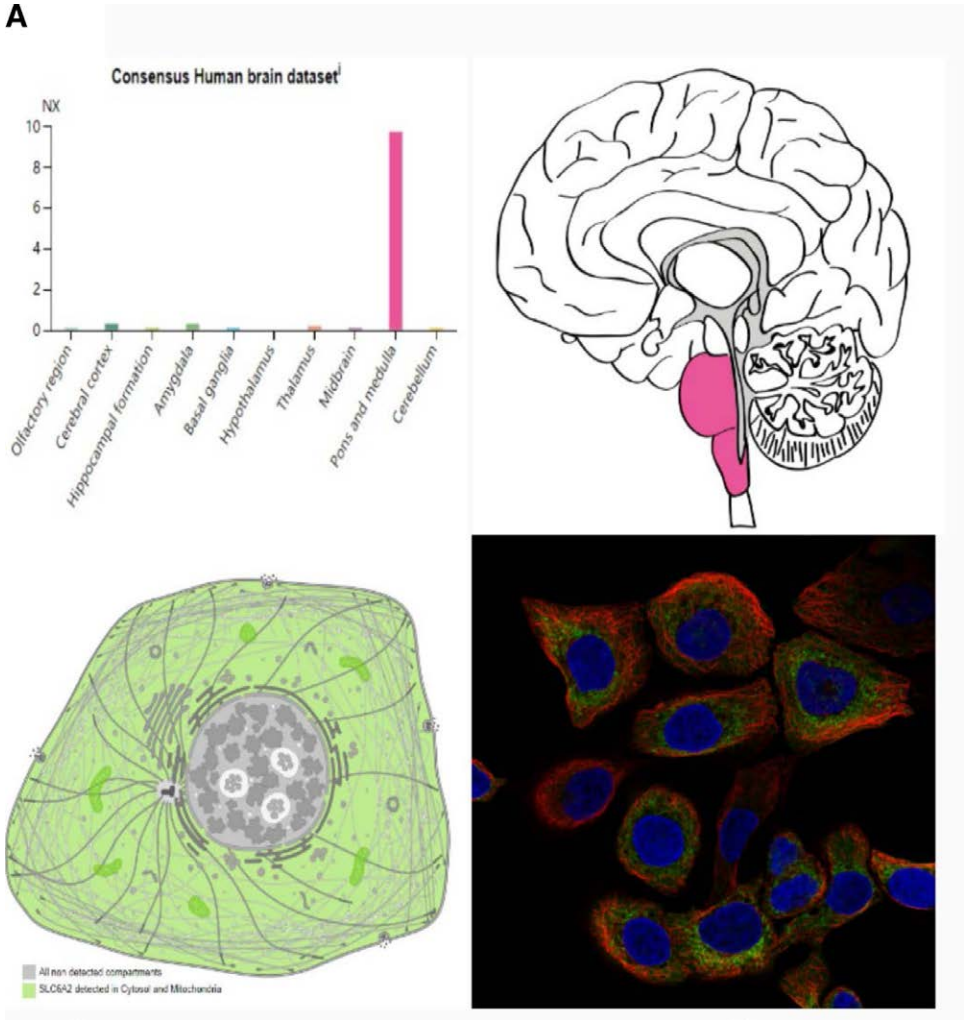

Figure 3 Wayne diagram. Effective oral compound corresponding target data set, effective topical compound corresponding target data set and disease corresponding target data set, and the intersection of the three data sets (A); There are 6 oral effective compounds corresponding to SLC6A2, 77 topical effective compounds, 9 oral effective compounds corresponding to SLC6A3, 35 topical effective compounds, 9 oral effective compounds corresponding to SLC6A4 and 24 topical effective compounds. The intersection of the above six groups of compound data sets (B); The number in the coil is the number of targets. SLC6A2 = sodium-dependent noradrenaline transporter, SLC6A3 = sodium-dependent dopamine transporter, SLC6A4 = sodium-dependent serotonin transporter.

are neurotransmitter transport, sympathetic and transmission. Distribution map of key targets (Fig. 4).

3.5. PPI network and GO function, KEGG signal path, LinCS I1000 characteristic matrix analysis

To systematically explain the role of common key targets, we uploaded SLC6A3, SLC6A4, and SLC6A2 to the Genemania database and found that there were 23 nodes in the PPI network (Table S7, Supplemental Digital Content, http://links.lww.com/MD/H714). The nodes represent interrelated proteins, and each edge represents the interaction between the protein and the egg white. Among them, 67.64% were physical interactions, 13.50% were co-expressed, 6.35% were predicted, 4.35% were pathways, and 6.17% were co-localized, shared protein domains accounted for 0.59%, and genetic interactions accounted for 1.40%. The analysis results showed that these proteins are interrelated in biology, and the 23 targets in the network will pave the way for further enrichment analysis (Fig. 5A).

23 targets associated with the PPI network were identified and analyzed. GO function analysis (Fig. 5B) includes biological processes (BP), mainly involving neurotransmitter transport, monoamine transport, response to drug, amino acid transmembrane transport, chemical synaptic transmission, dopamine upload involved in synaptic transmission, domino biosynthetic process, transmembrane transport, amino acid transport, response to toxic substances, gamma aminobutyric acid transport, positive regulation of neurotransmitter secrets, adenohypophysis development, etc. (Table S8, Supplemental Digital Content, http://links.lww.com/MD/H715). Cell composition (CC) mainly involves the integral components of the plasma membrane, plasma membrane, synaptic vesicle membrane, synaptic vesicle, and other aspects (Table S9, Supplemental Digital Content, http://links.lww.com/MD/H716). Items related to molecular function (MF) mainly include neurotransmitter: sodium symporter activity, amino acid transporter activity, monoamine transporter activity, dopamine: sodium symporter activity, gamma aminobutyric acid: sodium symporter activity, protein N-terminal binding, etc. (Table S10, Supplemental Digital Content, http://links.lww.com/MD/H717).

The enrichment results of the signal pathway (KEGG) (Table S11, Supplemental Digital Content, http://links.lww.com/MD/H718) were mainly distributed in Parkinson's disease, cocaine addition, amphetamine addition, and the cAMP signaling pathway (Fig. 5C). According to the multi-group enrichment results of the database, Huangqi Guizhi Wuwu decoction, whether orally or topically, plays a role in the prevention and treatment

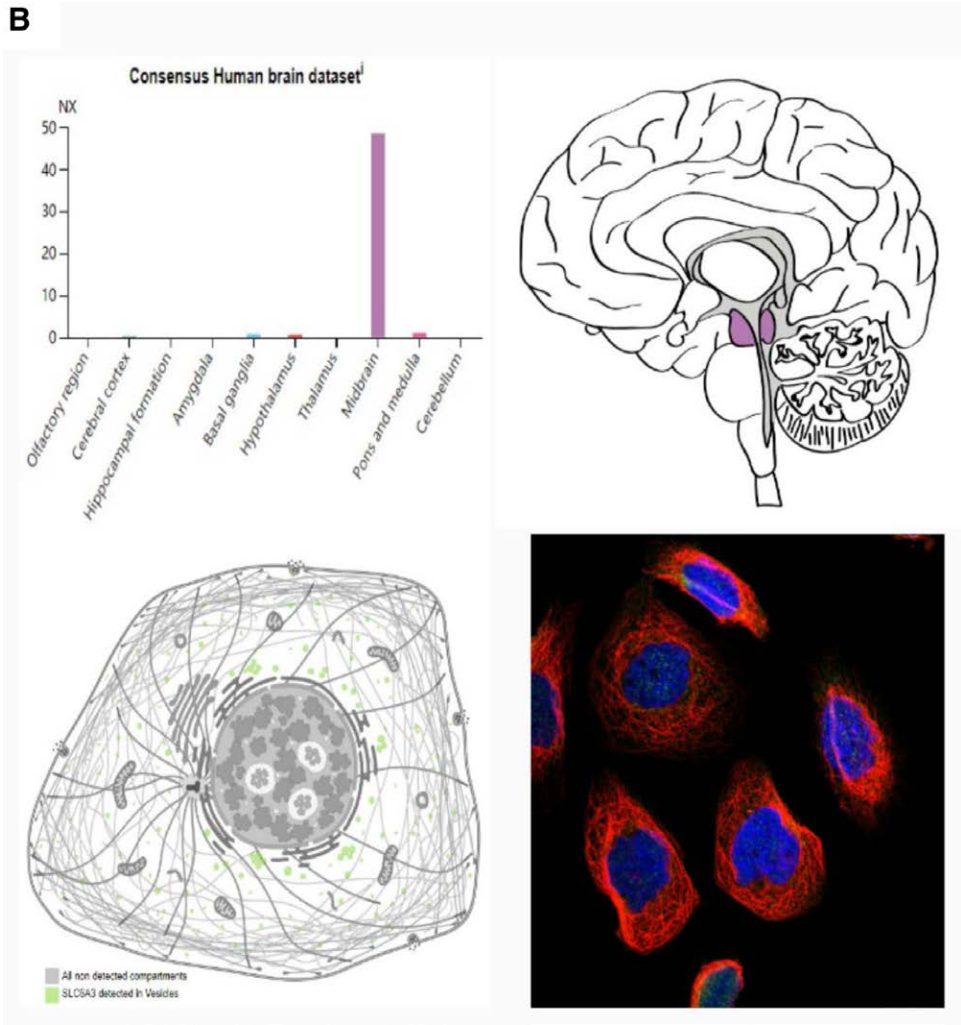

Figure 3 Continued

of peripheral neuropathy caused by chemotherapy through multiple mechanisms, such as affecting the secretion or transmission of neurotransmitters or amino acids and the function of neuron-related structures.

L1000 kinase and GPCR perturbation matrix analysis (Table S12, Supplemental Digital Content, http://links.lww.com/MD/H719) of genes corresponding to 23 proteins, including 350 associated genes involved in downregulation and 460 associated genes involved in upregulation, The characteristics of L1000 perturbation genes show that they have a certain impact on the expression of these kinase genes such as salermide, trichostatin a, HDAC6 inhibitor ISOX, and vorinostat (Fig. 5D).

3.6. Molecular docking and analysis

It was modeled with the dopamine transporter template and ChemBio3D Ultra 12.0, (ChemBridge Corp., San Diego, CA) to draw the structures of compounds 6-Gingerol and nuciferin, then transformed into three-dimensional structures with ChemBio3D Ultra 12.0, and optimized using the MMFF94 force field.[31] The three-dimensional structure of SLC6A4 (PDB ID: 5i74) was downloaded from the RCSB Protein Data Bank (https://www.rcsb.org/) as a docking protein. Proteins SLC6A2, SLC6A3, SLC6A4 and compounds 6-Gingerol and nuciferin were transformed into pdbqt format files using AutoDocktools 1.5.6.[32,33] Enter AutoDock Vina 1.1.2 for molecular docking. To increase the accuracy of the calculation, we set the parameter Exhaustivity to 20 and the other parameters adopted the system default values. Finally, we selected the conformation with the highest score and used the Free Maestro 11.9 (https://www.schrodinger.com/Maestro/) Analyze the results.

We docked compounds 6-Gingerol and nuciferin to the active pockets of proteins SLC6A2, SLC6A3, and SLC6A4 (Fig. 6). The affinity scores are presented in Table 1. The affinity is the molecular docking score. Generally, a negative value indicates successful docking. These interactions are the main forces between protein molecules and compounds.[34] The lower the negative affinity score, the closer the docking and the greater the impact of the compound on the protein molecular structure.[35] Therefore, it is speculated that all these interactions lead to the formation of stable complexes between SLC6A2, SLC6A3, SLC6A4 and compounds 6-Gingerol and nuciferin (Fig. 6).

4. Discussion

Peripheral neurotoxicity is one of the most common side effects of oxaliplatin, but there is still a lack of effective treatment. Calcium, magnesium, and reduced glutathione can reduce the symptoms of neurotoxicity to a certain extent; however, their reliability needs to be further verified in the clinic. Traditional Chinese medicine plays an increasingly important role in the prevention and treatment of oxaliplatin peripheral neurotoxicity.

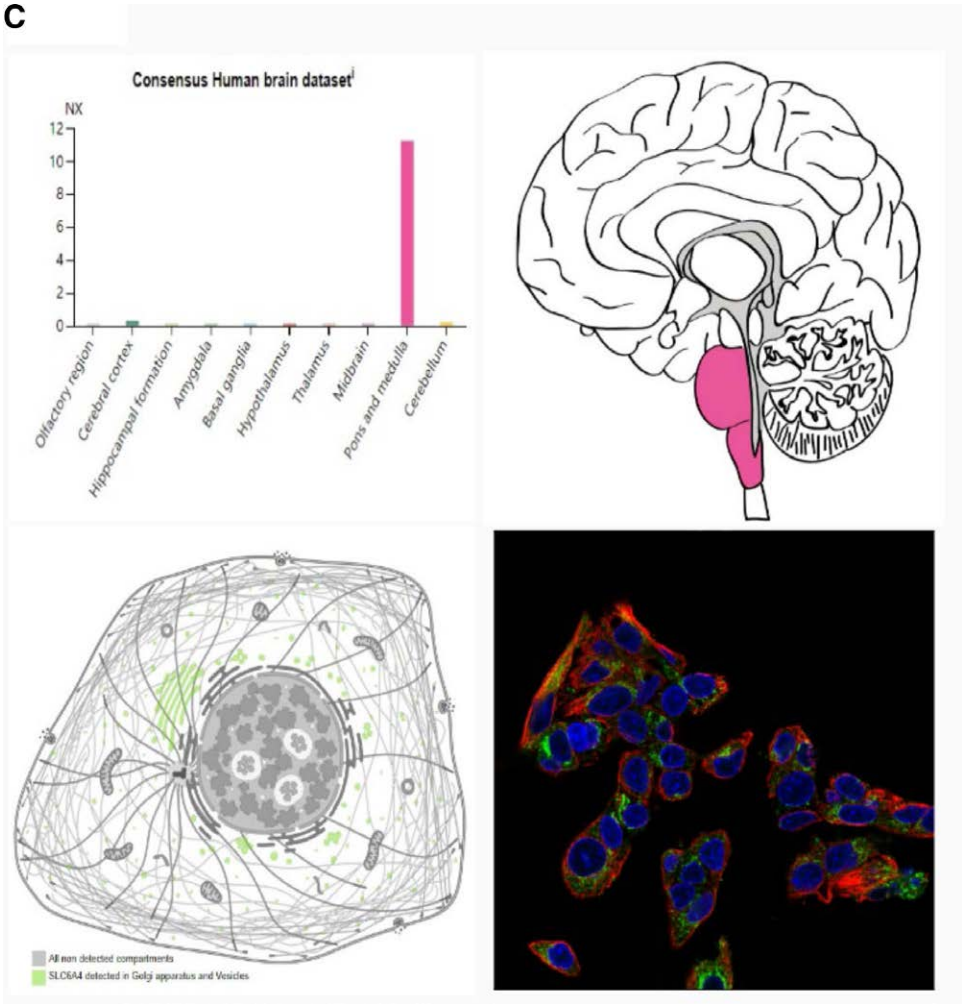

Figure 3 Continued

To further reduce the incidence of oxaliplatin-induced peripheral neurotoxicity, this study determined the effective components and possible detailed molecular mechanism of Huangqi Guizhi Wuwu decoction, an effective traditional Chinese medicine prescription for the treatment of oxaliplatin-induced peripheral neurotoxicity, in existing clinical trials. This study provides a basis for the wide application of the Huangqi Guizhi Wuwu decoction in the treatment of oxaliplatin-induced peripheral neurotoxicity and further mechanistic research.

First, according to the OB and DL values of each component in the five traditional Chinese medicine decoction pieces, the effective oral compounds and action target data sets were determined. According to the alogp and Caco-2 values, the effective topical compounds and action target dataset were determined. Through multiple public databases, the corresponding target data set of disease is established. This study identified two main effective components of Huangqi Guizhi Wuwu Decoction in the treatment of oxaliplatin induced peripheral neurotoxicity: 6-Gingerol and nuciferin, and three main targets: SLC6A2, SLC6A3, and SLC6A4. Previous studies mainly focused on the anti-inflammatory effect of Huangqi Guizhi Wuwu decoction and the role of rheumatoid arthritis, radicular cervical spondylosis, and diabetic peripheral neuropathy.[36–38]

6-Gingerol, a potential natural drug extracted from herbal medicine, can effectively regulate aging and degenerative diseases.[39] Gawel Kinga et al[40] showed in a study in 2021 that 6-Gingerol had strong dose-dependent anticonvulsant activity in zebrafish hyperkinetic convulsions induced by pentylenetetrazole (PTZ). As an inhibitor, 6-Gingerol may interact with the amino terminal domain, glutamate-binding site, and ion channel of the NR2B N-methyl-D-aspartate (NMDA) receptor to regulate the concentration of neurotransmitters and achieve anticonvulsant effects. Relevant experiments conducted by Liu et al[41] in 2020 showed that 6-Gingerol inhibited microglia mediated neuroinflammation by down regulating the akt-mtor-stat3 pathway, thereby improving cerebral ischemic injury. As an alkaloid extracted from pure natural herbal medicine, nuciferin reduces blood lipid levels and has anti-inflammatory and antioxidant properties.[42] Chen et al[43] In a cell experiment in 2020, it was found that lotus leaf alkali may promote the proliferation of vascular endothelial cells by upregulating the expression of the SDF-1/ CXCR4 signaling pathway, which is dose-dependent, and is most obvious in the 50 mg/L lotus leaf alkali group. Xiong et al[44] In their 2021 research report, lotus leaf alkaloids increased the relative abundance of Alloprevotella, Turicibacter, and Lactobacillus, improved intestinal flora, reduced obesity, and downregulated adipose tissue inflammatory factors IL-6, IL-1β, and TNF-α gene expression, thus improving intestinal permeability to alleviate chronic inflammation and play a role in the treatment of obesity. Therefore, 6-Gingerol and nuciferin are good drugs for anti-aging, anti-inflammatory reactions, and the control of chronic degenerative diseases. However, the application of 6-Gingerol and nuciferin in the study of oxaliplatin-induced peripheral neurotoxicity has not been reported, and the mechanism of 6-Gingerol and nuciferin in oxaliplatin-induced peripheral neurotoxicity is not clear.

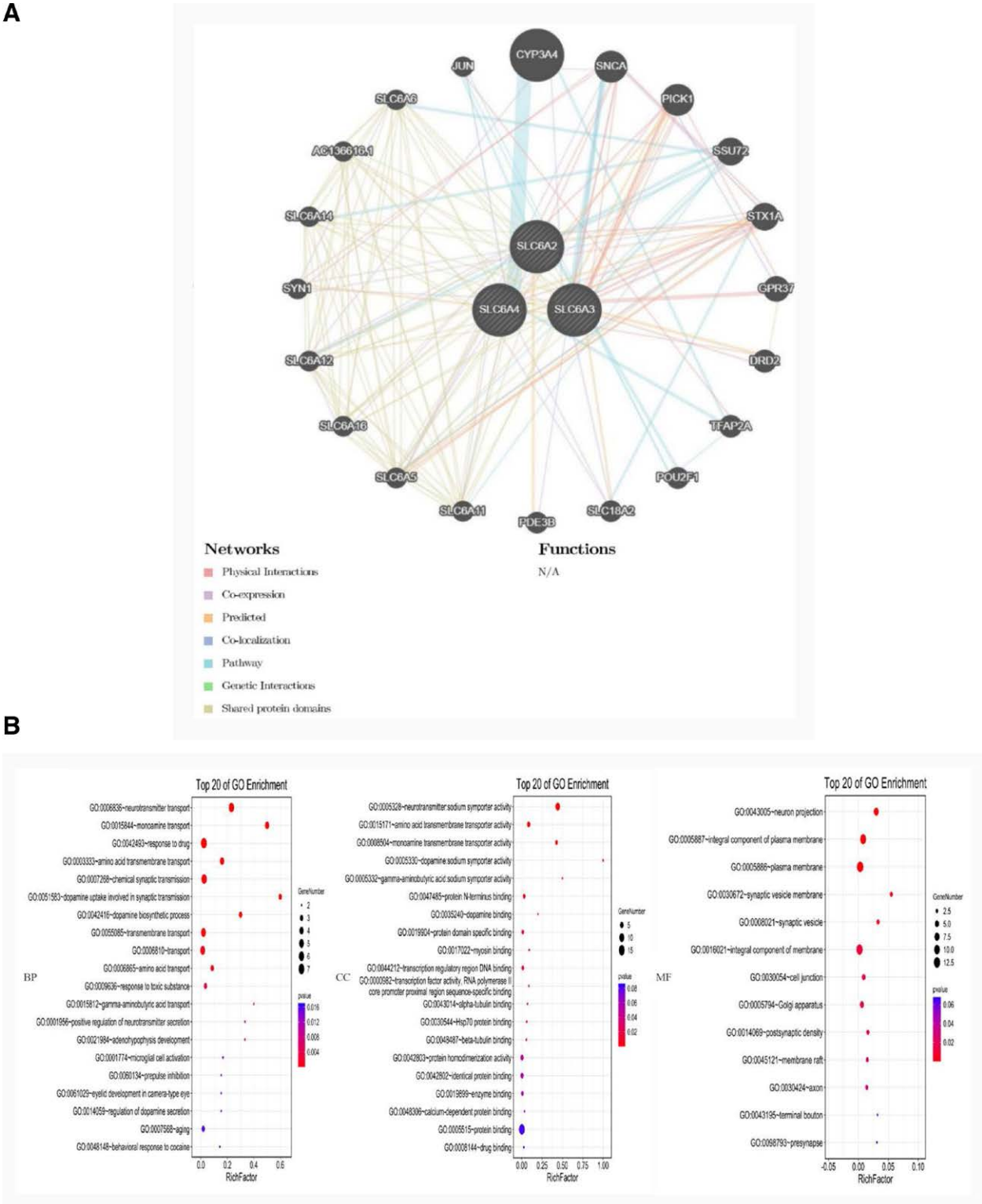

Figure 4 Distribution map of key targets. Expression of key targets SLC6A2, SLC6A3, and SLC6A4 found in HPA database in different human tissues and organs. (A) expression of SLC6A2 in different human tissues and organs. (B) expression of SLC6A3 in different human tissues and organs. (C) expression of SLC6A4 in different human tissues and organs. SLC6A2 = sodium-dependent noradrenaline transporter, SLC6A3 = sodium-dependent dopamine transporter, SLC6A4 = sodium-dependent serotonin transporter.

In 2014, Biederman J et al[45] studied neurobiology and genetics by taking SLC6A2 and SLC6A4 as ADHD risk genes. Harikrishnan et al[46] Gene expression in neuronal cortical cells was found to be involved in the increase in histone hyperacetylation of the SLC6A2 promoter. In a 2017 study, SLC6A2 and SLC6A3 were associated with major depression in the Chinese Han population.[47]

The Genemania database was used to obtain the PPI network related to SLC6A3, SLC6A4, and SLC6A2, and the associated target genes in the network were used to obtain the enrichment

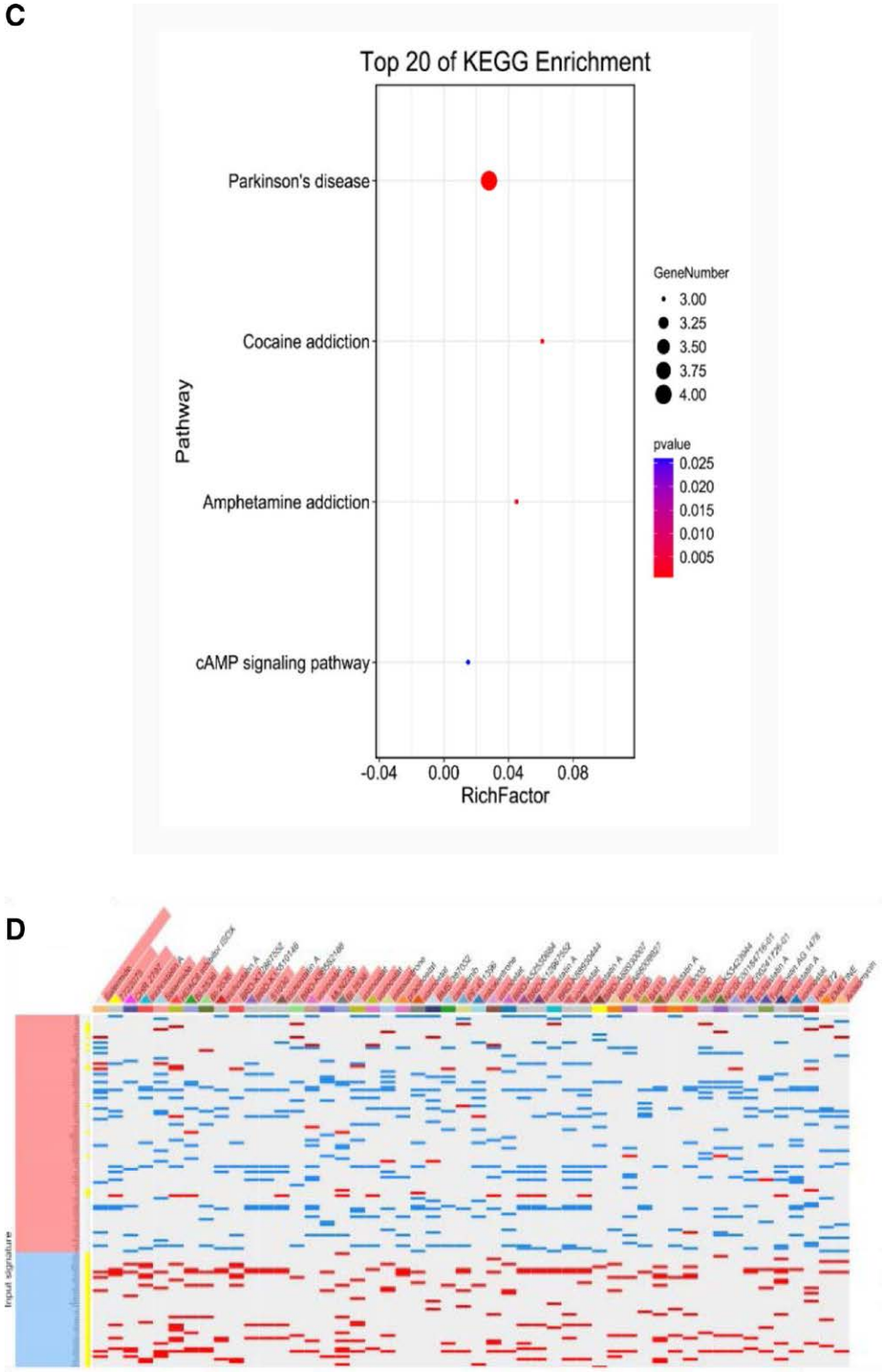

Figure 4 Continued

map of GO and KEGG pathway analysis (Fig. 3). Figure 3 shows that the enrichment information related to GO or KEGG pathway related to the target involves neurotransmitters, dopamine and γ-aminobutyric acid transport, neurotransmitters, dopamine, and γ-aminobutyric acid-related sodium transporter activity, related pathways of drug response, cAMP signaling pathway, etc. The kinase disturbance of the corresponding genes mainly involves trichostatin A, the HDAC6 inhibitor ISOX, and vorinostat.

A study in 2006 showed that in the differential gene expression of transcriptome analysis of mouse central and peripheral nervous systems, the gene functions rich in mouse dorsal root ganglion, including the G protein coupled receptor protein signaling pathway, potassium transport, sodium transport, signal transduction connected with sensory perception and cell surface receptors, synaptic transmission, and organic acid transport genes related to neurotransmitter transport and circulation, are enriched in the mouse lumbar spinal cord.[48]

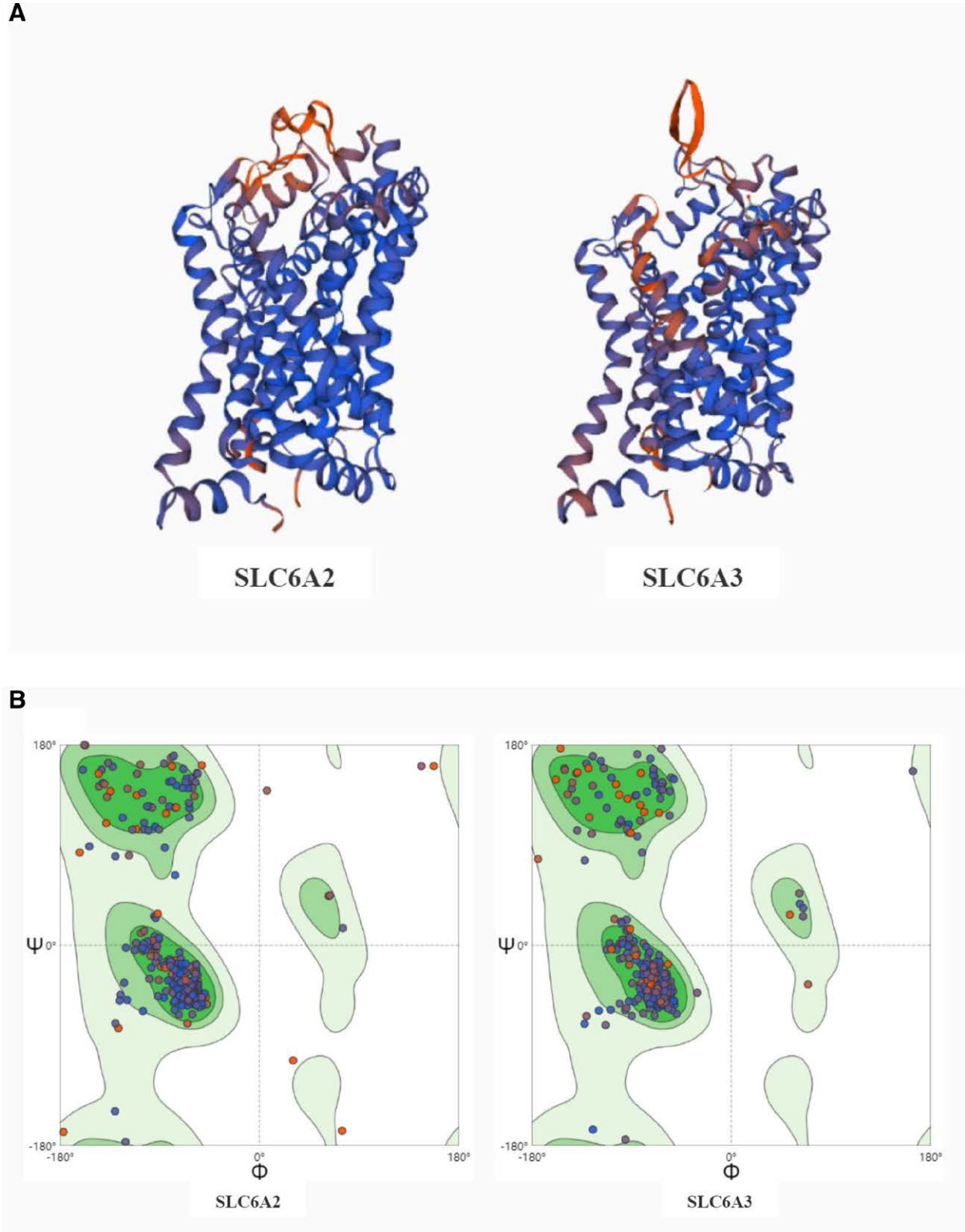

Figure 5 Enrichment analysis diagrams. (A) shows PPI network, (B) shows the enrichment of go function, (C) shows the enrichment of KEGG signal pathway, (D) shows LinCS L1000 characteristic matrix analysis. GO = gene biological process, KEGG = Kyoto Encyclopedia of Genes and Genomes, PPI = protein-protein interaction network.

In the process of searching the literature, no dopamine and γ-The in-depth research results of aminobutyric acid in the peripheral nervous system and the role of the cAMP signaling pathway in peripheral nervous system diseases has not been studied. The main effect of the cAMP signaling pathway is to activate target enzymes and activate gene expression, which is completed by protein kinase. Therefore, whether the above target genes and pathways are related to the predicted kinases,

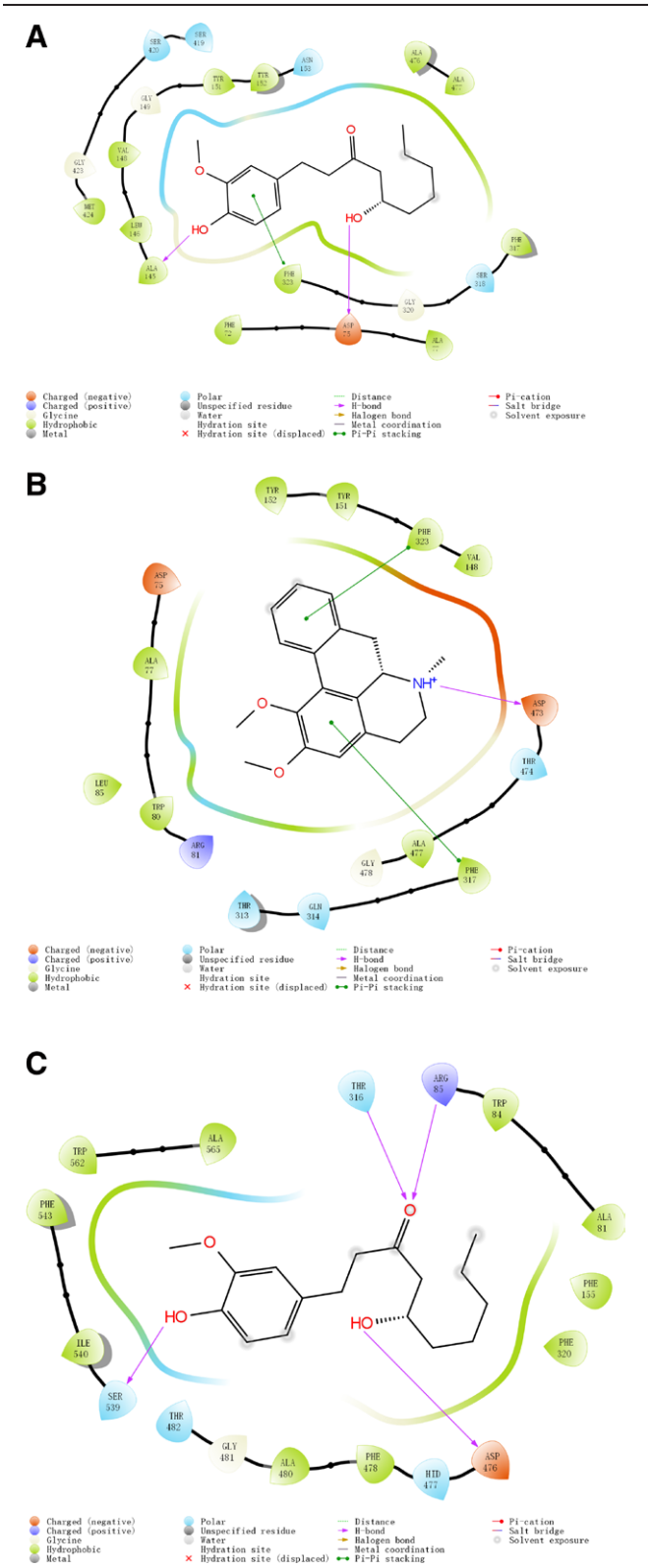

Figure 6 Compounds 6-Gingerol and nuciferin dock to the active pockets of proteins SLC6A2, SLC6A3, and SLC6A4. (A) 6-Gingerol-SLC6A2, (B) nuciferin-SLC6A2, (C) 6-Gingerol-SLC6A3, (D) nuciferin-SLC6A3, (E) 6-Gingerol-SLC6A4, and (F) nuciferin-SLC6A4. SLC6A2 = sodium-dependent noradrenaline transporter, SLC6A3 = sodium-dependent dopamine transporter, SLC6A4 = sodium-dependent serotonin transporter.

such as trichostatin A, HDAC6 inhibitor ISOX, and vorinostat, has not been retrieved in previous studies and needs to be further verified.

Figure 6 Continued

5. Conclusion

In the process of using multiple databases and various software and technical platforms to predict the mechanism of Huangqi Guizhi Wuwu decoction in the prevention and treatment of oxaliplatin-induced peripheral neuropathy, we can see that a variety of compounds contained in Huangqi Guizhi

Table 1

Molecular docking score.

Compound	Target	Bind energy (kcal/mol)
6-Gingerol	SLC6A2	–7.2
	SLC6A3	–5.8
	SLC6A4	–7.3
Nuciferine	SLC6A2	–7.5
	SLC6A3	–7.1
	SLC6A4	–8.5

SLC6A2 = sodium-dependent noradrenaline transporter, SLC6A3 = sodium-dependent dopamine transporter, SLC6A4 = sodium-dependent serotonin transporter.

Wuwu decoction prevented and controlled oxaliplatin-induced peripheral neuropathy through multi-target, multi-channel, and multi-system regulation. In the course of the study, we found that 6-Gingerol and nuciferin are the key compounds for the joint action of oral and topical use of the decoction when soaking hands and feet. For the prediction mechanism, we selected SLC6A2, SLC6A3, and SLC6A4 as the key targets of the Huangqi Guizhi Wuwu decoction for the treatment of diseases. The above three targets, provides reference and ideas for future clinical and basic research. We are looking forward to a more in-depth and comprehensive study of the prediction results in the near future, with the permission of science and technology and funding, to provide a reliable reference basis for the molecular mechanism of Huangqi Guizhi Wuwu Decoction in the prevention and treatment of oxaliplatin-induced peripheral neurotoxicity.

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Author contributions

TC conceived of and designed the experiments. WS contributed significantly to the analysis and preparation of this article. TC wrote the article with the input of other coauthors.
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Data curation: Yabo Shi, Wenchuan Shi.
Formal analysis: Yabo Shi.
Software: Yabo Shi.
Validation: Wenchuan Shi.
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Writing – review & editing: Tingting Chen.

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