Exploring the Mechanism of Action of Gastrodin in Parkinson's Disease Based on Network Pharmacology

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Abstract [**Objectives**] To explore the mechanism of action of gastrodin in the treatment of Parkinson's disease (PD) by employing network pharmacology technology, and to provide a scientific theoretical basis for the rational clinical application of gastrodin. [**Methods**] The target of gastrodin was identified through a search of the SwissTargetPrediction database. The keyword "Parkinson Disease" was employed to identify the pertinent targets of PD in the GeneCards and OMIM databases. The relationship between gastrodin and PD was elucidated, and a Veen map was constructed to identify the genes that were common to both. A total of 52 common drug targets associated with PD as identified in the Wayne chart were imported into the String database (https://string-db.org/) for protein-protein interaction prediction. Subsequently, Cytoscape 3.9.1 software was employed to construct a "drug-target" network. The potential targets of gastrodin in the treatment of PD were then imported into the DAVID database, where GO analysis and KEGG enrichment results were obtained. [**Results**] A total of 22 core targets and 53 related pathways of gastrodin were identified as potentially beneficial for the treatment of PD. [**Conclusions**] Gastrodin may be a potential therapeutic agent for the treatment of PD by modulating the biological process of apoptosis, affecting the relevant pathways such as the IL-17 signaling pathway and the TNF signaling pathway, and acting on GAPDH, EGFR, CASP3, MMP9 and other targets.

Key words Parkinson's disease, Network pharmacology, Gastrodin

1 Introduction

A common type of central nervous system degenerative disease in the elderly is Parkinson's disease (PD), which is distinct from Alzheimer's disease. The primary clinical manifestations of PD are static tremor and most of them are accompanied by slow movement, rigid gait and posture change[1]. As the global population continues to age, PD has emerged as the most rapidly growing neurodegenerative disorder affecting the central nervous system. The prevailing hypothesis regarding the pathogenesis of PD is that the loss of dopamine transmitters from the striatum in brain tissue is the primary factor[2]. Although anticholinergics and dopamine analogs have been developed for the treatment of PD, their side effects and related efficacy have been unsatisfactory[3]. Therefore, the development of drugs for the treatment of PD and related target studies remain a pressing necessity.

Gastrodin is the first phenolic glycoside compound isolated from *Gastrodia elata*, and it is the principal active component of *G. elata*[4]. It has been demonstrated that gastrodin can regulate the related cells of central, vascular, immune, metabolic and other systems, and the side effects are minimal[5]. However, the exact mechanism of action remains unclear. Network pharmacology technology establishes a network model by connecting nodes with corresponding targets, thereby transforming abstract and single entities into complex networks. Subsequently, through further concrete analysis, the composition relationship and characteristics of complex networks are completed, the key targets of drugs are mastered, the potential value of traditional Chinese medicine is predicted, and finally the systematic identification of organisms is realized[6]. In this study, network pharmacology was employed to predict the mechanism of action of gastrodin in the treatment of PD, in order to provide a scientific basis for its clinical application.

2 Materials and methods

2.1 Data sources

2.1.1 Target prediction for gastrodin. In this study, the target of gastrodin was predicted by searching the SwissTarget Prediction database (http://swisstargetprediction.ch/)[7].

2.1.2 PD target acquisition. A search was conducted using the keywords "Parkinson Disease" in the GeneCards (https://www.genecards.org/) and OMIM (https://omim.org/) databases. The resulting targets were then screened to remove duplicate values, thus obtaining the disease targets[8].

2.2 Analysis method

2.2.1 Drug-disease target prediction results. The obtained drug targets and disease targets were mapped to each other to create the Veen map. The intersection of the two was identified as the potential target of gastrodin in the treatment of PD.

2.2.2 Construction of target protein interaction network. In order to further study the interaction between proteins in the treatment of PD by gastrodin, the intersection targets of gastrodin and PD were transferred to the String database[9] for the construction of a protein-protein interaction network (PPI). The species selected was "Homo sapiens", and the remaining parameters in the database were not adjusted, with the default settings maintained. The results of the generated gastrodin and PD were stored in a tab-separated value (TSV) format and imported into the dedicated oper-

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ating software Cytoscap 3.9.1 for analysis. Subsequently, the size of nodes and the use of different colors served to reflect the degree. The size of the node was positively correlated with the degree value generated. The size of the combine score was determined by the pre-set value of the thick and thin edge. The greater the thickness of the edge, the larger the comprehensive score. Finally, the core target was selected, and a diagram of the interaction network for gastrodin in relation to the PD protein was constructed.

2.2.3 GO function and KEGG pathway enrichment analysis of key targets. The identified core targets were uploaded to the DAVID database, with the species restricted to "Homo sapiens". Subsequently, the data underwent analysis through GO and KEGG pathway enrichments. The GO functional enrichment analysis encompassed three categories: cellular components (CC), molecular functions (MF), and biological processes (BP). The top 20 entries for each category were visualized in the enrichment outcomes. Furthermore, the metabolic pathways predominantly associated with the key target genes were analyzed, annotated, and presented in a visual format, with the top 20 pathways in the enrichment results highlighted. The outcomes of the enrichment analysis were imported into the microbiology platform for graphical representation and visualization.

3 Results and analysis

3.1 Prediction results of gastrodin targets The result of the gastrodin target prediction conducted using the SwissTargetPrediction database yielded 100 potential targets for gastrodin.

3.2 Prediction results of PD targets The gene information of PD target was obtained by conducting a search in the GeneCards and OMIM databases. The results showed that 9 717 AR-related targets were obtained from GeneCards, and 202 PD-related targets were obtained from OMIM. After the removal of redundant targets and the application of a median screening process, a total of 2 342 PD-related targets were identified.

3.3 Drug-disease target prediction results The 100 active component targets of gastrodin and the 2 432 related targets of PD were imported into the Venn 2.1.0 mapping platform for mapping. This process yielded 52 potential targets of gastrodin in the treatment of PD, which were subsequently depicted in the Venn diagram (Fig. 1).

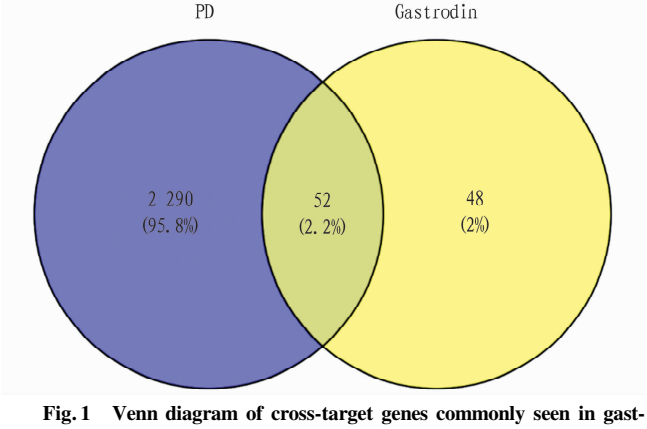
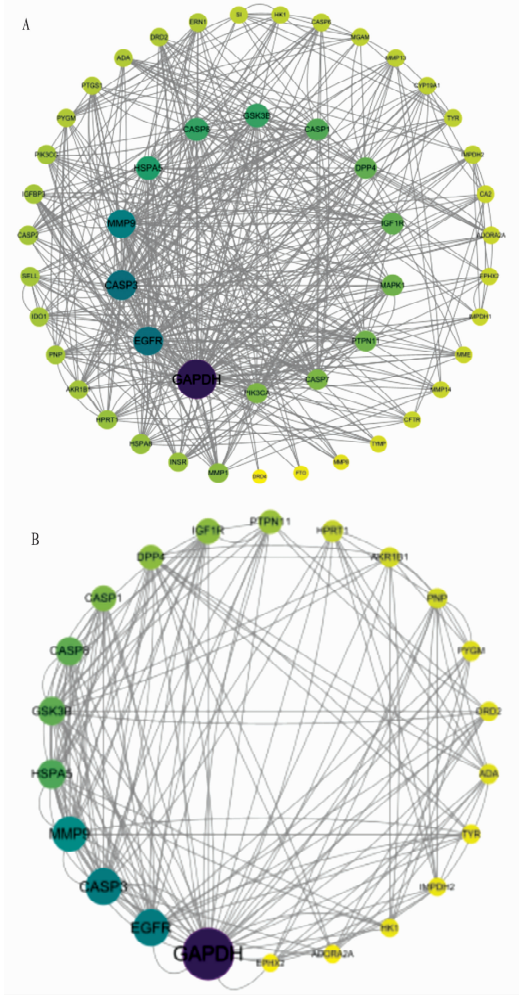


Fig. 1 Venn diagram of cross-target genes commonly seen in gastrodin and PD

3.4 Core target and network interaction The drug targets obtained by Venn diagram and the 52 common targets related to PD were imported into the String database for PPI prediction, with the species set as Homo sapiens. Upon clicking "Continue", the minimum interaction threshold was set to the highest confidence level (0.4). Unconnected proteins should be hidden and other settings should be retained in their default state. The network file should be saved in TSV format and imported into Cytoscape 3.9.1 software for the purpose of drawing the PPI network between gastrodin and PD. Furthermore, a topological analysis of the network should be carried out. The degree value was employed to reflect the dimensions and color of the target, while the combined score value was utilized to reflect the thickness of the edge. Subsequently, the interdependent relationship between proteins was elucidated. The network comprised a total of 52 nodes and 235 edges. The topological features of Centiscape 2.2 were evaluated using the values of DC, BC, and CC. According to the criteria of DC≥10, BC≥15, and CC≥0.421, 22 key targets were identified, as shown in Fig. 2.

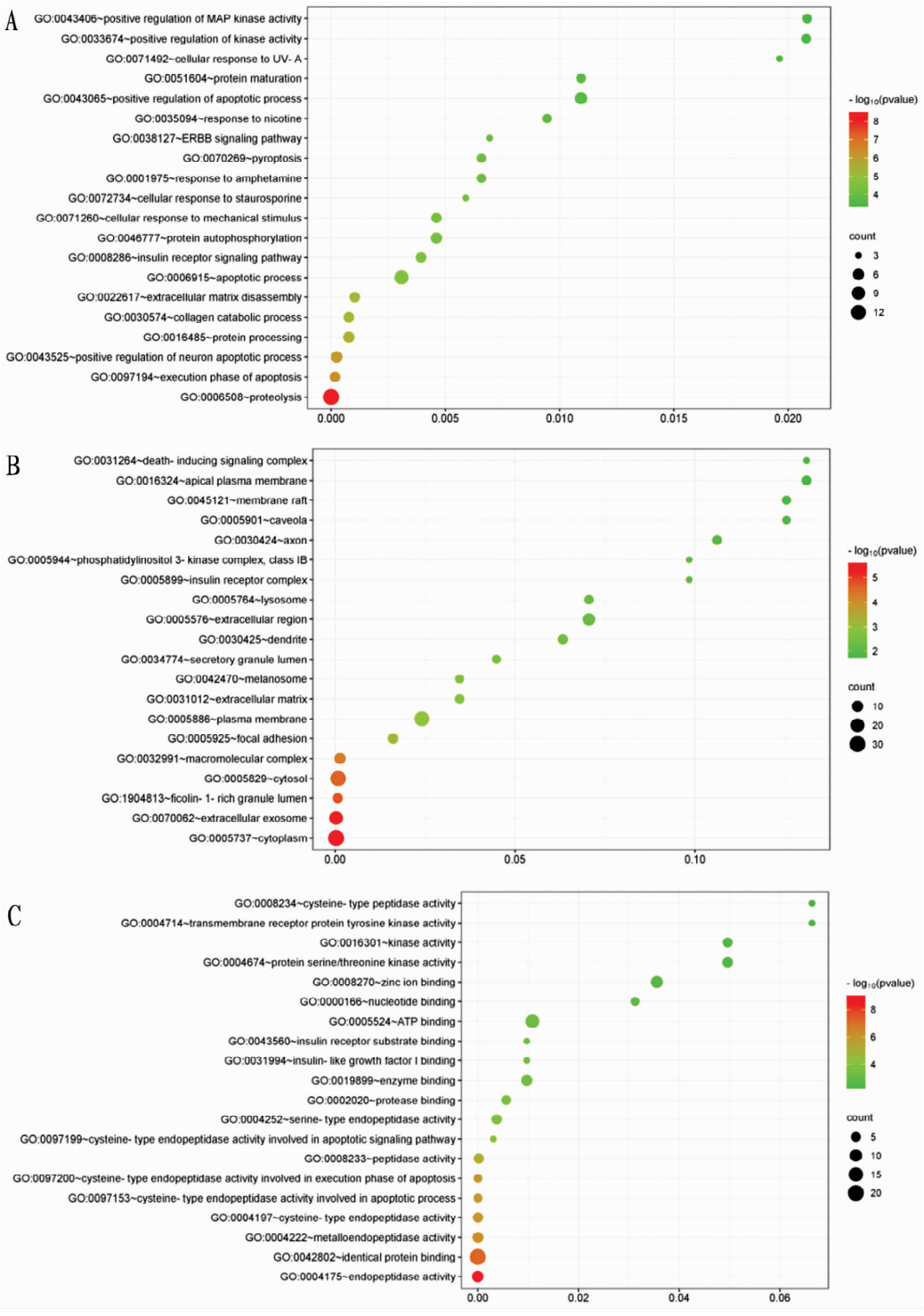


NOTE A. PPI network visualization of 52 target genes; B. Gene maps of the first 22 core targets from the PPI network.

Fig. 2 PPI networks of 52 target genes in PPI network and the top 22 target genes in PPI network

3.5 Biological function enrichment analysis

3.5.1 GO enrichment analysis. A total of 240 GO items were selected by using the DAVID library, of which 157 were classified as BP, 34 were identified as CC, and 49 were designated as MF. The top 20 items from the aforementioned biological processes should be selected to create a bar chart. BP is involved in the positive regulation of MAP kinase activity, cell response to mechanical stimulation, positive regulation of kinase activity, *etc.* CC primarily encompasses the plasma membrane, cytoplasm, dendrites, *etc.* MF is primarily associated with protein serine/threonine kinase activity, cysteine-type endopeptidase activity, protease binding, *etc.* , as illustrated in Fig. 3.



NOTE A. GO-BP enrichment analysis of 52 targets; B. GO-CC enrichment analysis of 52 targets; C. GO-MF enrichment analysis of 52 targets.

Fig. 3 GO analysis of 52 core therapeutic targets

3.5.2 Enrichment analysis of KEGG pathway. A pathway enrichment analysis was conducted using the DAVID database[8], resulting in the identification of 53 pathways related to gastrodin for the treatment of PD. Pathways related to GsRb1 for the treatment of PD were screened out according to $P < 0.01$. Pd-related pathways such as apoptosis, IL-17 signaling pathway, HIF-1 signaling pathway, and Alzheimer's disease were screened, as shown in Fig. 4.

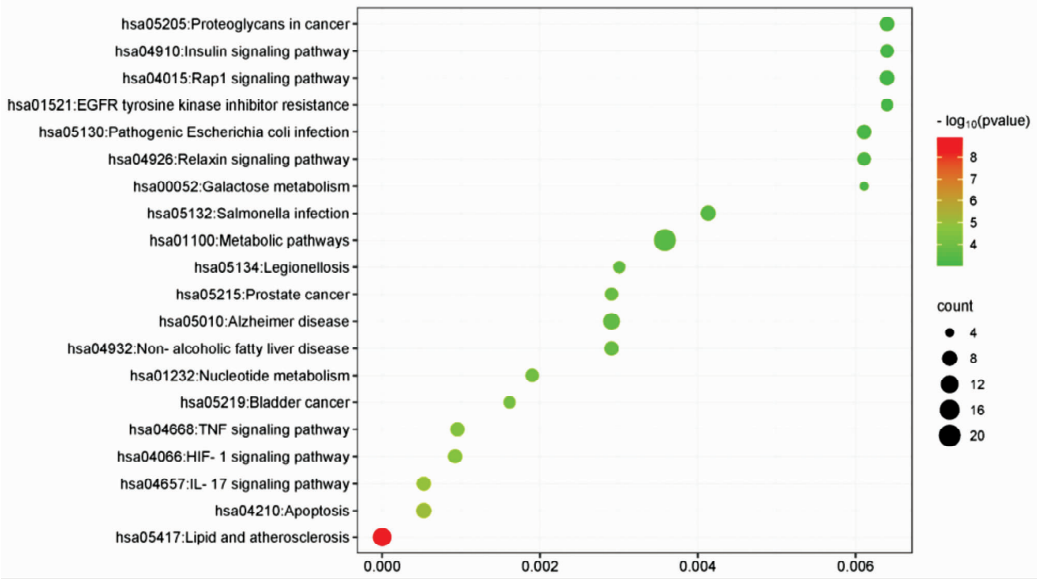


Fig. 4 KEGG pathway enrichment analysis of 52 core therapeutic targets

4 Discussion

In this study, a total of 52 potential targets of gastrodin for PD were identified through an intersection of drugs and disease targets. This suggests that gastrodin may be involved in regulating multiple targets, potentially playing a therapeutic role. The established PPI main target network encompassed 52 nodes and 235 edges, including core targets such as GAPDH, MMP9, and CASP3. GAPDH was initially presumed to be a straightforward steward gene and internal reference protein involved in glycolysis. Recent studies have demonstrated that GAPDH is not a single functional protein. In addition to its role in glycolysis, GAPDH is also involved in a multitude of cellular physiological processes, such as membrane fusion and transport, DNA repair, nuclear RNA output, cytoskeletal homeostasis, calcium homeostasis regulation, apoptosis and tumorigenesis[10-12]. GAPDH is associated with a number of neurodegenerative disease proteins, including α-synuclein and β-amyloid precursor protein (β-APP), huntingtin and others. These proteins interact with each other and participate in nerve cell apoptosis induced by various factors. The evidence is mounting that GAPDH is intricately linked to neurodegenerative disorders such as PD[13]. Caspase-3 is a pivotal enzyme in the process of apoptotic process, and typically exists in a dormant state. It is only when the protein is cut and activated as a heterodimer that the apoptotic signaling pathway is activated, inducing programmed cell death and thus promoting the occurrence and development of PD[14]. The findings demonstrate that the network pharmacological approach can provide a scientific basis for the treatment of PD with gastrodin by identifying and prioritizing key targets.

In summary, gastrodin may impede the emergence and subsequent progression of GO function and KEGG pathway enrichment. The main pathways for the treatment of PD by gastrodin encompass the TNF signaling pathway, the IL-17 signaling pathway, the HIF-1 signaling pathway, and the Alzheimer's disease signaling pathway. The TNF signaling pathway and IL-17 signaling pathway are both involved in the pathogenesis of inflammatory diseases[15]. The IL-17 signaling pathway plays a crucial role in host defense, tissue repair, and the pathogenesis of inflammatory diseases. The IL-17 signaling pathway is primarily regulated by the IL-17 family. IL-17A and IL-17F are two major pro-inflammatory cytokines that bind to IL-17RA and IL-17RC, respectively, to form receptor complexes, thereby initiating the transcription of inflammatory target genes[16]. HIF-1 is a hypoxia-inducing factor. Studies have found that the activation pathway of HIF can mitigate an excessive inflammatory response by inhibiting the NF-κB pathway, which is of considerable importance for reducing neuroinflammatory response and tissue damage[17]. Consequently, gastrodin may influence the occurrence and development of PD by regulating these key pathways.

In conclusion, gastrodin may impede the emergence and progression of PD by modulating multiple targets. However, the precise mechanism of action and associated regulatory pathways remain to be validated through further experimentation.

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to generate multiple possible binding conformations.

Analysis of results: score the generated docking conformations, and select the conformation with the lowest binding free energy for analysis, and display the ligand-protein binding mode and key interactions by visualization software (such as PyMOL).

4.2.2 Molecular docking prediction results. The interaction between the main active components of *G. jasminoides* and its key target proteins was verified by molecular docking analysis. The following are the docking results of some important active components:

Quercetin and MMP-9: Quercetin stably bound to the active site of MMP-9 through hydrogen bonds and hydrophobic interactions, with a binding free energy of −8.5 kcal/mol, indicating that it has a high binding affinity, which helps to inhibit the enzymatic activity of MMP-9, thereby slowing down the eye skin photoaging process[15].

Kaempferol and NF-κB: Kaempferol bound to NF-κB mainly through hydrogen bonding and π-π stacking, and the binding free energy was −7.8 kcal/mol. This binding mode may contribute to the inhibition of the activation of NF-κB signaling pathway by kaempferol, thus exerting its anti-inflammatory effect.

Baicalin and ERK1/2: The binding between baicalin and ERK1/2 protein showed multiple hydrogen bond interactions with a binding free energy of −8.2 kcal/mol, suggesting that baicalin may play an antioxidant and anti-inflammatory role by interfering with ERK1/2 signaling pathway.

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