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Network Pharmacology and Molecular Docking Analysis on Mechanisms of Isobavachalcone in Parkinson's Disease

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Abstract

Objective: To use bioinformatics methods to identify the target genes of isobavachalcone (ISO) in Parkinson's disease (PD), and to construct pharmacology network to characterize the underlying mechanism of ISO in PD. Methods: Potential targets of ISO, as well as related genes of PD were obtained from the public databases, the potential targets and signaling pathways were determined by protein-protein interaction (PPI), gene ontology (GO) and KEGG pathway enrichment analyses. And the network among ISO, PD and their co-targets was constructed using Cytoscape 3.3.0. AutoDock Tools and PyMOL software were applied for molecular docking. Results: 34 potential targets of ISO related to PD were predicted using the public databases. PPI network showed that AKT1, PTGS2, EGFR, HSP90AA1, APP, SNCA, ACHE, BACE1, AKR1B1, MAOB, ABCB1 and PTGES were considered to as hub genes. Through enrichment analysis, ISO was found to exert its potential therapeutic effects on PD through several pathways. Molecular docking showed that ISO might bind to the key PD-associated amyloid protein, α-synuclein. Conclusion: Altogether, this study preliminarily investigated the pharmacological effects of ISO on PD and its potential underlying therapeutic mechanisms mediated by multiple targets and pathways.

Keywords

Isobavachalcone; Parkinson's Disease; Network Pharmacology.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease in the world, affecting approximately 0.3% of the global population and more than 1% of people over the age of 60 and 4% of people over the age of 80 [1]. With the aging of the population, it is estimated that the number of PD patients will continue to rise in the next few years, and will bring huge medical challenges and economic burden to the society. Due to the lack of dopamine, PD patients often show motor symptoms such as bradykinesia, rigidity, resting tremor, postural instability and gait disorders, as well as clinical manifestations of non-motor symptoms such as sleep disorders, olfactory disorders, autonomic nervous dysfunction, cognitive and mental disorders. PD is characterized by high disability rate and slow progression, and with the progression of the disease, the symptoms of PD gradually worsen, greatly affecting the living standard and quality of PD patients [2]. However, there is no drug to effectively cure PD currently. Therefore, drug research and development for PD is particularly important.

Isobavachalcone (ISO), a plant-derived flavonoid compound, is composed of the main active components in the Chinese herbal medicine Psoralea corylifolia Linn [3]. ISO structurally contains two benzene rings with three different hydroxyl groups attaching to them. Functionally, studies in vitro and in vivo have revealed that ISO possesses a variety of pharmacological effects, including anti-cancer, anti-bacterial, anti-inflammatory, anti-oxidant, etc. [4]. More importantly, studies have revealed a neuroprotective effect of ISO in neurodegenerative diseases, including Alzheimer's disease (AD) and PD, possible through

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decreasing inflammation and the activation of microglia cells [5] or inhibiting AD-associated amyloid protein [6, 7]. As a result, ISO is a promising therapeutic agent for neurodegenerative diseases. However, the study of ISO in PD is still insufficient and the underlying mechanism also remains to be explored.

In the present study, we aim to explore the potential molecular mechanisms and pathways of ISO on PD though bioinformatics, network pharmacology and molecular docking. These results might offer a new insight to understand the potential molecular mechanism for ISO to overcome PD through possible candidate targets.

2. Materials and Methods

2.1. Prediction of Targets of ISO

For target prediction, the SMILES of ISO (CID: 5281255) from PubChem (https://pubchem.ncbi.nlm.nih.gov/) was employed to visualize the structure and predict the targets in the website of Swiss Target Prediction (http://www.swisstargetprediction.ch/).

2.2. Collection of Potential Target Genes for Parkinson's disease

The PD-related gene targets were downloaded from GeneCards (https://www.genecards.org/) database [8], using keyword "Parkinson's disease".

2.3. Pharmacology Network Construction

Pharmacology network among ISO, PD and their co-targets were visualized by Cytoscape 3.7.0 [9].

2.4. Construction of Protein–Protein Interaction (PPI) Network

Protein–protein interaction (PPI) network was constructed by the STRING (https://string-db.org/) database [10], using the overlap genes between ISO targets and PD targets, with the species limited to "Homo sapiens".

2.5. Gene Ontology (GO) and KEGG Pathway Enrichment Analyses

Database for Annotation, Visualization and Integrated Discovery [11] (DAVID, https://david.ncifcrf.gov/) was used for gene ontology (GO) and KEGG pathway enrichment analysis with requirement of false discovery rate (FDR) < 0.05. GO terminology was annotated including biological process (BP), cellular component (CC) and molecular function (MF) categories.

2.6. Molecular Docking

To obtain a deeper understand about the interaction between ISO and α-synuclein, molecular docking was performed to evaluate the binding affinity and mode of the interaction between ISO and α-synuclein. The crystal structure of α-synuclein (PDB code: 2N0A) were obtained from RCSB Protein Data Bank (PDB, http://www.rcsb.org/). The chemical structure of ISO (CID: 5281255) was prepared from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and converted into PDB format through OpenBabel3.1.1 [12]. A grid box with dimension of 126 × 126 × 126 Å3 and centre x = 97.487, y = 148.695 and z = −34,111, was set for molecular docking using AutoDock Tools (ADT, version 4.2.6) [13, 14], and PyMOL software (https://pymol.org) was applied for and visualization.

3. Results and Discussions

3.1. Structure of ISO

The two-dimensional chemical structure of ISO was obtained from PubChem (CID: 5281255). As showed in Figure 1, ISO contains two benzene rings with three different hydroxyl groups attaching to them in structure.

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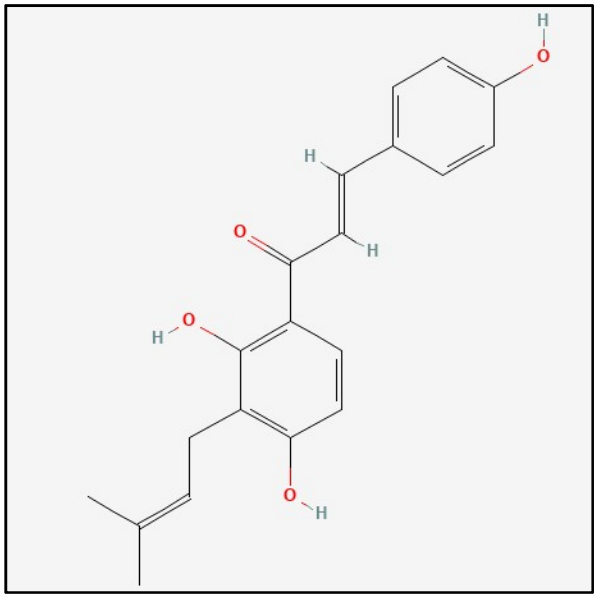

Figure 1. The chemical structure of ISO obtained from PubChem (CID: 5281255)

3.2. Putative Targets of ISO

To identify the potential protein targets of ISO, we input ISO on the website of Swiss Target Prediction to search its possible targets. According the results of Swiss Target Prediction, we found that the top 15 protein targets of ISO belonged to classes of enzyme (26.7%), oxidoreductase (20.0%), kinase (13.3%), cytochrome P450 (6.7%), ligand-gated ion channel (6.7%), membrane receptor (6.7%), surface antigen (6.7%), voltage-gated ion channel (6.7%) and protease (6.7%), indicating this small molecule may regulate different clusters of protein that possess different functions. To know about the exact protein that ISO targets to, we obtained 41 protein targets with the estimated probability larger than 0, where alpha-synuclein (α-synuclein) was included as a target with probability equal to 0.104671941 (Table 1).

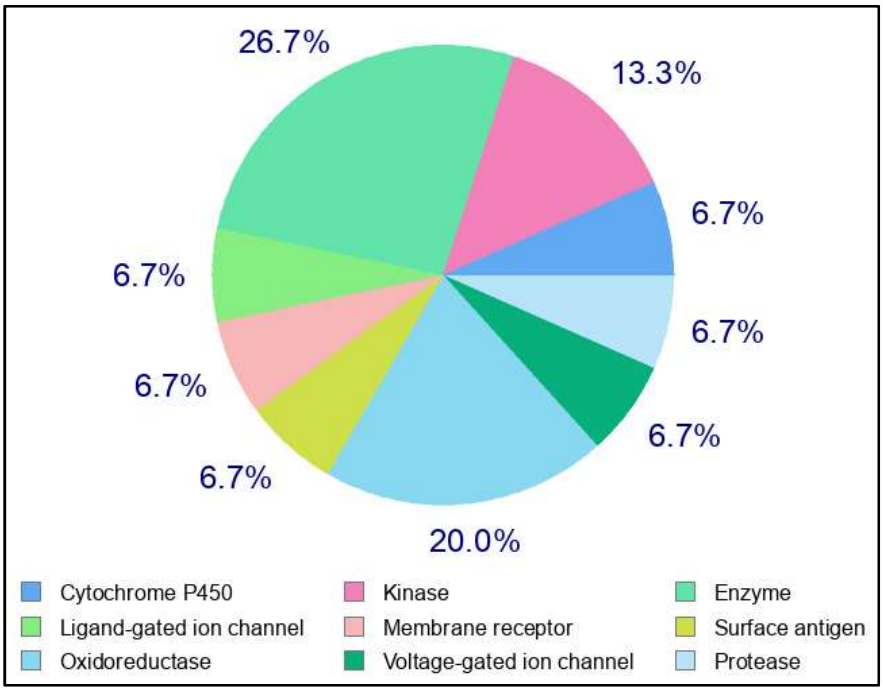

Figure 2. The classes of the top 15 targets of ISO based on Swiss Target Prediction

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Table 1. The top 20 possible targets of ISO predicted by Swiss Target Prediction.

Target	Common name	Uniprot ID	Target Class	Probability*
Cytochrome P450 19A1	CYP19A1	P11511	Cytochrome P450	0.217731
Epidermal growth factor receptor erbB1	EGFR	P00533	Kinase	0.161187
Aldose reductase	AKR1B1	P15121	Enzyme	0.161187
Neuronal acetylcholine receptor protein alpha-7 subunit	CHRNA7	P36544	Ligand-gated ion channel	0.153093
Nitric oxide synthase, inducible	NOS2	P35228	Enzyme	0.13697
3-phosphoinositide dependent protein kinase-1	PDPK1	O15530	Kinase	0.120824
Beta amyloid A4 protein	APP	P05067	Membrane receptor	0.112748
Coagulation factor VII/tissue factor	F3	P13726	Surface antigen	0.112748
Aldehyde dehydrogenase	ALDH2	P05091	Oxidoreductase	0.104672
Monoamine oxidase B	MAOB	P27338	Oxidoreductase	0.104672
Voltage-gated potassium channel subunit Kv1.3	KCNA3	P22001	Voltage-gated ion channel	0.104672
Beta-secretase 1	BACE1	P56817	Protease	0.104672
Arachidonate 15-lipoxygenase	ALOX15	P16050	Enzyme	0.104672
Arachidonate 5-lipoxygenase	ALOX5	P09917	Oxidoreductase	0.104672
Telomerase reverse transcriptase	TERT	O14746	Enzyme	0.104672
Alpha-synuclein	SNCA	P37840	Unclassified protein	0.104672
ATP-binding cassette sub-family G member 2	ABCG2	Q9UNQ0	Primary active transporter	0.104672
P-glycoprotein 1	ABCB1	P08183	Primary active transporter	0.104672
Aldo-keto-reductase family 1 member C3	AKR1C3	P42330	Enzyme	0.104672
Protein farnesyltransferase	FNTA FNTB	P49354 P49356	Enzyme	0.104672

3.3. Target Identification of ISO in PD

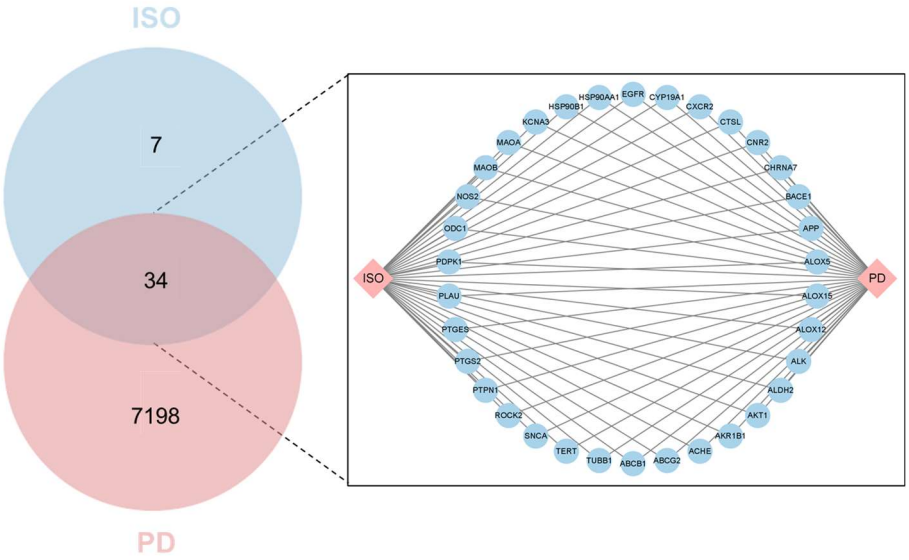

Figure 3. The venn diagram of ISO targets and PD targets and the network of ISO, PD and co-targets

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Next, we performed a comprehensive search on GeneCards using "Parkinson's disease" as a keyword and identified a total of 7198 genes that are associated with PD. When crossing the 41 targets of ISO with the 7198 PD-associated genes, 34 targets including α-synuclein encoding gene, SCNA, were found to be overlapped, which indicated that they were co-targets of ISO and PD. An overview of the network was established to unveil the correlation among ISO, PD and their co-targets. The network contained 36 nodes and 68 edges and clearly showed that ISO may play an important role in PD through targeting various PD-related molecules.

3.4. Target Interactional Network Analysis

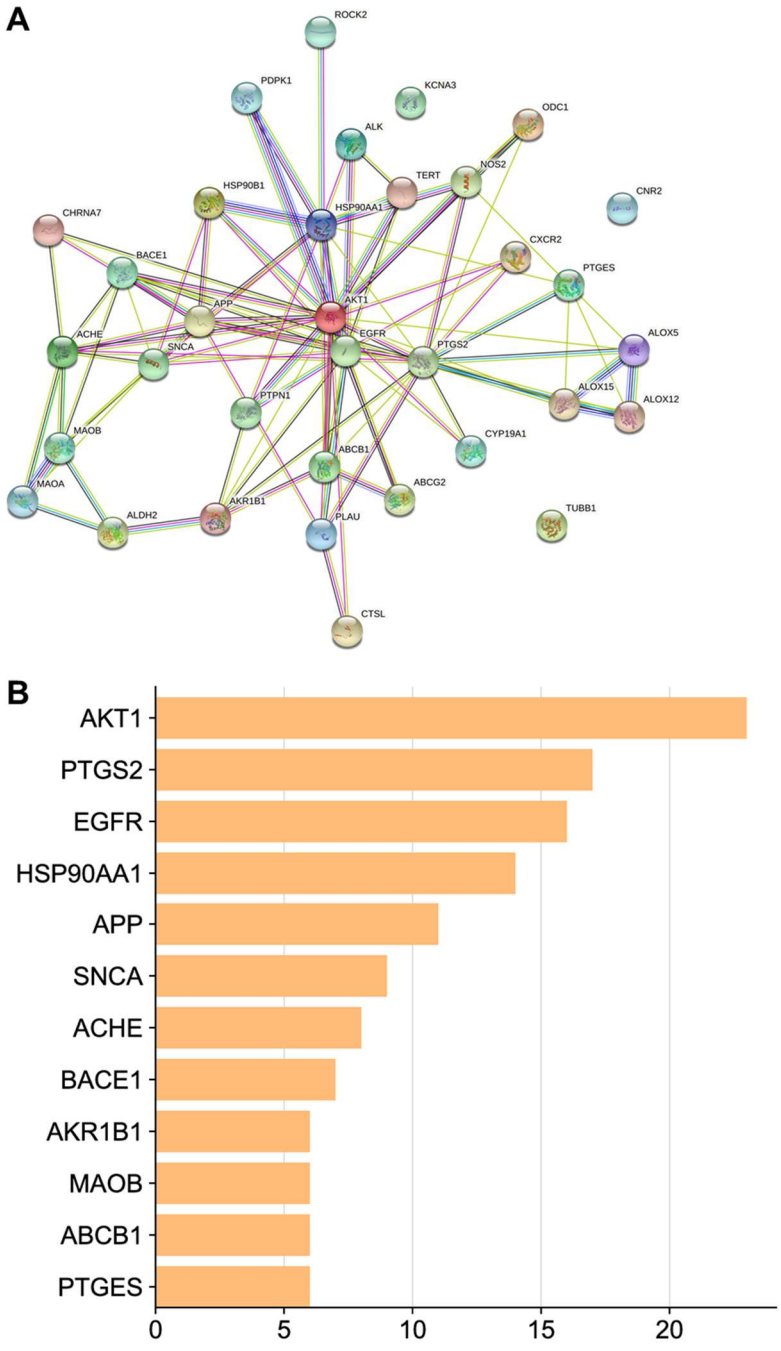

Figure 4. PPI analysis of ISO and PD co-targets. (A) The PPI network of ISO and PD co-targets. (B) Bar plot showing the top 12 proteins in the PPI network. X-axis represented the number of neighboring proteins of the target protein, and the y-axis represented the target protein

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The 34 potential co-targets were submitted to STRING to obtain PPI data that could be used to construct the PPI network, as shown in Figure 4A. The PPI network contained 34 nodes and 98 edges, which represented proteins and protein-protein relationships, respectively. Proteins with more edges were considered as the more significant targets in the network. As shown in Figure 4B, the top 10 proteins with multiple edges in the network were plotted. Among them, AKT1, PTGS2, EGFR, HSP90AA1, APP, SNCA, ACHE, BACE1, AKR1B1, MAOB, ABCB1 and PTGES contained 23, 17, 16, 14, 11, 9, 8, 7, 6, 6, 6 and 6 edges, respectively, indicating that they were possibly the most vital targets, through which ISO acted on PD.

3.5. GO Pathway Enrichment Analysis

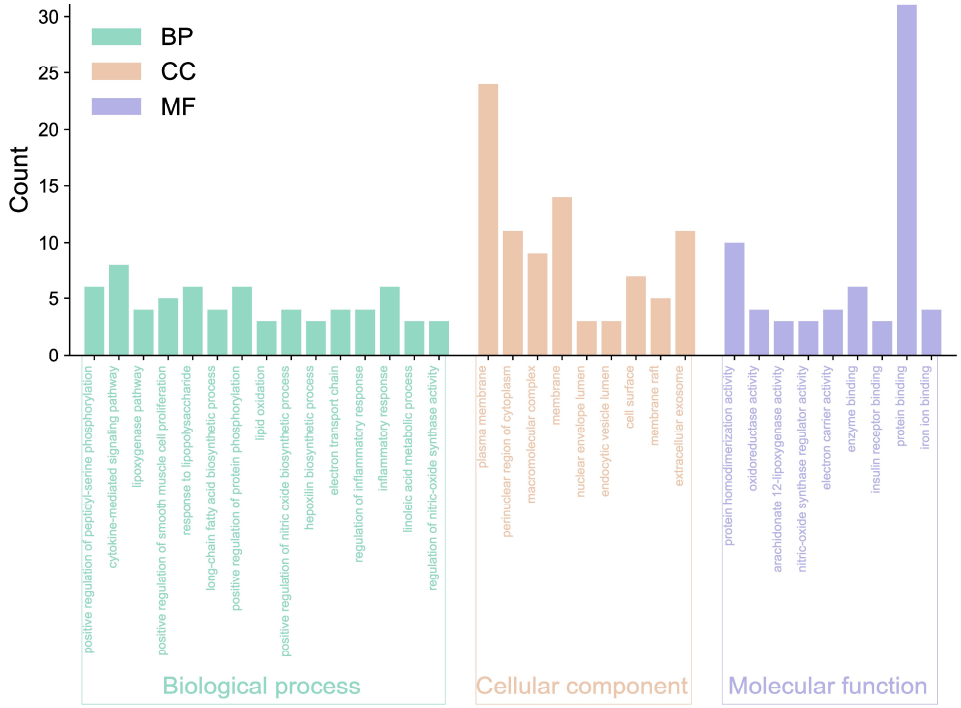

Figure 5. GO analyses of the 34 potential co-targets correlated with PD. The significantly enriched BP (Biological process), CC (Cellular component), and MF (Molecule function) terms (FDR < 0.05) are plotted on the x-axis; the count of the significantly enriched genes related to these terms is plotted on the y-axis

To explore the therapeutic mechanisms of putative co-targets of ISO on PD, the GO and KEGG pathway enrichment analyses were performed using DAVID online analysis. For the potential biological process (BP), cellular component (CC) and molecular function (MF) that the predicted co-targets may be involved in, 136 BP, 38 CC and 37 MF terms in total were acquired. After screening, 15 BP, 9 CC and 9 MF terms met the requirement of FDR < 0.05, and the identified significantly enriched GO terms in BP, CC, and MF were plotted in Figure 5. For BP, ISO may be involved in positive regulation of peptidyl-serine phosphorylation, cytokine-mediated signaling pathway, lipoxygenase pathway, positive regulation of smooth muscle cell proliferation, response to lipopolysaccharide, long-chain fatty acid biosynthetic process, positive regulation of protein phosphorylation, lipid oxidation, positive regulation of nitric oxide biosynthetic process, hepoxilin biosynthetic process, electron transport chain, regulation of inflammatory response, inflammatory response, linoleic acid metabolic process, regulation of nitric-oxide synthase activity. In addition, the cell components targeted by ISO contained plasma membrane, perinuclear region of cytoplasm, macromolecular complex, membrane, nuclear, envelope lumen, endocytic vesicle lumen, cell surface, membrane raft and extracellular exosome. Moreover, the significantly enriched MF terms by co-targets of ISO and PD included

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protein homodimerization activity, oxidoreductase activity, arachidonate 12-lipoxygenase activity, nitric-oxide synthase regulator activity, electron carrier activity, enzyme binding, insulin receptor binding, protein binding and iron ion binding. These results all together suggested that ISO possessed therapeutic activities in PD through effecting a variety of biological processes and molecule functions in different parts of cells.

3.6. KEGG Pathway Enrichment Analysis

To further reveal the underlying mechanism on involved pathways of ISO in PD, KEGG pathway enrichment analysis of the involved targets was conducted. Among all the 40 obtained enriched pathways, 7 pathways with FDR < 0.05 were predicted to be the most significantly enriched and were displayed in Figure 6. The result also indicated the top 3 pathways are pathways in cancer, Alzheimer disease, and serotonergic synapse with 9, 8, and 7 involved targets, respectively, which implied the potential value of ISO in the treatment of neurodegenerative diseases.

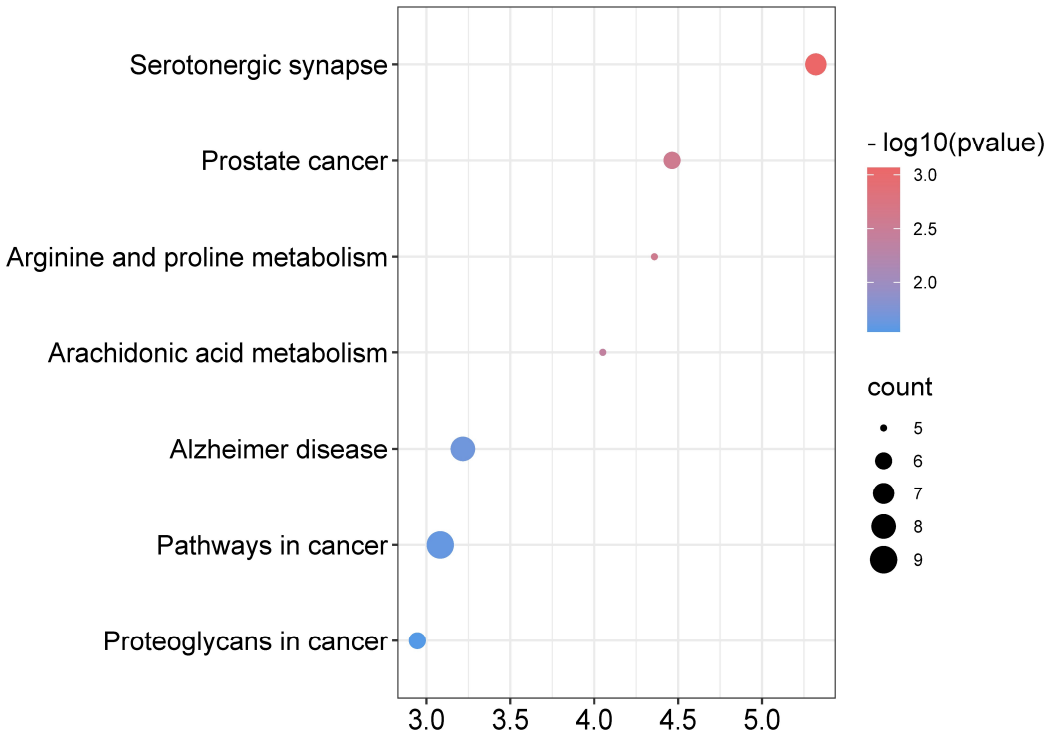

Figure 6. Dot plot showing the significantly enriched KEGG pathways (FDR < 0.05). X-axis represents the -log10(p-value); the y-axis represents the enriched pathways. The size of each dot corresponds to the number of genes annotated for the term. The blue dot corresponds to low -log10(p-value), and the red dot represents high -log10(p-value)

3.7. Interaction between ISO and α-synuclein

It is widely reported that the aggregation of amyloid α-synuclein protein is a hallmark of PD and is the crime culprit causing neuron damage and degeneration [15, 16]. Based on our present data, α-synuclein, encoded by SCNA gene, was predicted to be one of the most vital potential targets of ISO in PD. Therefore, in order to explore the interaction between ISO and α-synuclein, we performed molecular docking analysis using AutoDock Tools (ADT, version 4.2.6), and the computational study was based on the NMR structure of α-synuclein (PDB code: 2N0A). According to the similar study of Vittorio et al. [14], we defined the same region as the search space for the docking simulation. The docking results showed 10 protein-ligand binding conformations with binding energy < 0 kcal/mol (Figure 7A). Among them, 8 proposed binding poses had binding energy < -4 kcal/mol, and we chose the binding pose with minimum energy of -4.97 kcal/mol to visualize the binding mode between α-synuclein and ISO. As shown in

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Figure 7B, ISO mainly interacted with the NAC region of α-synuclein through forming two hydrogen bonds with Glu61 and Gly73. Our result was in well consistent with that of Vittorio et al., who identified that amyloid fibril inhibitors targeting to α-synuclein bind in the sites located between the N-terminal and the NAC domains of the protein [14]. Therefore, we assume that α-synuclein is the putative target of ISO that can be directly bound by ISO in PD.

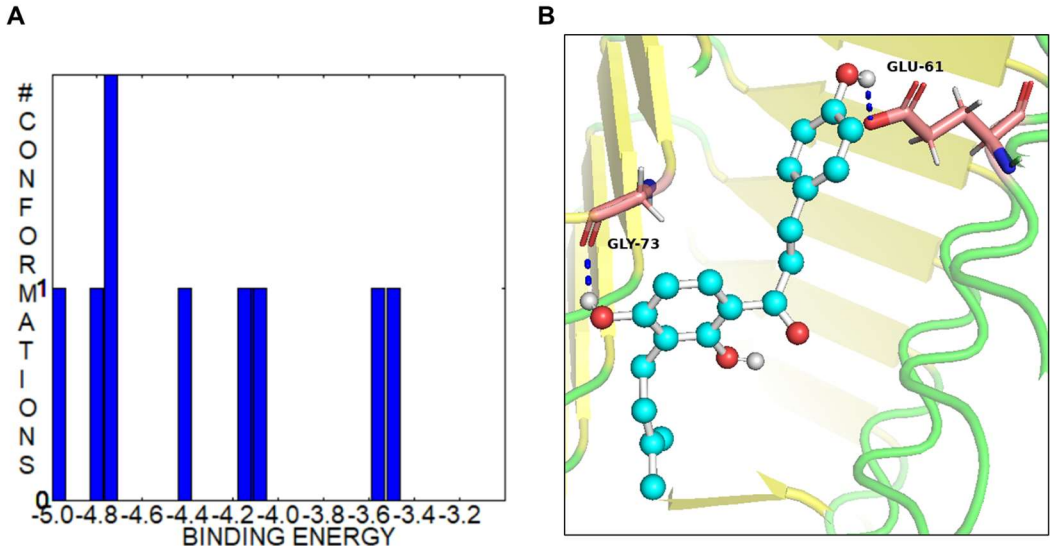

Figure 7. Molecule docking analysis of the interaction between ISO and α-synuclein. (A) The binding energy of each predicted binding conformation. (B) The proposed binding model of ISO (ball and stick). The interacting residues of the binding site are represented as sticks. The image is created by PyMOL software (https://pymol.org)

4. Conclusion

In the present study, we first identified 41 potential targets of ISO, 34 of which were further demonstrated to be the vital targets associated with PD. Next, we analyzed the interactions among these putative targets using PPI network. Moreover, GO annotation and KEGG pathway enrichment analyses were performed to characterize the potential biological functions of the targets of ISO in PD, and the results displayed that target genes of ISO in PD were associated with multiple pathways. We also predicted that ISO may have a direct interaction with PD-associated amyloid protein, α-synuclein. Altogether, we for the first time revealed the potential targets of ISO on PD based on network pharmacology and discovered that ISO may play a neuroprotective role in PD through various targets and pathways, which provided theoretical basis for the future studies on ISO's application on the treatment of PD.

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