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Therapeutic Targets and Molecular Mechanisms of Resveratrol in Amyotrophic Lateral Sclerosis: A Systematic Study of Network Pharmacology Incorporating Molecular Docking

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Abstract: In order to explore the therapeutic targets and molecular mechanisms of resveratrol in amyotrophic lateral sclerosis based on network pharmacology and molecular docking techniques. In this study, we obtained the active compounds of resveratrol from TCMSP Chinese Medicine System Pharmacology database and used the Swiss Target Prediction platform to predict the potential targets of the active compounds. The effective targets of amyotrophic lateral sclerosis were obtained from GeneCards database, and the intersection was taken with the potential targets of resveratrol. Protein interaction networks were performed using the STRING database, and further network topology analysis was performed using Cytoscape 3.9.1 software. Meanwhile, gene ontology (GO) functional analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed on the intersecting targets by Metascape database; finally, molecular docking validation was carried out by Pubchem and PDB databases as well as by Pymol and Autodocktools software. Resultly, forty-nine potential targets of resveratrol and 1599 potential targets of amyotrophic lateral sclerosis were obtained. The enrichment analysis showed that resveratrol could have therapeutic effects on amyotrophic lateral sclerosis through multi-targets and multi-pathways. The molecular docking results showed that the core components could bind to the core targets better. Resveratrol mainly acts on Pathways in cancer pathway, Proteoglycans in cancer proteoglycans, Relaxin signaling pathway, Lipid and atherosclerosis lipids and atherosclerosis pathway through the core targets such as EGFR, PTGS2, SRC, MMP9, IGF1R and so on. In conclusion, this study obtained the potential targets and molecular mechanisms of resveratrol for the treatment of amyotrophic lateral sclerosis, which provides a theoretical basis for promoting the development and clinical application of targeted drugs.

1. Introduction

Amyotrophic lateral sclerosis, also known as motor neuron disease, is a fatal and progressive neurodegenerative disease characterized by progressive lesions of upper and lower motor neurons in the brainstem and spinal cord [1-3], with clinical manifestations of progressively worsening muscular atrophy, weakness, and pyramidal fasciculations, and ultimately, the majority of patients die from dysphagia and respiratory muscle weakness 3-5 years after diagnosis. Numerous studies have shown that the pathogenesis of ALS involves different cellular and molecular mechanisms, including mitochondrial dysfunction, ROS-related oxidative stress, protein misfolding and aggregation, autophagy, apoptosis, nucleoplasmic translocation, and glutamate-mediated excitotoxicity [4-8]. Although ALS involves different cellular pathways, disease progression inevitably leads to motor neuron death [2]. Currently, there is no effective treatment for ALS. Edaravone and riluzole are the only drug treatments approved to improve survival by 2-3 months and provide only mild symptomatic relief for patients [9, 10].

The treatment of many neurodegenerative diseases is hampered by the presence of the blood-brain barrier in the central nervous system, through which most drugs are unable to enter the central nervous system and act. However, most polyphenols can cross the blood-brain barrier, so they are widely used in the treatment of various neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis. Resveratrol (3, 5, 4'-trihydroxytrans-stilbene) is a polyphenolic compound of the stilbene family that is widely found in a wide variety of plants, fruits (e.g., grapes and berries), peanuts, tea, and red wine [11]. Several studies have demonstrated the antioxidant, anticancer, anti-inflammatory, anti-aging, neuroprotective and cardioprotective activities of resveratrol [12-17]. Due to its neuroprotective properties, it was thus found that resveratrol may delay the onset of ALS, increase survival and preserve spinal motor neuron function [18, 19]. This suggests that resveratrol has a potential therapeutic role in amyotrophic lateral sclerosis.

The traditional pharmacology concept of "single gene, single target, single disease" and drug research methodology can no longer be adapted to diseases formed by multiple genes and multifunctional protein interaction disorders, and is also unsuitable for the study of the complex mechanism of action of traditional Chinese medicine. With the rapid development of bioinformatics, systems biology and polypharmacology, and based on publicly available data, network pharmacology is a novel, promising and low-cost method for the discovery of biologically active ingredients, the prediction of drug targets, and the analysis of the mechanism of action of drugs, etc. At the same time, network pharmacology emphasizes the multichannel regulation of signaling pathways, and therefore, it is particularly suitable for the explanation of the mechanism of traditional Chinese medicines with a wide range of chemical compositions and molecular targets [20, 21]. Molecular docking is a drug design method based on the properties of receptors and the interactions between receptors and drug molecules [22]. In this study, we proposed to use network pharmacology and molecular docking technology to explore the pharmacological and material basis of resveratrol for the treatment of amyotrophic lateral sclerosis in a multidimensional manner from cytology to molecular, from pathway enrichment to biological process, so as to provide theoretical basis for the development of novel targeted drugs for the treatment of amyotrophic lateral sclerosis and lay the foundation for its clinical application.

2. Materials and methods

2.1. Collection and screening of potential targets of action of the chemical constituents of resveratrol

The active compounds of resveratrol were firstly obtained from TCMSP Traditional Chinese Medicine System Pharmacology database, and then its SMILES structure was obtained from Pubchem database (https://pubchem.ncbi.nlm.nih.gov/) and imported into Swiss Target Prediction platform (http://www.swis stargetprediction.ch/) to predict the target sites of active compounds, and the results obtained from the prediction were screened according to the likelihood (Probability>0).

2.2. Access to amyotrophic lateral sclerosis targets

The keyword "Amyotrophic lateral sclerosis" was searched in GeneCards (https:// www. genecards.org/) to obtain disease targets related to amyotrophic lateral sclerosis, and the valid targets were screened for their relevance score of ≥15. Valid targets with a relevance score of ≥15 were screened, and potentially relevant targets for amyotrophic lateral sclerosis treatment were obtained by intersecting resveratrol targets with amyotrophic lateral sclerosis-related targets using a Venn diagram.

2.3. Constructing protein-protein interaction (PPI) networks.

The intersection targets were imported into the STRING database (https:// string-db. org/), and "Homo sapiens" was selected for "Organization", "minimum required interaction score" was set to "medium confidence (0.4)", "network" was set to "medium confidence (0.4)", and "interaction score" was set to "medium confidence (0.4)". The "minimum required interaction score" is set to "medium confidence (0.4)", and the "network display options" is set to "medium confidence (0.4)". Display options" was selected as "hide disconnected nodes in the network", and the other parameters were kept at their default settings to derive the PPI relationship. This data was also imported into Cytoscape 3.9.1 [23] software for network topology analysis, and filtered according to Degree, Betweenness, and Closeness values using the CytoNCA [24] plug-in to identify core target proteins.

2.4. Gene Ontology (GO) functional enrichment analysis and Kyoto Encyclopedia of Genes and Genes (KEGG) pathway enrichment analysis

The intersection targets of resveratrol active ingredient targets and amyotrophic lateral sclerosis were imported into Metascape (https://metascape.org/) for GO gene and KEGG pathway enrichment analyses, and the results of the individual analyses were ranked from smallest to largest P values, and the top 10 analyzed results were all selected, and the corresponding data were subsequently imported into the online graphing tool of Microsin [25]. Subsequently, the corresponding data were imported into Microbiology [25] (http://www.bioinformatics. com.cn/) online mapping tool, and the GO functional analysis included three parts: biological process, cellular component and molecular function, and the network diagram of KEGG enrichment analysis, which were all presented in the form of bubble diagrams.

2.5. Construction of component-target-pathway networks

The intersecting targets obtained from the active ingredients of resveratrol and amyotrophic lateral sclerosis were combined with the KEGG pathway enrichment analysis data, and imported into

Cytoscape 3.9.1 to construct the component-target-pathway network, and analyzed the network topology parameters of the active ingredients and the targets with the function of AnalyzeNetwork, and the degree of connectivity (degree), betweenness, and closeness of the network topology parameters were analyzed. The network topology parameters of active components and targets were analyzed by AnalyzeNetwork, and the degree of connectivity, betweenness and closeness of the network topology parameters were integrated to determine the core components and core targets of resveratrol in the prevention of amyotrophic lateral sclerosis.

2.6. Molecular docking validation

The protein structures of the core targets were downloaded from the RCSB PDB database (http://www.rcsb.org/). The structures of the core components corresponding to the core targets were downloaded from PubChem database and converted to mol2 format using Chem3D 20.0 software. The proteins were pretreated with AutodockTools 1.5.7 software to remove water of crystallization, hydrogenation, etc. The pretreated protein structures and active ingredient structures were converted to pdbqt format, and molecular docking was carried out and the binding energy was counted with AutodockVina 1.2.0 software. The smaller the binding energy indicates that the docking is more stable. Finally, the molecular docking results were imported into PyMOL software [26] for visualization and analysis, and the corresponding docking pattern maps were derived. According to previous literature reports, binding energy less than -5.0 kcal/mol suggests good binding.

3. Results

3.1. Candidate resveratrol targets for ALS treatment.

Forty-nine targets were obtained by importing the SMILES structures of resveratrol active ingredients into the Swiss Target Prediction platform after prediction and removal of duplicates. 1559 validated targets for amyotrophic lateral sclerosis were screened from the GeneCard database. The intersection of resveratrol active ingredient-related targets and amyotrophic lateral sclerosis targets was taken, and the 20 common targets obtained were the predicted potential targets of resveratrol against amyotrophic lateral sclerosis, as shown in Figure 1.

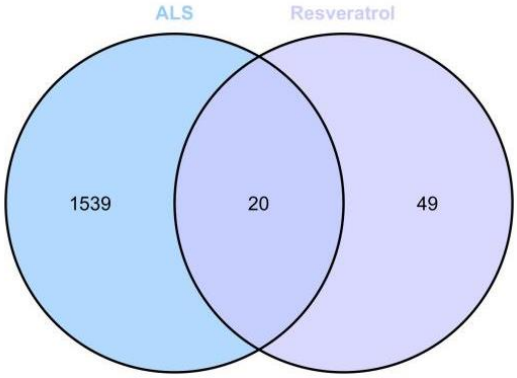

Figure 1: Wayne diagram of potential targets for resveratrol intervention in amyotrophic lateral sclerosis

3.2. Constructing a resveratrol-targeted PPI network for ALS treatment

Forty-six common targets of resveratrol and ALS were imported into the STRING database to obtain the PPI network relationships. The PPI network consisted of 62 edges (concentric circles in

descending order of relevance in the figure) in 19 nodes (circles). The topology analysis was performed in cytoscape 3.9.1 software, and the Degree values were sorted according to the network topology analysis, and the top 5 Degree values of EGFR, PTGS2, SRC, MMP9, and IGF1R were selected as the core targets for in-depth study and analysis, which are shown in Fig. 2 for details, with the color reflecting the strength of Degree relationship of the network topology analysis.

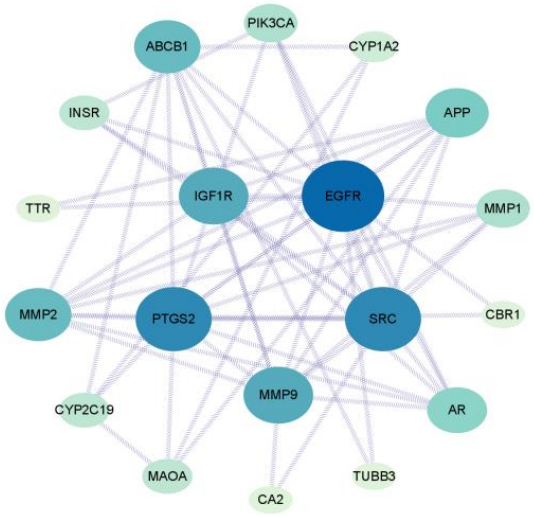

Figure 2: Interaction network diagram of potential target proteins for resveratrol intervention in amyotrophic lateral sclerosis

3.3. GO and KEGG enrichment analysis

Further GO functional analysis and KEGG pathway enrichment analysis were performed to elucidate the biological effects of resveratrol labeling and the signaling pathways associated with ALS treatment. The top 10 GO items were selected based on the P value, as detailed in Figure 3. Similarly, the top 20 KEGG pathway items were selected based on the P value and analyzed by category, as detailed in Figure 4.

GO enrichment analysis mainly involved biological processes such as positive regulation of smooth muscle cell proliferation, cell response to organic nitrogen compounds and Inorganic nitrogen compounds, positive regulation of phosphorylation and the signaling pathway of transmembrane receptor protein tyrosine kinase. And GO enrichment analysis mainly involved cellular components such as membrane raft, membrane microdomain, plasma membrane raft, neuronal cell body, neuronal cell body and so on. For molecular functions, it mainly involved hormone binding growth hormone binding protein, insulin receptor substrate, protein kinase activity, protein tyrosine kinase activity and so on.

KEGG enrichment analysis yielded a total of 60 pathways, which were mainly enriched in MAPK signaling pathway, PI3K-Akt signaling pathway, Pathways of neurodegeneration - multiple diseases, Leukocyte Pathways of neurodegeneration - multiple diseases, Leukocyte transendothelial migration and other pathways.

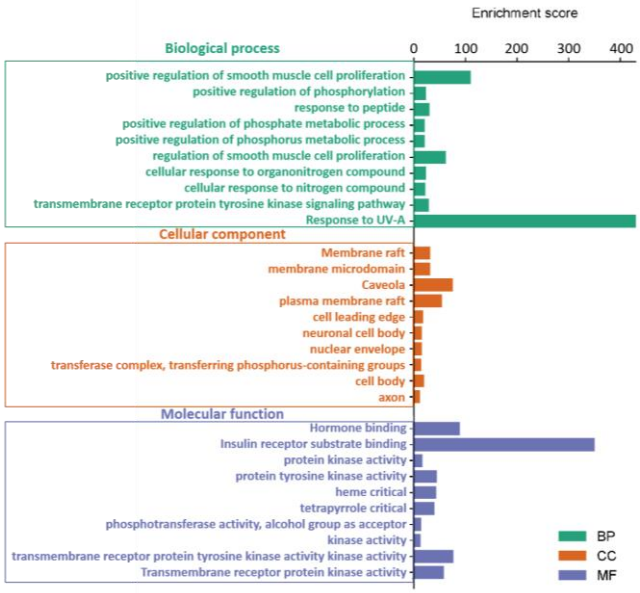

Figure 3: GO enrichment analysis

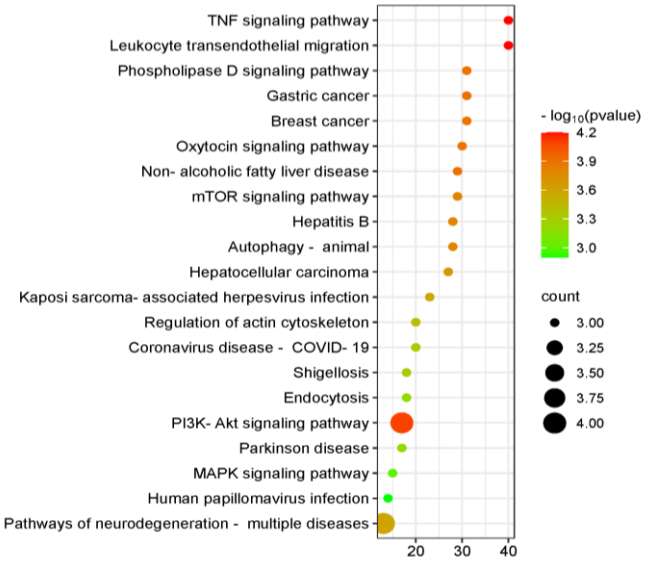

Figure 4: KEGG enrichment analysis

4. Analysis of molecular docking results

The interaction between PPIs and core proteins in the component-target-pathway network was verified by molecular docking simulations. In this study, resveratrol was used as the research vehicle, and the first five key targets of EGFR, PTGS2, SRC, MMP9, and IGF1R were used as the research targets for molecular docking. It is generally accepted that the lower the energy at which the ligand-receptor binding conformation is stabilized, the greater the likelihood of action occurring. A binding energy of less than 0 indicates that the ligand and receptor can bind spontaneously, and a binding energy of less than -5 kJ-mol-1 indicates that the ligand and receptor are tightly bound. The results showed that the binding energies of the key target and the active ingredient were all less than -5 k J-mol-1 (Table 1), indicating that the key target and the active ingredient have strong binding activity. See Figure 5 for details.

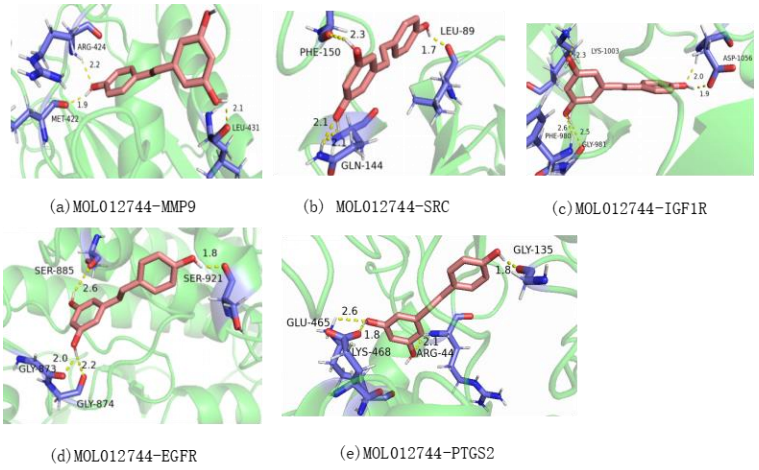

Figure 5: Molecular docking results

Table 1: Molecular docking energies

Binding energy/(kJ.mol-1)	EGFR	PTGS2	SRC	MMP9	IGF1R
MOL012744	-5. 15	-6. 97	-5. 30	-6. 23	-6. 07

5. Discussion

Amyotrophic lateral sclerosis, also known as motor neuron disease, is a devastating neurodegenerative disease in which degeneration of upper motor neurons in the motor cortex and lower motor neurons in the brainstem and spinal cord results in progressive denervation of random muscles. ALS occurs globally, with an incidence of approximately 2/100, 000 person-years and a prevalence of 6-9/100, 000 [27, 28], with a lifetime risk of approximately 1/350 [29]. Currently, there is no clinical cure for ALS, and only palliative care as well as symptomatic treatment is available. The only therapeutic agents approved for ALS treatment are the glutamate receptor antagonist riluzole and the free radical scavenger edaravone. Clinical trials have shown that riluzole extends median survival from 11.8 months to 14.8 months, delaying the use of alternative methods such as tracheotomy and mechanical ventilation. Edaravone modestly slowed the rate of disease progression and prolonged tracheotomy-free survival in ALS patients. Therefore, there is a need to aggressively explore additional drugs that are effective in the treatment of amyotrophic lateral sclerosis.

Previous studies have shown that resveratrol delays the onset of ALS, improves survival and preserves spinal motor neuron function [18, 19]. Resveratrol treatment upregulates factors associated with mitochondrial biogenesis, thereby improving the altered energy metabolism observed in ALS [30]. Resveratrol treatment also reduces mitochondrial damage and ROS production. In this regard, resveratrol prevents mitochondrial rupture by activating PGC1a mediated by the interaction between resveratrol and Silent Information Regulator 2-related enzyme 1 (sirtuin1 or SIRT1). Activation of SIRT1 by resveratrol reduces the toxic effects of misfolded SOD1 aggregate accumulation [31-34]. Many studies have reported neurotoxic properties in ALS patients with CSF and that this neurotoxicity is attributed to high glutamate concentrations in ALS/CSF, but resveratrol showed neuroprotection against glutamate toxicity in neuronal cultures and prevented the accumulation of high calcium concentrations [35].

In this study, by combining network pharmacology with molecular docking connection, we revealed several potential molecular mechanisms and possible therapeutic targets for amyotrophic lateral sclerosis. By integrating and organizing information from multiple databases, we identified 20 potential resveratrol targets involved in the pathology of ALS, and our results indicate that the

mechanism of therapeutic effect is multi-component, multi-target, and multi-pathway, which lays the foundation for subsequent in-depth study of the mechanism of resveratrol in the treatment of ALS.

In this study, we concluded that EGFR, PTGS2, SRC, MMP9, and IGF1R are potential core targets of resveratrol for the treatment of ALS.

Previous studies have shown that estrogen can inhibit the MAPK/ERK signaling pathway by reducing COX-2 encoded by PTGS2, thereby avoiding neurotoxic damage [36, 37]. This suggests that binding of estrogen analogs, soy sterol and sterol, to PTGS2 may negatively regulate PTGS2 expression, leading to reduced COX-2 levels and thus neuroprotection.

Recent studies have shown that SRC can regulate neurotransmitter release by phosphorylating synaptic vesicle proteins. In addition, it modulates protein phosphorylation to increase presynaptic Ca2+ channel activity, which promotes glutamate release and causes damage to neurons. Some researchers have found that Src/c-Abl inhibitors increase the survival of ALS iPSC-derived motor neurons, and that elimination of small interfering RNA (siRNA) for SRC or c-Abl will prevent motor neuron degeneration [38,39].

The results of KEGG enrichment analysis mainly include various pathways such as MAPK signaling pathway, PI3K-Akt signaling pathway, Pathways of neurodegeneration - multiple diseases. Therefore, it can be speculated that these genes mainly affect the MAPK signaling pathway, PI3K-Akt signaling pathway, etc. The PI3K-Akt signaling pathway regulates a variety of basic cellular functions involving proliferation, growth, survival, transcription and translation. Active Akt inhibits excessive autophagy activation and phosphorylates threonine from AKT via PDK1, thereby protecting neurons from death and promoting cell survival 0, 41]. MAPK signaling pathway mainly consists of 38 pathways including p3, ERK and JNK, which are involved in various cellular functions such as cell proliferation, differentiation and migration. Active MAPK can phosphorylate related proteins and regulate the expression of inflammatory factors that cause oxidative stress. Inhibition of the MAPK signaling pathway helps to alleviate inflammation and apoptosis and protect neurons [42-44]. Combining the results of enrichment analysis and the pathogenesis of ALS, the present study suggests that the estrogen receptor-related pathway is the main pathway for the treatment of ALS.

6. Conclusions

In this research, the molecular mechanisms of resveratrol treatment of amyotrophic lateral sclerosis were comprehensively analyzed by network pharmacology and molecular docking, and the diversity of related genes and pathways suggests that the mechanism of resveratrol treatment of ALS is multifactorial and multipathway. The strong association between resveratrol and MAPK signaling pathway, PI3K-Akt signaling pathway, as well as Fluid shear stress and atherosclerosis, and Lipid and atherosclerosis pathways, further confirms that resveratrol has anti-inflammatory and lipid metabolism regulation functions. This study provides a theoretical basis for new drug development and clinical application of the above targets.

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