

Mechanism of licochalcone A in the treatment of Alzheimer's disease based on network pharmacology

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Abstract. Objective: To systematically analyze the potential therapeutic mechanism of licochalcone A against Alzheimer's disease by network pharmacology. Methods: The Comparative Toxicogenomics Database (CTD) and GeneCards database were screened, and Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were performed on the overlapping genes of Alzheimer's disease and licochalcone A, followed by Protein-Protein Interaction (PPI) analysis and visualization. Results: A total of 64 potential overlapping targets between licochalcone A and Alzheimer's disease were identified, among which CASP3, AKT1 and BCL2 were core targets with high degree values. The annotated genes were enriched in signal transduction, metabolism and immune system. Conclusion: Licochalcone A may exert anti-Alzheimer's disease effects by targeting and regulating inflammatory response, participating in biological processes such as signal transduction and energy metabolism, which provides a theoretical basis for the development of multi-target drugs for Alzheimer's disease.

Keywords: Licochalcone A, Alzheimer's disease, network pharmacology

1. Introduction

Alzheimer's Disease (AD) is a neurodegenerative disease of the central nervous system characterized by progressive memory impairment, cognitive dysfunction, language impairment and other neuropsychiatric symptoms. At present, more than 50 million people worldwide suffer from AD, and the number is estimated to triple by 2050 [1]. The rapid aging of China's population also poses a major challenge, with an estimated 30.03 million AD patients in China by 2050 [2]. Traditional therapeutic drugs for AD mostly focus on A β clearance or cholinesterase inhibition, and single-target drugs (such as Donepezil) often fail to sustain efficacy in the treatment of AD due to their inability to cover multiple mechanisms of the disease [3]. Existing studies have shown that different drugs can simultaneously promote disease progression through inflammation, oxidative stress, metabolic disorders and synaptic dysfunction [4, 5]. Therefore, it is urgent and important to discover and study novel multi-target drugs for the treatment of Alzheimer's disease.

Licochalcone A (Lico A) is a natural highly active flavonoid extracted from licorice roots, which usually exists in plants in glycoside or free form [6]. Its unique structural properties enable licochalcone A to exert anti-inflammatory, antioxidant [7] and neuroprotective effects [8]. At present, the Flavor and Extract Manufacturers Association of the United States has confirmed its safety [9], and it is widely used in pharmaceuticals and dietary supplements due to its stability and effectiveness. Studies have explored the application effects of licochalcone A in other disease models [8, 10], but its potential application in the treatment of Alzheimer's disease has not been fully explored. In this study, GO enrichment analysis and KEGG pathway analysis were performed on genes related to Alzheimer's disease and licochalcone A using the Comparative Toxicogenomics Database (CTD), GeneCards database and STRING database, and key genes with potential interactions between Alzheimer's disease and licochalcone A were screened. Bioinformatics analysis was used to reveal the potential mechanism of licochalcone A in Alzheimer's disease at the molecular level, providing a new theoretical basis for the research of intervention and therapeutic drugs for Alzheimer's disease, helping to discover new molecular markers, and offering a new research perspective for the intervention and treatment strategies of Alzheimer's disease.

2. Materials and methods

2.1. Data source and screening

"Licochalcone A" was entered under the "Chemicals" entry and "Alzheimer's disease" was searched under the "Diseases" entry in the CTD database (<https://ctdbase.org/>) to obtain their respective gene sets; "Alzheimer's disease" and "Licochalcone A" were separately searched in the GeneCards database (<https://www.genecards.org/>) to obtain their respective gene sets. The intersections of corresponding genes in the two databases were selected, and matching screening was performed only based on gene names without setting specific thresholds.

2.2. Data analysis

2.2.1. Construction of venn diagram

The gene sets of licochalcone A and Alzheimer's disease were imported into R Studio (R 4.3.1), and the genes in the two datasets were selected to draw a Venn diagram using VennDiagram.

2.2.2. Construction of PPI network

The screened gene set was imported into the STRING database (<https://cn.string-db.org/>), with the species selected as "homo sapiens", to analyze and construct a Protein-Protein Interaction (PPI) network. The data were imported into Cytoscape 3.8 for degree value sorting, and a topological graph was drawn.

2.2.3. GO enrichment analysis and KEGG pathway analysis

The screened overlapping genes were input into the CTD database for analysis to obtain the sets of GO analysis and pathways. The gene set was imported into R Studio (R 4.3.1), and data processing was performed using dplyr. The "Biological Process", "Cellular Component" and "Molecular Function" sections were extracted by grouping according to "Ontology", and the last ten items of each group were selected after sorting by gene ratio from small to large. GO plots and KEGG pathway dot plots were drawn using ggplot2.

2.2.4. Statistical methods

Statistical analysis was performed based on the built-in stats package of R, and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Gene intersection analysis

The Venn diagram showed the overlapping range between Alzheimer's disease and licochalcone A, and a total of 64 common genes were found (Figure 1A). By integrating the overlapping genes of licochalcone A and Alzheimer's disease, a PPI network was constructed in the STRING database (Figure 1B). The data were imported into Cytoscape 3.9 software and its Network Analyzer (NCA) plug-in to optimize the PPI network, and the interaction pattern between these proteins was constructed (Figure 1C).

The results of Subgraph sorting showed that Caspase-3 (CASP3), B-cell lymphoma 2 (BCL2) and AKT serine/threonine kinase 1 (AKT1) were highlighted as core nodes in the PPI network, and functioned together with genes such as Tumor Necrosis Factor (TNF) and interleukin 1 beta (IL-1 β). The sorting indicated that licochalcone A may play an important role in the response of apoptosis regulatory proteins, inflammation regulation and cell survival signaling proteins in the onset of Alzheimer's disease. Based on the results of PPI network construction and analysis, we believe that the common genes of licochalcone A and Alzheimer's disease not only play a regulatory role in the development of the disease, but also their encoded proteins may become potential targets for the treatment of Alzheimer's disease.

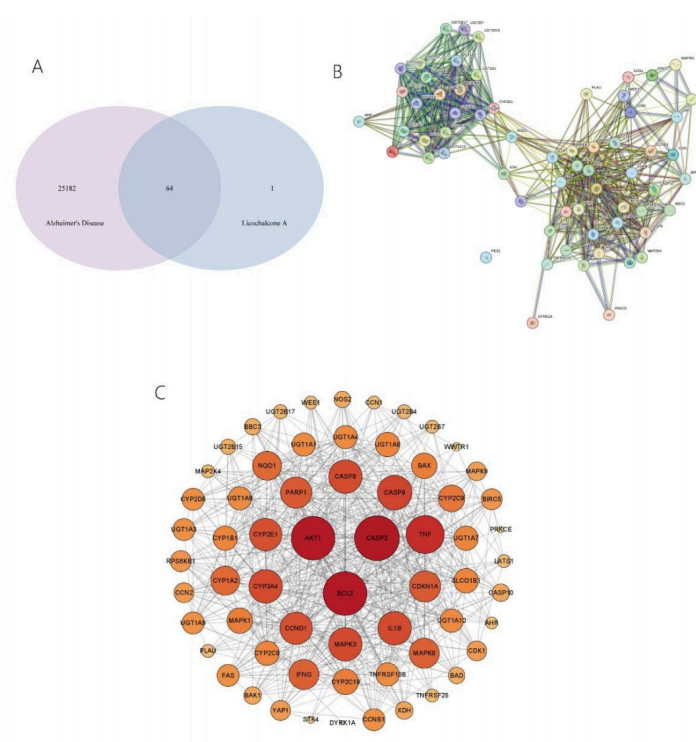


Figure 1. Venn diagram of genes between licochalcone A and Alzheimer's disease (A); PPI network (B); key targets of Alzheimer's disease and licochalcone A (C)

3.2. Results of GO and KEGG analysis

After exporting the analysis results of GO enrichment and biological pathways from CTD, we visualized the data using R 4.3.1 software. The GO plot (Figure 2A) intuitively showed the enrichment degree of key biological processes, cellular components and molecular functions related to licochalcone A and Alzheimer's disease. Cellular component analysis highlighted the significant enrichment of "Membrane", "Cytoplasm" and

"organelle", suggesting that cell membrane proteins, cytoplasmic signaling molecules and organelle functions are key links in the pathogenesis of Alzheimer's disease. Licochalcone A may maintain the stability of neuronal membrane and intracellular signal transduction by acting on membrane receptors and cytoplasmic signaling pathways. Molecular function analysis further revealed that the functions of "Ion Binding" and "Protein Binding" play a crucial role in the regulation of cell signal transduction, and functions such as catalytic activity and heterocyclic compound binding are also enriched to a certain extent. These results collectively indicate that licochalcone A may regulate intracellular signal transduction, ion homeostasis and enzymatic catalytic activity by binding to molecules such as ions and proteins, thereby interfering with Alzheimer's disease-related molecular pathways, maintaining the normal physiological functions of neurons and exerting neuroprotective effects.

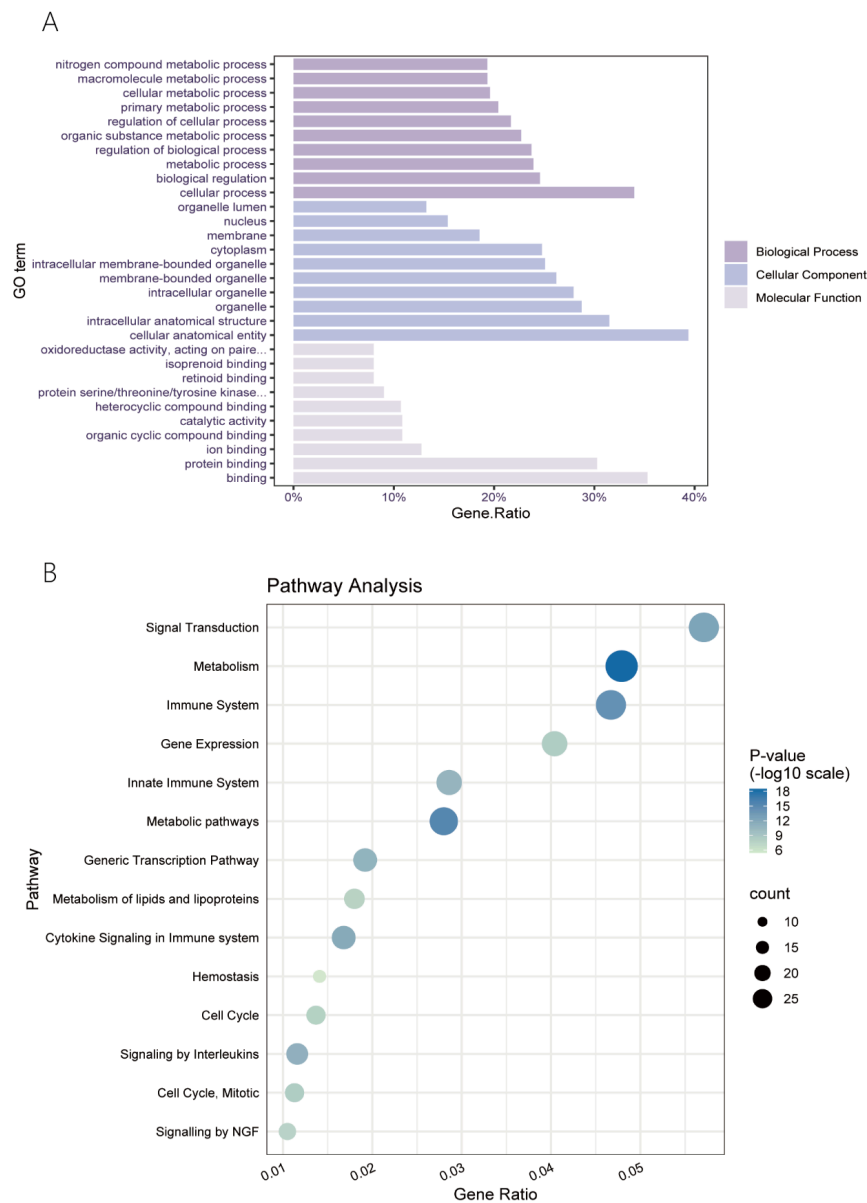


Figure 2. GO functional analysis (A) and KEGG pathway analysis (B)

In the bubble plot drawn by KEGG analysis (Figure 2B), the significant enrichment of 27 annotated genes in Metabolism confirmed the core position of metabolic regulation in the disease state, among which sub-pathways such as Metabolism of lipids and lipoproteins and Metabolic pathways were significantly enriched, suggesting disorders of nervous system energy homeostasis, lipid metabolism and metabolite balance in Alzheimer's disease. Licochalcone A may improve neuronal metabolic abnormalities and delay disease progression by regulating core metabolic pathways. The Signal Transduction pathway contained 23 annotated genes, covering sub-pathways such as Signaling by Interleukins and Signalling by NGF, involving multiple biological processes such as neuronal excitability regulation, cell proliferation and differentiation, and inflammatory factor secretion, reflecting the activity of intercellular signal transduction in the disease state, suggesting that licochalcone A may regulate neuronal function and immune cell activation by interfering with multiple signal transduction pathways. The Immune System pathway was enriched with 23 annotated genes, including sub-pathways such as Innate Immune System and Cytokine Signaling in Immune system, reflecting the activation state of the immune system in the pathological process of Alzheimer's disease, indicating the potential pathological mechanism of licochalcone A in interfering with disease immunity by regulating immune response and inhibiting excessive inflammation.

4. Discussion

With the rapid aging of China's population, the prevention and treatment of Alzheimer's disease are facing major challenges. Alzheimer's disease is a neurodegenerative disease characterized by progressive cognitive decline, β -amyloid ($A\beta$) deposition in the brain and hyperphosphorylation of tau protein, and its pathogenesis involves multiple links such as neuroinflammation, apoptosis, oxidative stress and energy metabolism disorders. The results showed that the degree values of CASP3, AKT1 and BCL2 were significantly higher than those of other targets, which were the core targets of licochalcone A against Alzheimer's disease. CASP3 is a key execution factor of the apoptosis pathway, and its overactivation leads to massive neuronal apoptosis. Studies have shown that the expression of activated CASP3 is significantly increased in neurons of the hippocampus and cortex in APP/PS1 mutant mice, which is positively correlated with the degree of neuronal apoptosis [11]. Licochalcone A may effectively inhibit the activation of CASP3 by targeted regulation, thereby reducing neuronal apoptosis and alleviating brain nerve cell damage. As a core kinase of the phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) signaling pathway, AKT1 plays a key role in the regulation of cell proliferation, apoptosis, metabolism and inflammation. Abnormal expression or altered phosphorylation level of AKT1 leads to excessive $A\beta$ deposition, aggravated tau protein phosphorylation and activation of neuroinflammation [12]. Licochalcone A may activate the AKT1 signaling pathway to reduce $A\beta$ deposition and inhibit neuroinflammation induced by Alzheimer's disease. BCL2 is an anti-apoptotic gene that regulates the permeability of the mitochondrial outer membrane and blocks the release of apoptotic factors such as cytochrome C, thereby inhibiting neuronal apoptosis. Overexpression of BCL2 can inhibit $A\beta$ -induced cell death and exert neuronal protective functions [13]. Studies have also shown that overexpression of BCL2 in AD model mice can reduce $A\beta$ deposition and improve AD symptoms [14]. The discovery of the three core targets suggests that licochalcone A may exert intervention and therapeutic effects on Alzheimer's disease by regulating key pathological processes such as neuronal apoptosis and inflammatory response.

The results of GO enrichment analysis and KEGG pathway analysis showed that the overlapping genes of licochalcone A and Alzheimer's disease were mainly enriched in biological processes related to signal transduction, metabolism and immune system. Abnormal activation of the immune system, namely neuroinflammation, is one of the core mechanisms of Alzheimer's disease pathogenesis. Excessive activation

of microglia in the brain is the main cause of neuroinflammation, and after activation, microglia release a large number of pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6), aggravating neuronal damage and A β deposition, forming a vicious cycle of "inflammation-damage" [15]. The high enrichment of annotated genes in the immune system confirmed that the main mechanism of licochalcone A against AD is the regulation of inflammatory response. Therefore, it is speculated that licochalcone A may inhibit the excessive activation of microglia by targeting immune-related genes, interrupt the inflammatory response in AD pathology, and thus reduce neuronal damage and A β deposition. In addition, the enrichment results of signal transduction and metabolic processes suggest that licochalcone A can also exert anti-Alzheimer's disease effects by regulating intracerebral neural signal transduction pathways and improving neuronal energy metabolism disorders. Studies have shown that the glucose utilization rate of the cerebral cortex is decreased in A β 42 mice and AD patients, which is related to the insufficient transport of GLUT1 to the plasma membrane in brain parenchymal cells [16]. Licochalcone A can restore energy homeostasis by activating adenosine monophosphate-activated protein kinase and inhibiting mammalian target of rapamycin complex 1 in other diseases [17]. Licochalcone A may target and regulate metabolism-related targets to alleviate energy metabolism disorders and provide energy support for the normal physiological activities of nerve cells.

The regulation of inflammatory response in Alzheimer's disease involves the interaction of multiple signaling pathways, such as PI3K/AKT, MAPK, NF- κ B, etc. Among them, AKT1, the core target in this study, as the core target of the PI3K/AKT pathway, can significantly inhibit the nuclear translocation of the NF- κ B pathway [18], reduce the release of pro-inflammatory cytokines, up-regulate BCL2 expression and inhibit CASP3 activation [19], achieving the dual effect of "anti-inflammatory and anti-apoptotic". Licochalcone A may activate the PI3K/AKT signaling pathway by targeting core targets such as AKT1, and block neuronal apoptosis at the same time, so as to block multiple links of the "inflammation-damage-apoptosis" chain in the pathological process of AD, and realize the synergistic regulation of inflammatory response and neuronal apoptosis in Alzheimer's disease.

Meanwhile, this study has certain limitations. As a predictive study of network pharmacology, all results are based on the information analysis of public databases and have not been verified by *in vitro* and *in vivo* experiments. Whether licochalcone A directly regulates the related targets or is the result of the co-regulation of multiple targets still needs to be confirmed by experimental research. In the process of target screening, only CTD and GeneCards databases were selected, and traditional Chinese medicine target databases such as TCMSP and SwissTargetPrediction were not combined, which may lead to the omission of some potential targets. The *in vivo* metabolism of licochalcone A was not considered in this study; it may undergo metabolic transformation after entering the body, and the resulting metabolites may also exert anti-Alzheimer's disease effects. In view of the above limitations, subsequent studies can be improved from the following aspects: combine *in vitro* cell experiments and animal *in vivo* experiments to verify the regulatory effect of licochalcone A on core targets and inflammatory pathways, as well as the improvement effect on pathological characteristics and cognitive function of Alzheimer's disease; integrate multiple databases for target screening to expand the scope of target screening and improve the comprehensiveness of results; combine pharmacokinetic studies to clarify the *in vivo* metabolic characteristics and active metabolites of licochalcone A, and improve its molecular mechanism against Alzheimer's disease.

5. Conclusion

In conclusion, this study identified 64 potential overlapping targets of licochalcone A in the treatment of Alzheimer's disease by network pharmacology, confirmed that CASP3, AKT1 and BCL2 are core targets, and proved that it exerts anti-AD effects mainly by regulating inflammatory response, participating in biological processes such as signal transduction and energy metabolism, which provides a theoretical basis for the development of licochalcone A as a multi-target therapeutic drug for Alzheimer's disease.

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