

Potential Mechanism of Andrographolide in Coronary Heart Disease Treatment Based on Network Pharmacology and Molecular Docking

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Abstract: The potential mechanism of andrographolide in the treatment of coronary heart disease was explored through network pharmacology and molecular docking. The potential targets of andrographolide were predicted by the PharmMapper platform, and the related targets of coronary heart disease were searched using GeneCard, OMIM, and other websites. The intersection of the two was obtained to determine the common targets of andrographolide and coronary heart disease, and the common targets were analyzed. Finally, 10 core targets of andrographolide in the treatment of coronary heart disease were obtained. The PDB database was accessed to obtain the core target structure and molecular docking was performed between the core target protein structure and andrographolide structure. The structure with the largest absolute affinity was further analyzed. A total of 117 proteins were obtained as potential targets for andrographolide in the treatment of coronary heart disease. Further analysis showed that 10 proteins (IGF1, ESR1, HSP90AA1, SRC, MAPK1, MAPK8, MAPK14, PIK3R1, PTPN11 and EGFR) were obtained as core targets. After molecular docking, the above targets had high affinity for andrographolide, and andrographolide could bind to the above coronary heart disease-related proteins. Andrographolide can treat coronary heart disease through a variety of ways. This experiment verifies the potential mechanism of andrographolide in the treatment of coronary heart disease, but it still needs long-term animal and clinical trials to further verify the accuracy of this experiment.

Keywords: Coronary heart disease; Andrographolide; Network pharmacology; Molecular docking; Traditional Chinese Medicine System Pharmacology

1. Introduction

Coronary heart disease (CHD) is a common disease that seriously endangers human health. According to American Heart Association data from 2019, about 2396 people die every day from cardiovascular diseases. [1] It is generally believed that coronary atherosclerosis is closely related to an inflammatory response. [2] Andrographolide, the main component of *Andrographis paniculata*, is a type of labdane diterpenoid, with the molecular formula of C₂₀H₃₀O₅. Andrographolide has anti-inflammatory, [3] anti-platelet aggregation, [4] improving vascular remodeling [5] and anti-tumor [6] effects, which can be used for antiviral infection [7] and antibacterial treatment. [8] Andrographolide is commonly used in the treatment of diseases such as upper respiratory tract infection and bacillary dysentery as an effective component of traditional Chinese medicine. Since *A. paniculata* is a natural compound with anti-inflammatory effects, it is speculated that it also has a potential anti-atherosclerosis effect. Andrographolide has been shown to inhibit inflammatory cell infiltration by down-regulating the expression of cytokines and integrins, thereby delaying the progression of abdominal aortic aneurysm. [9] In this paper, network pharmacology and molecular docking technology were used to explore the potential mechanism of andrographolide in the treatment of CHD, as well as provide new ideas and methods for the treatment CHD with traditional drugs.

2. Materials and methods

2.1. Target prediction of andrographolide

The PubChem database (<https://pubchem.ncbi.nlm.nih.gov>) was searched using “andrographolide” as the keyword to obtain information about andrographolide and download its two-dimensional structure in SDF format. The structure of andrographolide was uploaded to the PharmMapper platform (lilab-ecust.cn/pharmmapper/index.html) for target prediction,^[10] and the species was limited to Homo sapiens. The results of z' value > 0 were used as screening conditions for potentially related targets of andrographolide. Using the Retrieve/ID mapping function of the Uniprot website [<https://www.uniprot.org/>], UniProtKB AC/ID is mapped to Gene Symbol, and unverified target genes are removed in this process.^[11]

2.2. Acquisition of CHD related genes

Acquisition of CHD related genes was obtained through accessing the GeneCard (<https://www.genecards.org/>) and OMIM (<https://www.omim.org/>) databases, with “coronary heart disease” as the keyword. to greater than the median relevance score as screening conditions, CHD related genes.

2.3. Acquisition of common targets

The Venn diagram of the PharmMapper predicted target and GeneCard database related genes was drawn by the ggplot2 package of R language (version 4.0.5), and the intersection of the two was used as a common target for andrographolide and CHD (Figure2).

2.4. Enrichment analysis

In order to understand the function of common targets and the role of each target protein in the corresponding signaling pathway, the common targets of andrographolide and CHD were enriched and analyzed by the gene ontology (GO) database (<http://geneontology.org/>) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (<https://www.genome.jp/kegg>) through the Cluster Profiler package^[12] of R language.

2.5. Protein Interaction Analysis

The common targets were uploaded to the String database (<https://string-db.org/>) for online protein interaction analysis^[13] and the interaction relationship between the common targets was mined to find the core genes of andrographolide in the treatment of CHD. With interaction scores > 0.7 , the isolated proteins that do not interact with other proteins were hidden, and the results were saved and imported into Cytoscape software (version 3.9.0) for visualization. The built-in NetworkAnalyzer module of the application software was used to analyze the protein interaction network. The top 10 target proteins of degree were selected as the core targets of andrographolide in the treatment of CHD.

2.6. Molecular docking

In order to verify the accuracy of the above predicted results, the structure of andrographolide was obtained from the PubChem website, and the mechanical structure of andrographolide was optimized by Chem3D software (version 20.1.1), to ensure that its energy was minimized and the file was generated in mol2 format from the output. The structures of the top 10 core targets above were obtained by accessing the PDB database (<https://www.rcsb.org/>), and were saved as files in PDB format. The protein ligands and solutes were further removed by PyMol software (version 2.5.2). After hydrogenation of the core targets by AutoDockTools software (version 1.5.7), the optimized andrographolide structure was docked by AutoDock Vina software (version 1.2.0).^[14] The output results were analyzed to find the structure with the lowest affinity as the optimal mechanical structure for the binding of the most receptors and ligands, and the structure model after docking was output. PyMol software (version 2.5.2) was used to confirm the optimal receptor-ligand binding mode, and further uploaded to the Proteins Plus website to output the two-dimensional pattern map after docking.^[15]

3. Results

3.1. Common targets

After removing the unverified target genes, 163 potential targets of andrographolide were predicted by PharmMapper platform. By accessing GeneCard database and OMIM database, a total of 3814 related targets of CHD were searched and 117 common targets (Figure 1) were obtained as potential targets for andrographolide in the treatment of CHD.

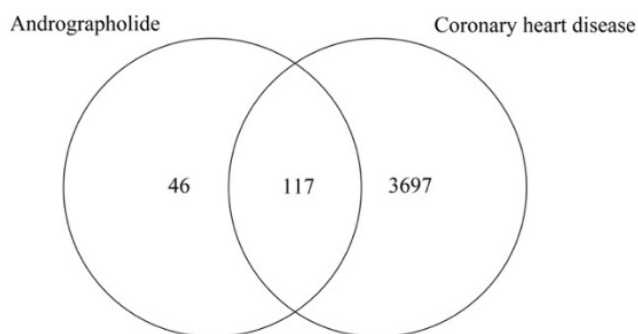


Figure 1: Common targets

3.2. Enrichment analysis results

The top 10 pathways of GO database enrichment analysis results are shown in Figure 2. In the biological process, the related targets are mainly enriched in the intracellular receptor signaling pathway, steroid metabolic process, transcription initiation from RNA polymerase II promoter, response to steroid hormone, and DNA-templated transcription initiation. On the cellular component, the related targets are enriched in dicolin-1-rich granules, dicolin-1-rich granule lumen, vesicle lumen, secretory granules lumen, and the cytoplasmic vesicle, among others. At the molecular function level, the related targets are mainly enriched in nuclear receptor activity, ligand-activated, transcription factor activity, and drug binding.

The results of the KEGG enrichment analysis are shown in Figure 3. The related targets are enriched in the progesterone-mediated oocyte maturation, chemical carcinogenesis-reactive oxygen species, chemical carcinogenesis receptor activation, metabolism of xenobiotics by cytochrome P450, lipid and atherosclerosis, Ras signaling pathway and other related pathways.

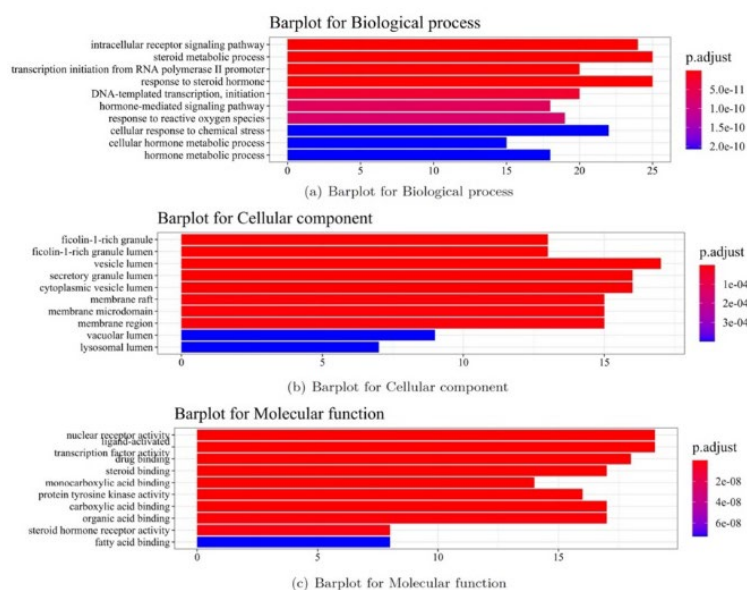


Figure 2: GO database enrichment analysis results. (a) Barplot for biological process, (b) barplot for cellular component, and (c) barplot for molecular function.

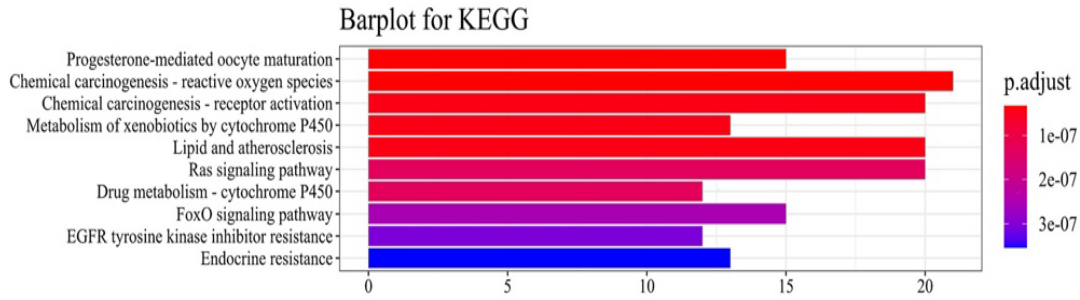


Figure 3: KEGG database enrichment analysis results

3.3. Protein Interaction Analysis

Figure 4 shows the results of the String database network analysis. Table 1 shows the parameters of the protein interaction network. Among them, the average number of adjacent nodes is 7.535, the network heterogeneity is 0.886, the network density is 0.077, and the network radius is 4. The top 10 proteins were selected as the core targets of andrographolide against CHD (Table 2).

Protein-Protein Interaction Networks

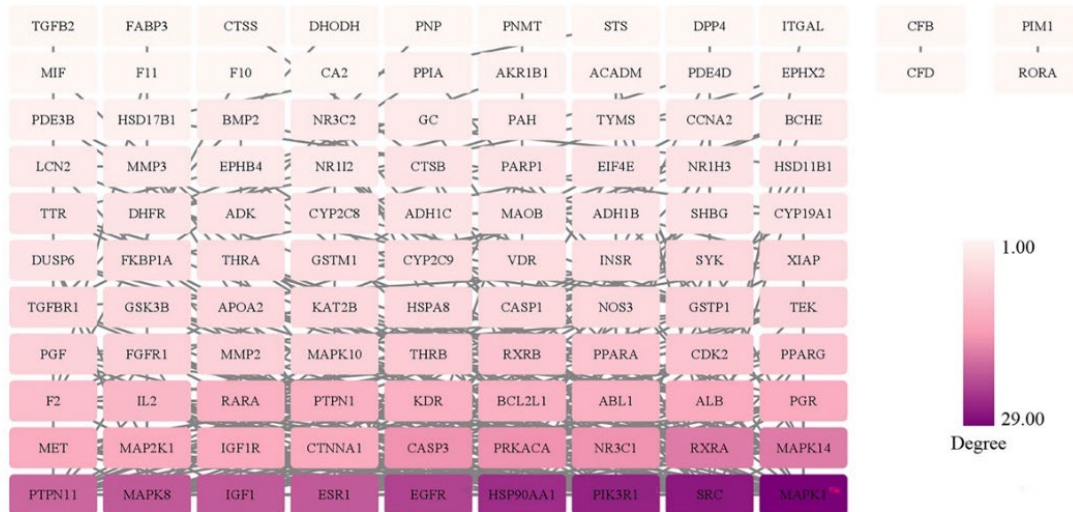


Figure 4: Protein-protein interaction network

Table 1: Protein Interaction Network Parameters

Parameters	Value
Avg. number of neighbors	7.535
Characteristic path length	3.015
Network centralization	0.224
Network density	0.077
Network heterogeneity	0.886
Network radius	4
Number of edges	375
Number of nodes	103

Table 2: Core targets

Gene Symbol	Degree	Betweenness Centrality
MAPK1	29	0.108486
SRC	27	0.118562
PIK3R1	26	0.076361
HSP90AA1	25	0.111579
EGFR	23	0.045118
MAPK8	21	0.129662
IGF1	21	0.049447
ESR1	21	0.061872
PTPN11	20	0.019196
MAPK14	18	0.023549

3.4. Molecular docking

The core targets were subjected to molecular docking with andrographolide structure by AutoDock Vina software to further verify the reliability of target prediction. The docking results are shown in Table 3, and some docking patterns are shown in Figure 5. The docking results showed that the absolute values of the affinity between andrographolide and the core targets were all greater than 5.0 kcal/mol, indicating that andrographolide had high affinity with the core targets, further indicating that andrographolide had good binding ability with the core targets and played a role in the treatment of CHD. Among them, andrographolide had the highest affinity with EGFR (-7.6kcal/mol) and PTPN11 (-7.3kcal/mol).

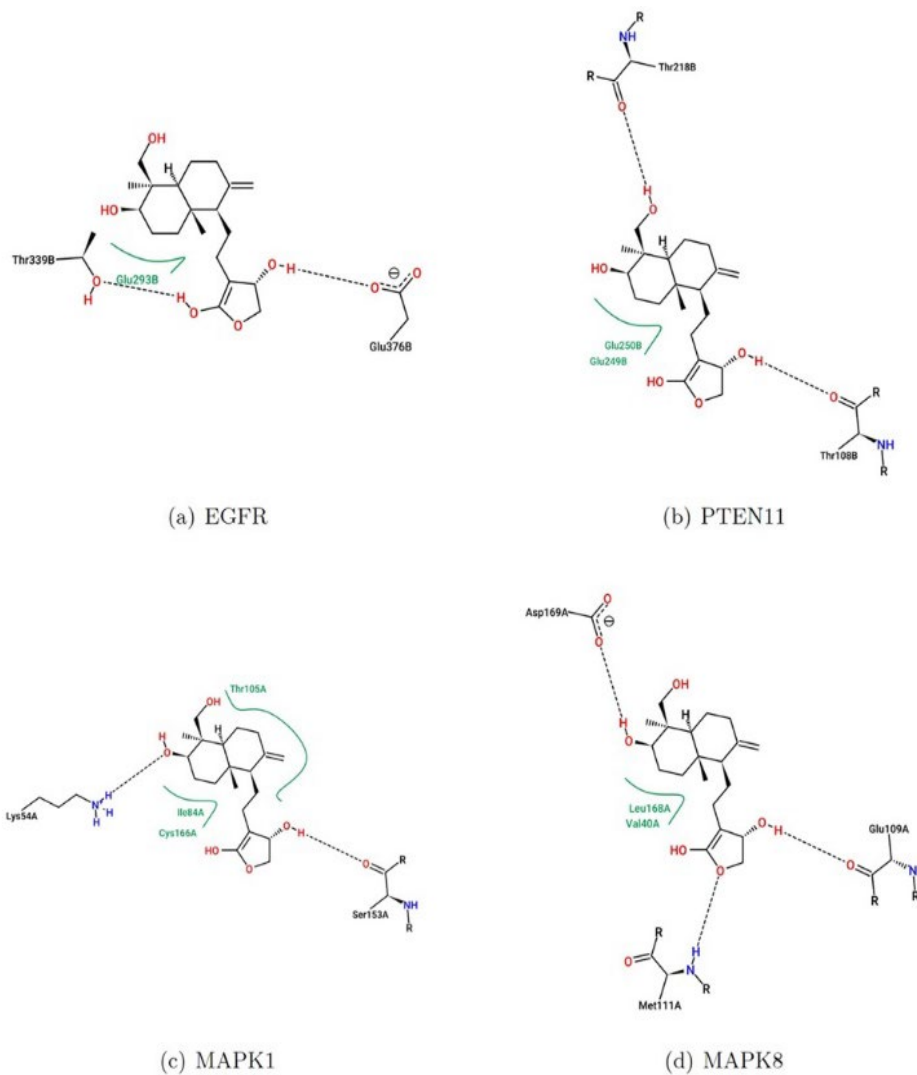


Figure 5: Patterns of andrographolide docking with EGFR, PTPN11, MAPK1 and MAPK8

Table 3: Molecular docking affinity

Gene name	Uniprot ID	Target name	Degree	Affinity
IGF1	P05019	Insulin-like growth factor I	21	-5.5
ESR1	P03372	Estrogen receptor	21	-6.3
HSP90AA1	P07900	Heat shock protein HSP 90-alpha	25	-6.5
SRC	P12931	Proto-oncogene tyrosine-protein kinase Src	27	-6.7
MAPK14	Q16539	Mitogen-activated protein kinase 14	18	-6.8
PIK3R1	P27986	Phosphatidylinositol 3-kinase regulatory subunit alpha	26	-6.8
MAPK8	P45983	Mitogen-activated protein kinase 8	21	-7.0
MAPK1	P28482	Mitogen-activated protein kinase 1	29	-7.3
PTPN11	Q06124	Tyrosine-protein phosphatase non-receptor type 11	20	-7.3
EGFR	P00533	Epidermal growth factor receptor	23	-7.6

4. Discussion

At present, the research of traditional Chinese medicine is constantly developing, and the treatment of hyperlipidemia by traditional Chinese medicine has gradually become an effective way, and the cost is low, the effect is remarkable, the curative effect is reliable, and there is broad development prospect. At present, the combined application of traditional Chinese medicine and Western medicine for lipid-lowering has been used in China, such as traditional Chinese medicine *Gastrodia elata*, *Polygonum multiflorum*, *Chuanxiong*, *Danshen*, *Pueraria lobata* and their active ingredients.^[16] Although PCSK9 inhibitors have emerged as a new class of drugs that are effective in reducing LDL-C and preventing major vascular events, they are expensive and require long-term medication, which poses a heavy financial burden on patients.

Andrographolide, the main extract of *Andrographis paniculata*, has a small toxicity. It is reported that for adult male mice, the median lethal dose is 1.46g/kg.^[17] At the same time, the results of pharmacokinetic studies suggest that andrographolide is easily absorbed by oral administration, and its oral bioavailability is $9.27 \pm 1.69\%$ and T_{max} is 0.42 ± 0.14 . After intravenous injection and oral administration, it can be quickly removed by plasma. The half-life of the drug ($t_{1/2}$) is 1.86 ± 0.21 h and 3.30 ± 0.35 h, respectively.^[18] Andrographolide can be used in patients with CHD to improve its efficacy in combination with other drugs.

Here, the potential molecular mechanism of andrographolide in the treatment of CHD was verified by network pharmacology and molecular docking technology. A total of 117 related targets of andrographolide in the treatment of CHD were obtained by target prediction combined with database searching. Through GO database enrichment analysis, we found that related targets are closely related to steroid hormone response and metabolism, RNA / DNA transcription, chemical stress and hormone metabolism. The results of the KEGG enrichment analysis showed that the related targets were closely related to CHD. Some genes were enriched in the KEGG pathway and involved in cell apoptosis, cell injury, foam cell formation and inflammatory response. Further analysis revealed that andrographolide might treat CHD related mechanisms.

The results of protein interaction analysis further suggested that IGF1, ESR1, HSP90AA1, SRC, MAPK1, MAPK8, MAPK14, PIK3R1, PTPN11, and EGFR were the core targets of andrographolide in the treatment of CHD. Among them, insulin-like growth factor 1 (IGF1) is generally believed to be involved in the occurrence and development of CHD; ^[19] IGF1 can act on potassium channel or nitric oxide synthesis pathways, so that vascular endothelial cells and smooth muscle cells release nitric oxide to promote vascular endothelial cell relaxation, thereby increasing myocardial oxygen supply in CHD. ^[20] IGF1 and heat shock protein 60 (Hsp60), as target genes of miR-1, ^[21-22] are involved in the process of ischemia-reperfusion injury and myocardial injury aggravated by infarction. Heat shock protein 90 (Hsp90) is the most abundant inducible molecular chaperone in the body; it is involved in induced cell stress response, cell cycle control and proliferation/anti-apoptosis signal. ^[23] Estrogen (ESR) has a protective effect on the cardiovascular system, which can reduce the risk of CHD in women of childbearing age. Src family protein tyrosine kinase (SFK) is widely expressed in various types of cells. Previous studies have shown that SFKs are mainly involved in cardiac signal transduction in physiological and pathological processes, and their activity is closely related to maintaining homeostasis in the cardiovascular system. SFKs are also involved in conditions such as hypertension, CHD, ischemic

heart disease, myocardial ischemia reperfusion injury, arrhythmia and cardiomyopathy.^[24] The MAPK pathway receives extracellular signal stimulation and participates in the regulation of cell growth, differentiation, migration and inflammation through cascade phosphorylation.^[25] Studies have confirmed that MAPKs accelerate the progress of chronic inflammatory diseases of atherosclerosis by promoting the formation of foam cells.^[26] Animal experimentation has demonstrated that downregulation of the PIK3R1/Akt/mTORC1 pathway attenuates pathological autophagy induced by angiotensin II, thereby reducing oxidative stress and apoptosis in cardiomyocytes.^[27] PTPN11 is reported to play a crucial role in the regulation of myocardial remodeling by cardiomyocytes.^[28] Epidermal growth factor receptor (EGFR) is the convergence point of complex signal network and cell function regulation such as cell growth, differentiation, movement, survival and death. It plays an important role in various pathophysiological processes including atherosclerosis.^[29]

According to the literature and previous experience,^[30] if the binding energy is less than -1.2kcal/mol , we believe that the docking results are feasible, and the lower the binding energy between the receptor and the ligand, the more stable the binding conformation is, and the more likely the ligand and the receptor are to react. Molecular docking results show that andrographolide has good affinity to each receptor protein. Molecular docking results show that the absolute value of the binding energy between andrographolide and the core target is greater than 5.0kcal/mol , indicating that andrographolide has high affinity to the core target. Andrographolide and EGFR binding conformation is the most stable (-7.6kcal/mol). Molecular docking results suggest that this stable binding between andrographolide and the receptor protein plays a role in the treatment of CHD.

In recent years, breakthroughs have been made in the development of new lipid-lowering drugs with different mechanisms of action. Combined lipid-lowering therapy based on statins can achieve ideal cholesterol management and bring about a significant reduction in the risk of ASCVD and even all-cause mortality. Combined lipid-lowering therapy has gradually become a major trend in the management and control of ASCVD risk. The combination of andrographolide and statins is of great significance for the long-term management of patients with ASCVD.

The limitation of this paper is that we only use "Coronary heart disease" as keywords to search disease-related gene databases such as genecards.org, OMIM and other websites to search for coronary heart disease-related genes, and the search results obtained are clearly targeted. As for whether andrographolide can treat other atherosclerotic diseases such as cerebral infarction, arteriosclerotic obliterans, abdominal aortic aneurysm, etc., our research group has included follow-up research; andrographolide, as the main component of traditional anti-inflammatory Chinese medicine, has potential Therapeutic value, and after database analysis, it is found that the potential targets of andrographolide in the treatment of coronary heart disease and the targets of diseases such as cerebral infarction overlap to a certain extent. We believe that andrographolide has the potential to treat arteriosclerotic diseases, which is also the goal of our research group's follow-up research.

5. Conclusions

This paper verifies the potential mechanism of andrographolide in the treatment of CHD, and provides ideas for drug treatment of CHD and improvement of long-term prognosis of patients with CHD. However, long-term animal and clinical trials are still needed to further verify the accuracy of molecular docking results.

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