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Exploring the mechanism of astragalus membranaceus in the treatment of multiple system atrophy based on network pharmacology and molecular docking

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Abstract

Multiple system atrophy (MSA) is a fatal neurodegenerative disease, it causes functional degradation of multiple organs and systems throughout the body. Astragalus membranaceus (AM), a well-known traditional Chinese medicine, has been used to improve muscle wasting-related disorders for a long history. In this study, we used network pharmacology and molecular docking to predict the mechanism underlying AM for the treatment of MSA. We screened the active compounds of AM and its related targets, as well as the target proteins of MSA. We made a Venn diagram to obtain the intersecting targets and then constructed a protein-protein interaction network to find the core targets and build an active ingredient-target network map. After subjecting the intersecting targets to gene ontology and Kyoto encyclopedia of genes and genomes analysis, the binding ability of core compounds and core target proteins were validated by molecular docking. A total of 20 eligible compounds and 274 intersecting targets were obtained. The core components of treatment are quercetin, kaempferol, and isorhamnetin, and the core targets are TP53, RELA, and TNF. The main biological processes are related to cellular responses and regulation. Molecular functions are mainly associated with apoptosis, inflammation, and tumorigenesis. Molecular docking results show good and standard binding abilities. This study illustrates that AM treats MSA through multiple targets and pathways, and provides a reference for subsequent research.

Abbreviations: AM = astragalus membranaceus, BP = biological process, CC = cell composition, GO = gene ontology, KEGG = Kyoto encyclopedia of genes and genomes, MF = molecular function, MSA= multiple system atrophy, PPI = protein-protein interaction, TCMSP = traditional Chinese medicine systems pharmacology database.

Keywords: astragalus membranaceus (AM), molecular docking, multiple system atrophy, network pharmacology

1. Introduction

Multiple system atrophy(MSA) is an adult-attack, fatal neurodegenerative disease, with clinical features of progressive autonomic function failure, Parkinsonian features and assorted cerebellar and pyramidal characters.[1] According to the dominant characteristic symptoms, it is classified into parkinsonian and cerebellar subtypes.[2] The median survival of MSA is 6.2 to 7.5 years and decreases with increasing age of attack.[3,4] As the disease progresses, it can decrease the functions of the brain, cardiovascular system, respiratory system, urinary system, etc,[5] reducing the quality of life and increasing the burden on family and society.[6,7] The pathogenesis underlying MSA remains not fully understood. A loss-of-function mutation in the COQ2 gene encoding by coenzyme Q10 synthase is the only certified cause of monogenic MSA till now.[8] Neuronal and glial dysfunction contribute to MAS progression, and some scholars marked it as an oligodendrocyte neurological α-synucleinopathy.[9,10] Only symptomatic therapy is available currently, so the most urgent need is to develop a treatment option that can slow down its process.[2] Astragalus membranaceus(AM), a well-known traditional Chinese medicine, has been used to improve muscle wasting-related disorders for a long history. AM can reduce fatigue and enhance endurance to improve athletic performance,[11,12] and its components like formononetin and calycosin benefit muscle atrophy improvement and cardiovascular protection.[13,14] Network pharmacology is a method that combines laboratory

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval is not required because individual patient data and privacy were not involved in this study.

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and clinical investigations with data handling to study and clarify the mechanisms of drug actions. In this study, we utilized this approach to illuminate the underlying mechanisms of AM in MSA therapy.

2. Materials and Methods

The flowchart of this research process is displayed in Figure 1.

2.1. Screening of active pharmaceutical compounds and corresponding targets

The active compounds and targets of AM were inquired as screening criteria with oral bioavailability ≥ 30% and drug-likeness ≥ 0.18 via the traditional Chinese medicine systems pharmacology database (TCMSP, https://old.tcmsp-e.com/tcmsp.php). For the supplemented drug targets, the corresponding Canonical SMILES codes of active pharmaceutical ingredients can be queried on Pubchem(https://pubchem.ncbi.nlm.nih.gov/) through the InChIKey code and complemented by the Swiss Target Prediction(https://www.swisstargetprediction.ch/).

2.2. Acquiring of MSA-associated targets

The keyword "multiple system atrophy" was retrieved in the Online Mendelian Inheritance in Man(OMIM, https://omim.org), GeneCards(https://www.genecards.org), and Drugbank database (https://www.drugbank.ca) to obtain the MSA-associated targets.

2.3. Construction of active component–target network

All the acquired targets were normalized in UniProt (https://www.uniprot.org), with status set to "Reviewed" and species set to "Human." After eliminating duplicate targets, a Venn diagram was drawn through Venny 2.1 online software (http://jvenn.toulouse.inra.fr/app/example.html) to get the intersection targets of MSA and AM. The information on the components and targets was intuitively analyzed and shown in an active component–target network based on Cytoscape 3.9.1 software (https://cytoscape.org/). The main active compounds of AM in the treatment of MSA were predicted as per the degree value.

2.4. Construction and analysis of the protein-protein interaction (PPI) network

In order to identify the interaction of the joint genes, the intersection targets were submitted to STRING 11.0(https://string-db.org). After hiding free nodes, the results were channeled into Cytoscape 3.9.1 for the construction and analysis of PPI network. The species option was set to "Human," the minimum interaction threshold was opted to "High Confidence (≥0.9)," and other settings were default. The median and upper quartile values of degree (DC), betweenness (BC), cell composition (CC), eigenvector (EC), local average connectivity-based method (LAC), and network (NC) were used to select the core targets.

2.5. Gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis

GO and KEGG analysis of intersection targets were conducted in Metascape (https://metascape.org/), and the screening condition was $P < .01$. GO functional analysis can clarify the use of target proteins of herbal compounds in gene function, including biological process (BP), CC, and molecular function (MF). Kegg enrichment analysis captures the enriched signaling pathways in drug and disease targets. The top 10 results of biological functions and related signaling pathways were selected for visualization by Bioinformatics (https://www.bioinformatics.com.cn/).

2.6. Molecular docking

The structures of core targets were acquired from Protein Data Bank database (PDB, https://www.rcsb.org/). After being removed the water molecule by PyMol1.8 software (https://pymol.org/) and being imported into AutodockTools1.5.6 software (https://autodock.scripps.edu/) for hydrogenation, energy calculation, and atomic type setting, the structures were saved in PDBQT format. The MOL2 structures of the core components were obtained from TCMSP, and the rotatable key was set before saving in "PDBQT" format. AutodockTools software ran molecular docking, and PyMol software visualized the docking results.

3. Results

3.1. Screened active compounds and corresponding targets of AM

A total of 87 active ingredients of AM were searched in the TCMSP database. According to the filter criteria, 20 eligible ingredients were obtained. Their Mol ID, molecule name, oral bioavailability%, and drug-likeness are illustrated in Table 1. After standardization by UniProt and eliminating duplications, 325 potential targets of active ingredients were acquired.

3.2. MSA-associated targets and the intersecting targets

Eleven thousand four hundred thirty potential targets were retrieved from Drugbank, Genecards, OMIM databases after standardization and duplicates elimination. A Venn diagram was plot between the targets of drug and disease (Fig. 2), which shows 274 intersecting targets.

3.3. Network of active ingredients and targets

The screened active ingredients were entered into Cytoscape software with 274 intersecting targets to establish a network of the active ingredient-target of AM for treating MSA. In the network (Fig. 3), as the degree value increases, the target nodes become larger, and the higher the combined score the thicker the line. The top 3 components filtered by degree value are quercetin, kaempferol, and isorhamnetin.

3.4. PPI network analysis

Two hundred seventy-four intersecting targets were submitted to STRING and Cytoscape for PPI analysis. The preliminary PPI network graph contains 220 nodes and 975 edges. The upper median value of DC, BC, CC, EC, NC and LAC was filtered to get the network with 36 nodes and 179 edges, after the screening of greater than quartiles, 3 core targets were obtained, namely TP53, RELA and TNF. Figure 4 illustrates the PPI network and the screening process of the core targets.

3.5. GO analysis

Metascape was utilized for GO analysis of the 274 AM relevant targets of MSA. The top 10 items of BP, CC, and MF are visualized in Figure 5.

A total of 2337 BP items were enriched. The top 10 according to the degree value include response to inorganic substance, response to xenobiotic stimulus, cellular response to lipid, response to hormone, response to nutrient levels, regulation

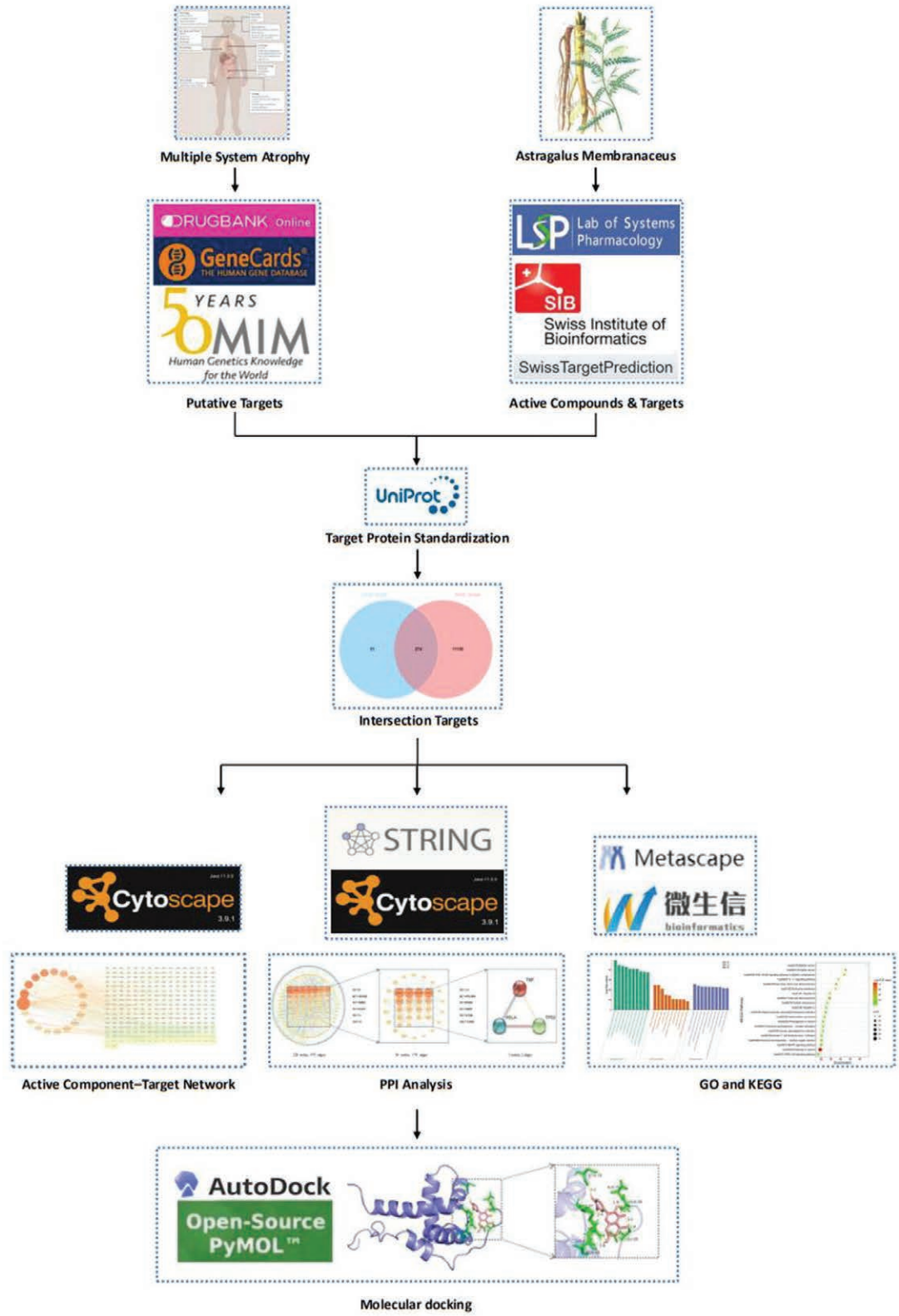

Figure 1. The flow chart of network pharmacology and molecular docking in this study.

of kinase activity, response to extracellular stimulus, cellular response to nitrogen compound, cellular response to organonitrogen compound, and positive regulation of protein phosphorylation.

A total of 160 CC items were enriched, and the results of the top 10 according to the degree value show that the genes mainly take effect in membrane raft, membrane microdomain, receptor complex, caveola, plasma membrane raft, transcription regulator complex, dendrite, dendritic tree, side of the membrane, and perinuclear region of the cytoplasm.

A total of 269 MF items were enriched. The results of the top 10 according to the degree value show that molecular functions

Table 1

Basic information on the main active ingredients of astragalus membranaceus.

Mol ID	Molecule name	OB%	DL
MOL000211	Mairin	55.38	0.78
MOL000239	Jaranol	50.83	0.29
MOL000296	Hederagenin	36.91	0.75
MOL000033	(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5-propan-2-yloctan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	36.23	0.78
MOL000354	Isorhamnetin	49.6	0.31
MOL000371	3,9-di-O-methylnissolin	53.74	0.48
MOL000374	5'-hydroxyiso-muronulatol-2',5'-di-O-glucoside	41.72	0.69
MOL000378	7-O-methylisomucronulatol	74.69	0.3
MOL000379	9,10-dimethoxypterocarpan-3-O-β-D-glucoside	36.74	0.92
MOL000380	(6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol	64.26	0.42
MOL000387	Bifendate	31.1	0.67
MOL000392	Formononetin	69.67	0.21
MOL000398	Isoflavanone	109.99	0.3
MOL000417	Calycosin	47.75	0.24
MOL000422	Kaempferol	41.88	0.24
MOL000433	FA	68.96	0.71
MOL000438	(3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol	67.67	0.26
MOL000439	Isomucronulatol-7,2'-di-O-glucosiole	49.28	0.62
MOL000442	1,7-dihydroxy-3,9-dimethoxy pterocarpene	39.05	0.48
MOL000098	Quercetin	46.43	0.28

DL = drug-likeness, OB = oral bioavailability.

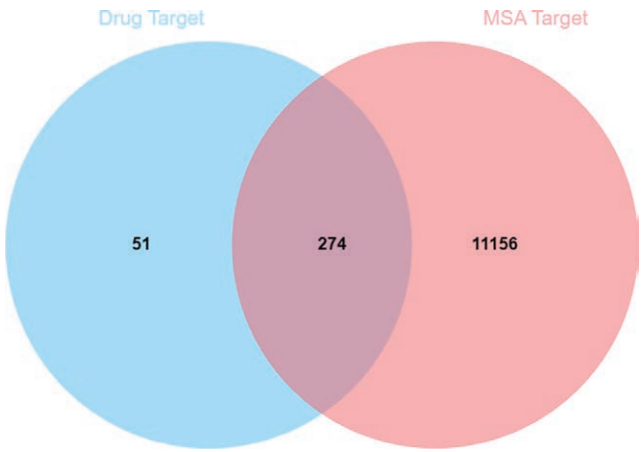

Figure 2. The Venn diagram for the targets of multiplesystem atrophy and astragalus membranaceus.

involved in the treatment mainly contain protein kinase activity, protein serine/threonine/tyrosine kinase activity, DNA-binding transcription factor binding, phosphotransferase activity, alcohol group as acceptor, transcription factor binding, kinase binding, oxidoreductase activity, RNA polymerase II-specific DNA-binding transcription factor binding, kinase activity, and protein kinase binding.

3.6. KEGG analysis

KEGG pathway enrichment analysis shows that the pharmacological process contains 160 signaling pathways. The bubble chart(Fig. 6) demonstrates the top 20 enriched pathways. The horizontal coordinate indicates the degree of enrichment, the bubble size represents the amount of enrichment, and the larger the *P* value the darker the color. The main pathways of enrichment include response to inorganic substance, response to xenobiotic stimulus, cellular response to lipid, response to hormone, response to nutrient levels, regulation of kinase activity, response to extracellular stimulus, cellular response to nitrogen compound, cellular response to organonitrogen compound, and positive regulation of protein phosphorylation.

3.7. Molecular docking

Finally, the possibility of core targets binding to AM-active compounds was verified by molecular docking. The Key targets TP53, RELA, and TNF were molecularly docked with the potent compounds of quercetin, kaempferol, and isorhamnetin, respectively. The results are shown in Table 2. We identified the binding activity as being standard, good, and strong when the binding affinity was <–4.25, <–5.0, and <–7.0 kcal/mol, respectively.[15] Molecular docking results show that TP53 and RELA have good binding ability with quercetin (docking score = –6.03; –5.79), kaempferol (docking score = –6.36; –6.39), and isorhamnetin (docking score = –6.15; –5.48). TNF has the standard binding ability with quercetin, kaempferol, and isorhamnetin (docking score = –4.96, –4.93, and –4.92). The docking results of good binding activity are visualized in Figure 7.

4. Discussion

The neurodegenerative changes caused by MSA affect the central and autonomic nerves as well as other parts of the peripheral nerves,[16] resulting in clinical manifestations of varying combinations of motor and non-motor deficits and functional degeneration in multiple systems throughout the body.[1] In traditional Chinese medicine, AM in treating chronic debilitating diseases and enhancing overall vitality has been a long time. It can play a role in immunomodulatory, anti-inflammatory, anti-oxidant, anti-viral, and so on.[17] This study explored the underlying mechanism of AM for treating MSA through network pharmacology and molecular docking.

The active ingredient-target network analysis reveals that the active compounds like quercetin, kaempferol, isorhamnetin, and

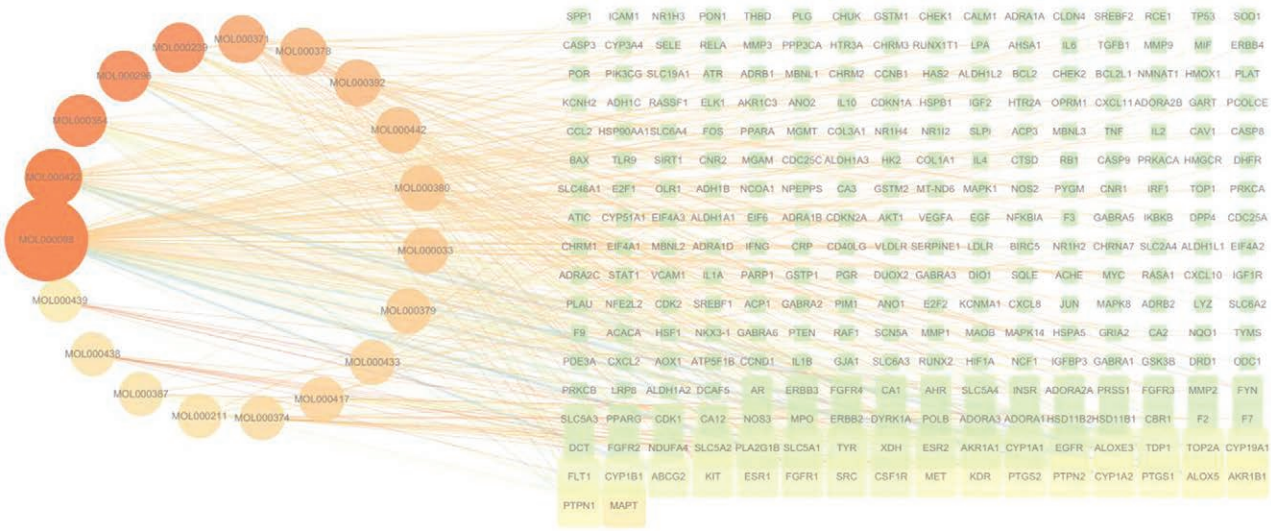

Figure 3. Active component–target network.

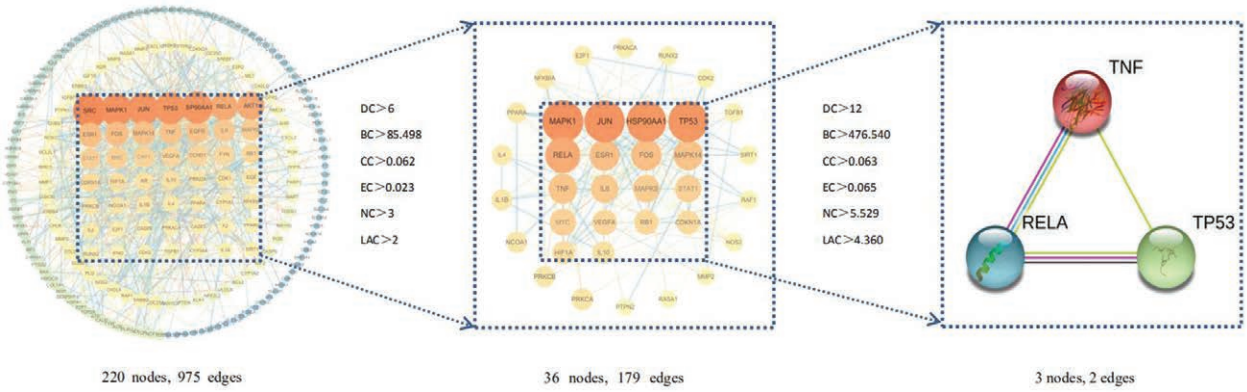

Figure 4. PPI network construction and screening. PPI = protein-protein interaction.

hederagenin in AM act on numerous targets, which implies that these ingredients may take an essential part in healing MSA and are worthy of further exploration. Quercetin can repress mitochondrion from releasing hydrogen peroxide and protect it from reduced biogenesis,[18] elevate the phosphorylation of Akt in addition to the expression of atrogenes,[19] and upregulate neuronal intrinsic growth capacity to accelerate functional recovery and postpone distal atrophy.[20] Kaempferol can reduce the accumulation of α-Syn, which is implicated in the pathogenesis of MSA, provide significant protection against α-Syn-related neurotoxicity and prevent neuronal cell death.[21] Amyloid β-protein is highly neurotoxic after precipitating and accumulating in the cell matrix, leading to neurodegenerative diseases, and destroying the blood-brain barrier. However, isorhamnetin can destroy the stability of amyloid β-protein aggregates and protect cells from Amyloid β-protein-induced cytotoxicity.[22] Isorhamnetin also improves the outgrowth of nerve growth factor-induced neurite and potentiates the antioxidant defense system, cholinergic signaling and synaptic plasticity.[23] Hederagenin stimulates autophagy, which prohibits the oligomerization of α-Syn and accelerates neurodegenerative diseases-related protein degeneration. It also has the potential for neuro-protection and improves motor degeneration.[24]

The initial PPI network has 220 targets, occupying two-thirds of the AM-related targets. SRC, MAPK1, JUN, TP53, and HSP90AA1 are the top 5 targets based on the degree value. TP53, RELA, and TNF are the targets that simultaneously satisfy the filter of upper quartiles of DC, BC, CC, EC, NC, and LAC. These proteins are involved in cellular senescence, apoptosis, regulation of immune function, inflammatory response, etc and may take an important effect in AM on treating MSA. It should be noted that SRC, MAPK1, JUN, and TP53 are all related to cancer and corresponding with the regulation of cell proliferation, oxidative stress, apoptosis, and so on. For instance, SRC as an oncogene is connected with pathways in regulating proliferation, angiogenesis, invasion, metastasis, and bone metabolism.[25] MAPK1-mediated signaling is related to cancer cell proliferation and apoptosis1,[26] and it is found that germline MAPK1 missense variants can cause neurodevelopmental disorders.[27] TP53 can induce cell cycle arrest and apoptosis,[28] and it promotes the regulation of neuronal differentiation and apoptosis.[29] Therefore, there is a need for further research on the mechanisms of the relationship between MSA and tumor-related targets, and this may provide new directions for the treatment of MSA. HSP90AA1 along with another target gene named miR-424-5p can regulate myogenesis progression.[30] In MSA-affected brain regions, nuclear translocation of RelA is a notable character.[31] Skeletal muscle atrophy can increase the expression of RelA.[32] Compared to healthy controls, TNF-α elevates obviously in the patients with MSA,[33] and RelA helps the expression of atrophy and inflammatory gene induced by TNF-α.[34] Accordingly, we deduce that quercetin, kaempferol, isorhamnetin and hederagenin, the major active components of AM, treat MSA mainly by controlling muscle atrophy and inflammation process.

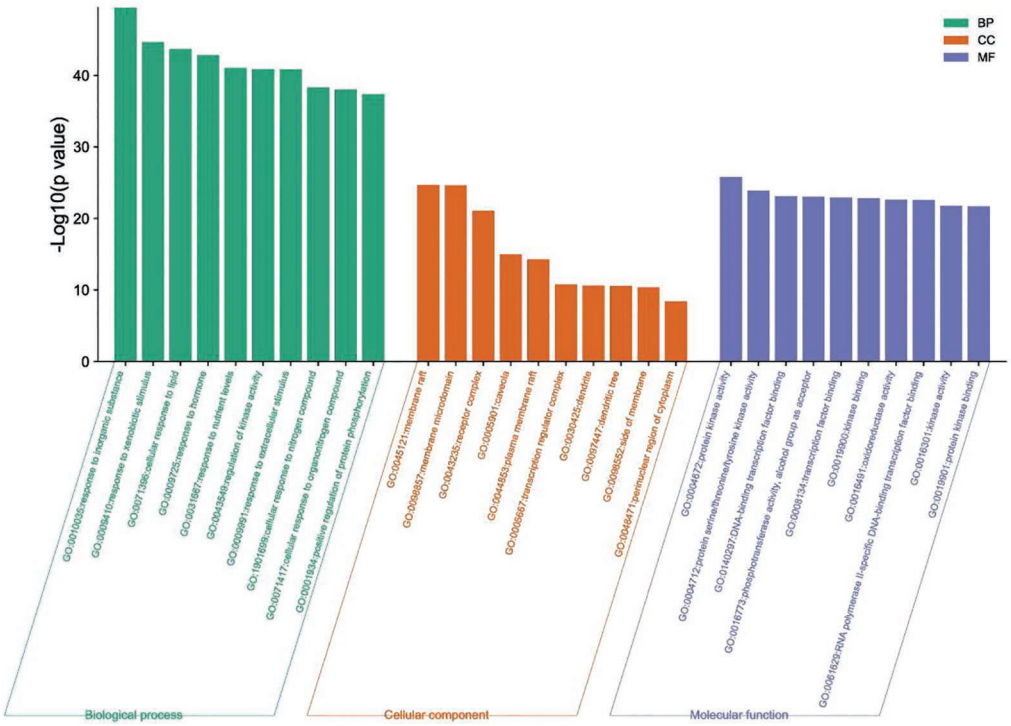

Figure 5. The top 10 items of BP,CC, and MF in GO analisis. GO = gene ontology, BP = biological process, CC = cell composition, MF = molecular function.

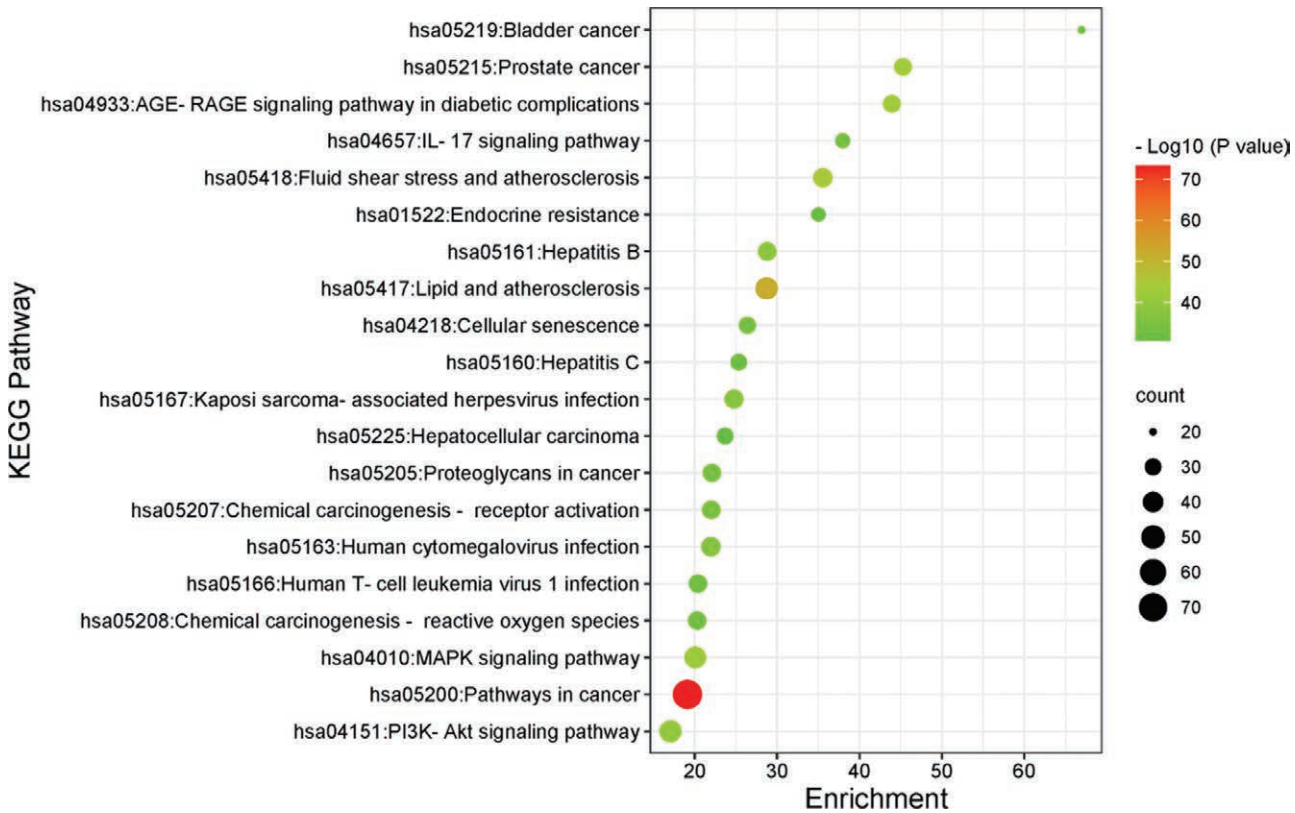

Figure 6. The bubble chart of the top 20 enriched pathways in KEGG analysis. KEGG = kyotoencyclopedia of genes and genomes.

GO analysis helps detect the possible therapeutic mechanism further. The most significant BP items are mainly relevant to cellular response and regulation. Glial cytoplasmic inclusion is the overriding neuropathological symbol of MSA, it is immunoreactive to tau, tubulin, ubiquitin, aB-crystallin, cyclin-dependent kinase 5, transferrin, Leu-7 and microtubule-associated protein 5.[35–38] Studies proved that α-Syn over-expression or cell-to-cell transfer, inflammation, and

Table 2

Binding capacity of core compounds to core proteins. (Kcal·mol – 1).

MOL ID	Compound	TP53	RELA	TNF
MOL000098	Quercetin	–6.03	–5.79	–4.96
MOL000422	Kaempferol	–6.36	–6.39	–4.93
MOL000354	Isorhamnetin	–6.15	–5.48	–4.92

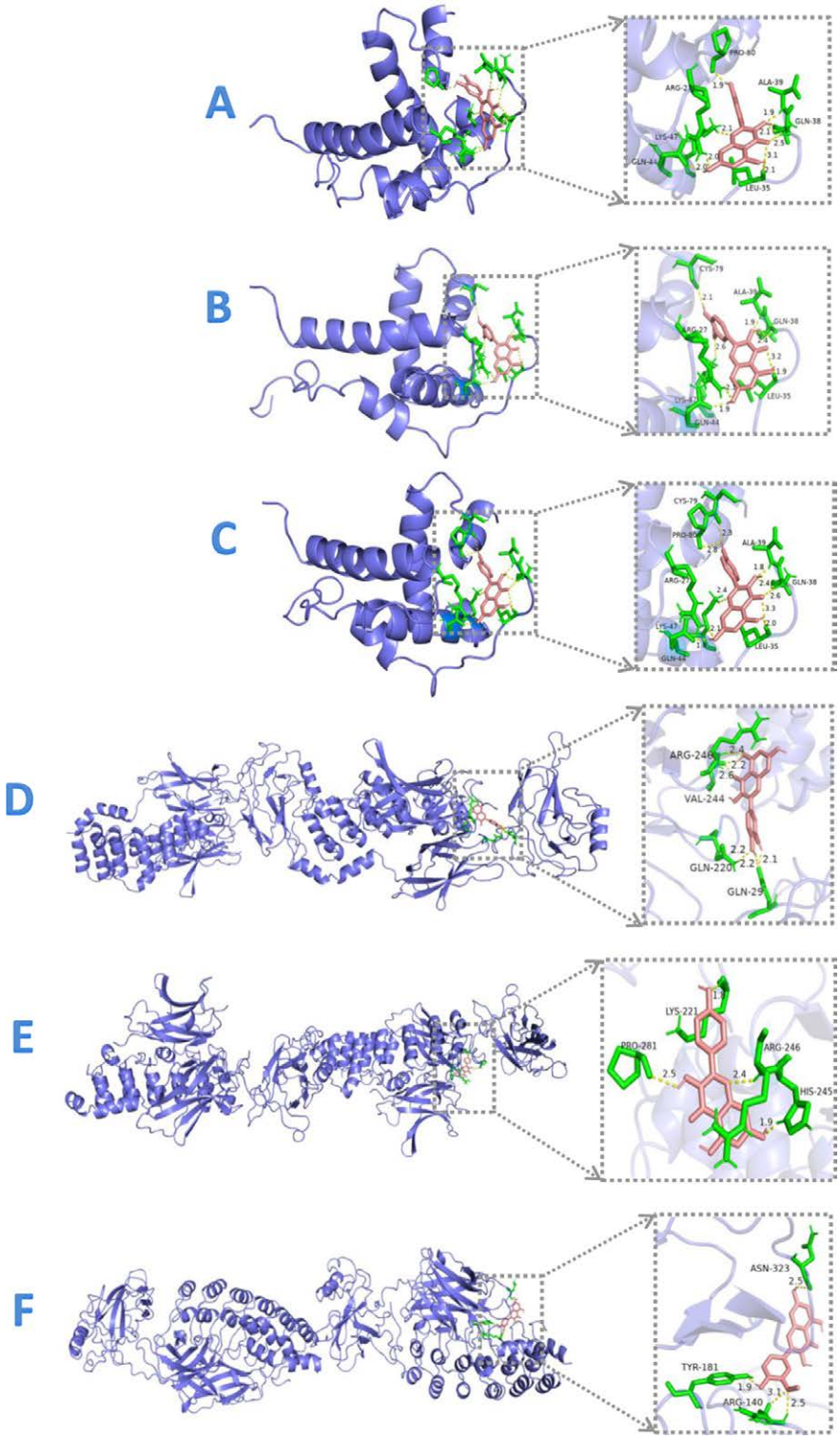

Figure 7. Molecular docking diagram of the results with good binding activity. The docking of (A) quercetin with TP53, (B) kaempferol with TP53, (C) isorhamnetin with TP53, (D) quercetin with RELA, (E) kaempferol with RELA, and (F) isorhamnetin with RELA.

mitochondrial functioning are related to MSA pathogenesis.[10] In MSA, NOS III-immunoreactive neurites and glial cells proliferate excessively.[39] Irregular expression of miRNAs in the serum may promote pathogenesis and serve as effective biomarkers.[40] MSA can lead to multiple regulatory disorders. For example, there are signs of early cellular dysfunction in those neural progenitor cells which derived neural cell adhesion molecules, like increasing the susceptibility to exogenous oxidative stress.[41] The expression of NLRP3 inflammasome-associated proteins is upregulated in the putamen of MSA patients and correlates with the neurodegenerative process.[42] The total iron level in the striatum is also increased in MSA patients.[43] Some MSA patients have inappropriate secretion of antidiuretic hormone.[44] This hints that the major targets are critical for various BPs. The enriched targets in MF mainly include protein kinase activity and binding, phosphotransferase activity, transcription factor binding, oxidoreductase activity, and protein serine/threonine/tyrosine kinase activity. The abnormal or ectopic expression of cyclin-dependent kinase 5 and mitogen-activated protein kinase gives rise to aberrant phosphorylation of microtubular cytoskeletal proteins, thus causing glial cytoplasmic inclusions formation in affected oligodendrocytes, which become the feature of MSA.[45] The targets involved in MFs mainly include GSK3B, MAPK14, PRKCB, SRC, and AKT1, which mainly participate in apoptosis, inflammation, and tumorigenesis. CCs enriched in targets mainly include membrane raft, membrane microdomain, receptor complex, caveola, and plasma membrane raft. These findings indicate the complexity of the potential mechanism of AM for treating MSA.

For further exploring of the latent treating mechanism, we conducted KEGG analysis of the 220 targets. The related pathways mainly include pathways in cancer, lipid and atherosclerosis pathway, virus infection pathways, and signaling pathways. The pathways in cancer are associated with the cancer-related targets involved in the PPI analysis above and need deeper studies. Advanced MSA patients have increased plasma leptin levels and fat accumulation with hypoalbuminemia and hypocholesterolemia.[46] There is a hypothesis that the unique neuropathology of MSA may be the oligodendrocytes-caused disorder of specific lipid metabolism associated with myelin synthesis and maintenance.[47] It is proved that viral α-Syn knockdown can prevent the spreading of synucleinopathy.[48] Based on the multiple pathways above, we speculate that AM for the treatment of MSA by the immune response, lipid regulation, and inflammatory and other processes.

Molecular docking results between 3 core proteins (TP53, RELA, and TNF) selected by PPI analysis and the top 3 components(quercetin, kaempferol, and isorhamnetin) filtered by degree are good and standard. It is theoretically proved that these target proteins may be effective in the treatment of MSA and be worthy of further research.

There are also some shortcomings in our study. Our exploration relies on numerous databases, so the depth of basic research and the accuracy and real-time updating of the datum in each database are particularly essential. We only explored the underlying mechanism through network pharmacology, therefore the results of this study still require further experiments to verify.

5. Conclusion

To summarize, the components, targets, and BPs involved in the pharmacological action of AM in the treatment of MSA are diverse. Among them, the core active compounds include quercetin, kaempferol, and isorhamnetin, the crucial target proteins involve TP53, RELA, TNF, SRC, MAPK1, and JUN, and the dominant BPs are related to cellular response and regulation. Molecular functions are mainly associated with apoptosis, inflammation, and tumorigenesis. The molecular docking results theoretically demonstrate the potential effectiveness of core target proteins for treating MSA. Our findings provide a reference for further studies on the therapeutic mechanism of AM in MSA.

Author contributions

Conceptualization: Ni Yang, Xianghua Qi.
Data curation: Ni Yang, Jing Hu, Jing Teng.
Methodology: Ni Yang, Jing Hu.
Software: Ni Yang, Jing Hu.
Supervision: Xianghua Qi, Chunlin Li.
Visualization: Ni Yang, Jing Hu,Yuangeng Wang, Jing Teng.
Writing – original draft: Ni Yang, Jing Hu.
Writing – review and editing: Yuangeng Wang, Xianghua Qi, Chunlin Li.

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