Exploring the Target and Molecular Mechanism of Astragalus Membranaceus in the Treatment of Vascular Cognitive Impairment Based on Systematic Pharmacology and Molecular Docking

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Abstract

Astragalus membranaceus (AM) is a kind of traditional Chinese herbal medicine that is extensively utilized in vascular cognitive impairment (VCI) treatment. Nevertheless, because of its complex ingredients, the exact molecular mechanism remains unclear. Therefore, the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP), STITCH, and SwissTargetPrediction have been utilized to gather the primary active ingredients of AM. The potential therapeutic targets of VCI were collected through GeneCards, OMIM, and DisGeNET databases. Then, the interactive network was drawn. The potential mechanism of AM in VCI treatment was demonstrated by means of enrichment analysis and network topology analysis of Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genome (KEGG) pathway. Subsequently, molecular docking predicted the binding properties of its potential active ingredients and targets. 20 effective compounds and 733 targets were screened from AM, among which 158 targets were identified as possible targets of AM in treating VCI. Protein-protein interaction (PPI) network and topology analysis revealed that MAPK3 and MMP9 were the key targets of AM intervention in VCI. Molecular docking studies indicated that calycosin and quercetin may be potential active compounds of AM in VCI treatment. Our study contributes to an in-depth understanding of the pharmacological actions of AM in VCI treatment, which also offers a scientific foundation for greater understanding of the molecular mechanism of AM in treating VCI.

Introduction

Vascular cognitive impairment (VCI) is a group of syndromes from vascular cognitive impairment no dementia (VCIND) to vascular dementia (VaD), which are provoked by cerebrovascular disease risk factors for example homocysteine, diabetes, hyperlipidemia and hypertension. Epidemiological studies show that with a large number of aging population, the morbidity, prevalence and mortality of VCI have been continuously increasing day by day across the globe, which can severely lower the patients' quality of life and be an economic burden to the patients and society (An and Li 2015; Smith 2017). Although modern medicine has made some progress in the diagnosis and treatment of VCI, its molecular mechanism has not been fully clarified (Calabrese et al. 2016). There are currently no drugs recommended for improving cognitive functioning in patients with VCI (Zhang et al. 2018). Therefore, VCI has become a research hotspot, and it is of great social implication to explore the therapeutic methods of VCI.

Astragalus membranaceus (AM) which is commonly used in treating VCI is a traditional Chinese herbal medicine. Modern pharmacological studies have confirmed that AM mainly includes astragalus polysaccharides, astragalus saponins, astragalosides and other ingredients, which have the functions of anti-inflammatory, anti-oxidative (Li et al. 2022), anti-atherosclerosis, regulating nerve growth factor (Wu et al. 2020), improving neuronal function (Tian et al. 2021), delaying neurodegenerative diseases, and so on (Fu et al. 2014). Furthermore, our previous experimental research disclosed that the compound containing AM could improve the cognitive function of VCI (Chen et al. 2020; Wu et al. 2015). More importantly, further analysis of the effective ingredients, targets and molecular mechanisms of AM on VCI by systematic pharmacology could provide the basis for clinical treatment and future scientific research.

Methods

Construction and screening of the active ingredients database of AM

The potential active compounds of AM were retrieved from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, https://old.tcmsp-e.com/tcmsp.php) (Ru et al. 2014). Subsequently, the potential active ingredients were filtered out following the screening standard of drug likeness (DL) ≥ 0.18 and oral bioavailability (OB) ≥ 30% (Xu et al. 2012).

Predicting target genes for active ingredients

Firstly, we obtained the SMILES formulas of active ingredients through the PubChem database(https://pubchem.ncbi.nlm.nih.gov/). Secondly, we imported the potential active compounds of AM into STITCH (http://stitch.embl.de/) (Szklarczyk et al. 2016) and SwissTargetPrediction database (http://www.swisstargetprediction.ch/) (Daina et al. 2019) in SMILES format, and the species was chosen as "Homo Sapiens". At last, we got the action targets of potential active ingredients in AM after searching for the potential targets of each active compound of AM and deleting the repeated targets.

Screening of VCI targets

We logged into GeneCards database (https://www.genecards.org/) (Stelzer et al. 2016), OMIM database (https://omim.org/), and DisGeNET disease database (https://www.disgenet.org/home/) (Piñero et al. 2021), and collected all VCI related disease targets with "vascular cognitive improvement" as the search term. After deleting duplicate and invalid genes, we obtained VCI disease targets. Then, we integrated

potential targets of AM and VCI related disease targets to obtain intersection genes. Finally, the Wayne diagram was made by tbtools software (version 1.09852) to obtain the potential target of AM acting on VCI.

Building protein-protein interaction (PPI) and herbs-active ingredients-targets-signaling pathways-diseases network diagram

Intersection genes were input into the STRING database (https://string-db.org) (Szklarczyk et al. 2021) with a confidence score of 0.4 as the screening criterion. Then, we obtained the interaction relationship between proteins and constructed a PPI network diagram. Visualization of the network was then performed by using Cytoscape software (https://cytoscape.org) (Otasek et al. 2019). Ultimately, the network topology was calculated through the CytoHubba plugin of Cytoscape software, and the "degree" was used as the screening criterion to obtain the top ten key targets of AM interfering with VCI.

Through the Metascape database(https://metascape.org) (Zhou et al. 2019), the core targets of AM acting on VCI were analyzed by module, and the key targets with the top ranked connectivity degree in each module were obtained. Then, we performed enrichment analysis on each module. Besides, we jointly analyzed the obtained key pathways and core targets to build herbs-active ingredients-targets-signaling pathways-diseases network diagram.

Gene Ontology (GO) biological function and Kyoto Encyclopedia of Genes and Genome (KEGG) pathway enrichment analysis

R language (version 4.0) was utilized to analyze the core target of AM acting on VCI, including the GO functional enrichment analysis and KEGG pathway enrichment analysis. Then, from the perspectives of biological process (BP), cellular component (CC) and molecular function (MF), relevant signaling pathways and biological processes were obtained and displayed visually.

Molecular docking analysis

Molecular docking technology is based on simulating the interaction between ligands and receptors (such as electrostatic interactions, hydrogen bonding interactions, hydrophobic interactions, van der Waals interactions, and so on) to predict the binding mode and affinity between protein and protein or small molecules and protein, thereby virtual screening of drug targets and predicting the effective ingredients. The key targets in the important signaling pathways obtained above were linked with the main active ingredients of AM by molecular docking.

The three-dimensional (3D) structures of the key target proteins were retrieved and downloaded from the RCSB PDB databases (http://www.rcsb.org/) (Rose et al. 2018). At the same time, the files of the 3D structures of active ingredients were downloaded from the PubChem database(https://pubchem.ncbi.nlm.nih.gov/). Prior to docking, the energy of the acceptor and ligand needed to be minimized, the water molecules of the acceptor (PDB files) needed to be deleted, polar hydrogen atoms needed to be added, and the magnetic field and charge needed to be added. By docking the active ingredients with the receptor protein molecules, the results of systematic pharmacological screening were evaluated and verified by using Autodock Vina and Autodock molecular docking software respectively. The flow chart of research is shown in Fig. 1.

Result

Screening of active ingredients and target prediction results of AM

There were 87 active ingredients of AM selected from the TCMSP database, and effective ingredients of AM were obtained by using OB and DL (OB $\geq$ 30%, DL $\geq$ 0.18). The data are given in Table 1.

Table 1
The major bioactive ingredients of AM

Mol ID	Molecular 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

The intersection genes of AM and VCI

We obtained the active ingredient targets of AM through the PubChem and SwissTargetPrediction databases. Eventually, there were 733 corresponding gene targets received after duplicates had been deleted. Simultaneously, we received 1017 genes of VCI related disease targets from GeneCards, OMIM, and DisGeNET disease databases. After the intersection between AM active ingredient targets and VCI disease-related targets, 158 potential target genes of AM acting on VCI were obtained (Fig. 2).

Constructing PPI networks and screening of key targets

We input 158 common targets into the STRING data platform to construct the interaction PPI network between the key targets of AM acting on VCI. The network consists of 158 nodes, and there are 2622 edges in the association between target points, and the expected number of edges is 911. According to the network topology parameters of Cytoscape software, the top ten genes with connectivity include AKT1, TNF, IL6, SRC, EGFR, MAPK3, CASP3, HSP90AA1, JUN and MMP9, which may be the important therapeutic targets of AM to treat VCI (Fig. 3).

Module analysis

In the results of module analysis, 86 targets involving 6 modules may play a more important role (Fig. 4). Each module corresponds to a specific biological function. All modules were ranked by log10 (P value), and then the top-ranked items were selected for analysis. Among them, module 1 and module 2 are the most significant cancer-related pathways. Module 2 also addresses RANKL/RANK signaling pathway and endocrine resistance. Here, we note that Module 3 is the most significant in circadian genes and involves the negative regulation of cell differentiation and the regulation of small molecule metabolic processes. Module 4 is the most significant drug resistance in EGFR tyrosine kinase inhibitors, and involves the glioblastoma signaling pathway. Module 5 is the most significant in the cell's response to UV-A. Module 6

is the most significant in chloride ion transmembrane transport. Eventually, we discovered that the top key targets in each module are: PIK3CA, APP, MAPK3, PRKCD, MMP1/MMP2/MMP3/MMP9, CFTR/GABRB2/GABRG2. Combined with the ranking results of connectivity, MAPK3 and MMP9 are identified as the key targets of AM to treat VCI.

GO and KEGG enrichment analysis

The 158 intersection genes of VCI treated by AM were analyzed with R language, and the top 10 results of GO enrichment analysis were screened out (Fig. 5A). The results showed that BP is significantly enriched in cellular response to chemical stress, response to oxidative stress, neuron death, regulation of protein kinase B signaling, phosphatidylinositol 3-kinase signaling and other biological processes. CC is significantly enriched in membrane raft, membrane microdomain, neuronal cell body, glutamatergic synapse, focal adhesion, and other cellular components. MF is significantly enriched in protein serine/threonine kinase activity, protein tyrosine kinase activity, phosphatase binding, transmembrane receptor protein kinase activity, growth factor binding and other molecular functions. In addition, the discovery of the top 30 of KEGG enrichment analysis indicated that the top signaling pathways include Lipid and atherosclerosis, Apoptosis, PI3K/Akt, MAPK, Rap1, Ras, HIF-1, TNF, IL-17, and VEGF signaling pathways (Fig. 5B).

Construction of herbs-active ingredients -targets-signaling pathways-diseases network diagram

Two key targets (MAPK3 and MMP9), 10 related active ingredients and 12 key pathways (Apoptosis, PI3K/Akt, Rap1, MAPK, Ras, HIF-1, TNF, IL-17, VEGF, FoxO, Toll-like receptor, and mTOR signaling pathway) of AM treatment for VCI were jointly analyzed. Then we utilized Cytoscape software to construct a herbs-active ingredients-targets-signaling pathways-diseases network diagram of AM for VCI treatment, as shown in Fig. 6.

Molecular docking

MAPK3 and MMP9, the two key targets of AM acting on VCI, were docked with 10 related drug active ingredients, among which MAPK3 was molecularly docked with calycosin, hederagenin and (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 to screen out the compounds with the strongest binding ability to MAPK3 (Fig. 7). In addition, the molecular docking of MMP9 with jaranol, isorhamnetin, (6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol, bifendate, kaempferol, FA, and quercetin was used to screen the compounds that have the closest interaction with MMP9 (Fig. 8).

The results indicated that the docking scores of 10 effective ingredients of AM and 2 targets were all less than − 4.0, indicating that they had certain binding activities. Among them, the binding energies of MAPK3 and calycosin are the strongest, and the binding energies of MMP9 and quercetin are also the strongest (Table 2). Therefore, calycosin and quercetin may be the potential active compounds of AM for VCI treatment.

Table 2
The molecular docking scores of the effective ingredients of AM and the target

Compound	CAS/PubChem ID	Ligand	Receptor	PUB ID	Binding energy
Calycosin	5280448	Conformer3D_CID_5280448	MAPK3	10.2210/pdb6GES/pdb	-9
Hederagenin	73299	Conformer3D_CID_73299	MAPK3	10.2210/pdb6GES/pdb	-6.3
(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	15976101	Conformer3D_CID_15976101	MAPK3	10.2210/pdb6GES/pdb	-7.2
Jaranol	5318869	Conformer3D_CID_5318869	MMP9	10.2210/pdb5TH6/pdb	-4.8
Isorhamnetin	5318645	Conformer3D_CID_5318645	MMP9	10.2210/pdb5TH6/pdb	-4.2
(6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol	14077830	Conformer3D_CID_14077830	MMP9	10.2210/pdb5TH6/pdb	-4.1
Bifendate	108213	Conformer3D_CID_108213	MMP9	10.2210/pdb5TH6/pdb	-6.6
Kaempferol	5280863	Conformer3D_CID_5280863	MMP9	10.2210/pdb5TH6/pdb	-8.1
FA	6037	Conformer3D_CID_6037	MMP9	10.2210/pdb5TH6/pdb	-7.1
Quercetin	5280343	Conformer3D_CID_5280343	MMP9	10.2210/pdb5TH6/pdb	-8.4

Discussion

The pathophysiological molecular mechanism of VCI is complex. The use of herbal compounds containing AM, AM and its extracts to treat VCI has been confirmed by experimental research and clinical trial research results (Li et al. 2017; Mei et al. 2017), but the mechanism of AM and its main effective ingredients in treating VCI have not been fully clarified.

In this study, 87 active ingredients of AM were screened through TCMSP. The results of molecular docking displayed that among the 20 effective ingredients, calycosin and quercetin may be the core active compounds of AM for VCI treatment. Calycosin whose mechanism may be associated with anti-autophagy, anti-apoptosis and anti-inflammatory is a phytoestrogen isolated from AM (Wang et al. 2018). It has neuroprotective effects on cerebral ischemia-reperfusion injury. The experimental studies revealed that calycosin can alleviate the inflammatory reaction by suppressing MAPK and TLR/NF-κB signaling pathways, and improve sensory and movement disorders in degenerative diseases of the nervous system (Yang et al. 2019). Studies have also indicated that quercetin is a flavonol compound with all kinds of biological activities that can inhibit the MAPK signaling pathway and its downstream targets p-38 MAPK, p-ERK, and p-JNK. Meanwhile, quercetin can also reduce the levels of TNF-α, IL-6 and IL-1β, relieving neuroinflammatory reaction and neuralgia. Additionally, the molecular docking results of this study showed that AM has the strongest binding energy of calycosin and MAPK3, which may regulate the growth, differentiation, and neuroinflammation of nerve cells through the MAPK signaling pathway. In this study, the results are presented in accordance with the findings of Ye et al (Ye et al. 2021).

By intersecting the key targets of AM active ingredients and VCI disease targets, 158 key targets of AM interfering with VCI were obtained, so as to construct the PPI network diagram. Topology parameter calculation showed that the important potential active ingredients may be AKT1, TNF, IL6, SRC, EGFR, MAPK3, CASP3, HSP90AA1, JUN, MMP9, showing multi-target intervention in VCI. Further analysis of the combined module showed that the key targets are MAPK3 and MMP9. MMP9 can degrade the extracellular matrix, damage cell structure, cause apoptosis, increase cerebral vascular permeability, and destroy blood-brain barrier (Chakraborty et al. 2018). In parallel, it can also play the role of neuro-inflammatory mediators and reduce the stability of atherosclerotic plaques. Overall, MMP9 is a hazard factor for cerebral infarction and concurrent cognitive impairment (Yang et al. 2017). After cerebral ischemic injury, the MAPK family is highly phosphorylated, activating the MAPK signaling pathway, and up-regulating TNF-α, IL-6 ,IL-1β and other neuroinflammatory factors (Lee et al. 2015).

The KEGG enrichment analysis of this study indicated that the important pathways of AM in the treatment of VCI include Lipid and atherosclerosis, PI3K/Akt, MAPK, Rap1, and Ras signaling pathway, etc. Among them, PI3K/Akt signaling transduction pathway is a classic signaling transduction pathway, which can resist apoptosis, promote the survival of neuron cells and interfere with autophagy of neuron cells. In addition, the PI3K/Akt signaling transduction pathway, which can protect nerve cells and regulate neuronal cell apoptosis caused by

cerebral ischemia and hypoxia, is related to cognitive dysfunction caused by cerebrovascular diseases. Akt, a key regulator of the PI3K/Akt signaling pathway, binds to 14-3-3 by phosphorylation the Ser136 site of Bad, preventing it from inhibiting the anti-apoptotic protein Bcl-2 and decreasing neuronal apoptosis (Pang et al. 2020). Akt's subtype AKT1 is a serine/threonine kinase encoding protein that affects the regulation of nerve cells. Its oxidative modification can lead to a decline in synaptic function and cause cognitive dysfunction (Ahmad et al. 2017). Experimental studies have also confirmed that activating of Gi protein/c-Src/Pyk2/EGFR/PI3K/Akt/p42/p44 MAPK cascade can lead the activation of the pro-inflammatory factor activator protein-1 (AP-1) containing JUN, inhibit neuroinflammation and reduce neuronal apoptosis (Yang et al. 2017; Liou et al. 2019).

MAPK signaling pathway regulates cell growth, differentiation, proliferation, cytoskeleton remodeling, and cell migration. It has extensive distribution and expression in the central nervous system. Furthermore, the MAPK signaling pathway is closely associated with cerebral ischemia injury and repair, which affects the long-term potentiation (LTP) of learning and memory formation in the hippocampus by regulating inflammatory factors and oxidative stress (Revest et al. 2014). The PI3K/Akt and MAPK signaling pathways are important downstream pathways of brain-derived neurotrophic factor (BDNF), and their common mediator is epithelial growth factor receptor (EGFR). EGFR can induce the small G protein Ras to activate the serine/threoninase Raf after activating SOS1. Ser338 site on Raf-1 can activate the downstream MAPK/ERK signaling, while Ser259 site can reversely inhibit MAPK/ERK pathway. The PI3K/Akt signaling pathway can activate Ser259 site to inhibit MAPK/ERK pathway activity (Park et al. 2011; Horn et al. 2015). It is worth noting that there is crosstalk among PI3K/Akt, MAPK and Ras signaling pathways, which are strongly correlated with the pathogenesis and treatment of VCI. The important pathway of AM in the treatment of VCI is closely related to neurotrophic, neuroinflammation, autophagy, and apoptosis.

Conclusion

To sum up, we analyzed the potential targets and mechanism of action of AM for VCI treatment through systematic pharmacology and molecular docking research methods, and found that the potential active compounds of AM for VCI treatment are effective active ingredients represented by calycosin and quercetin. The potential key targets of AM for VCI are MAPK3 and MMP9. The key signaling pathways are PI3K/Akt, MAP, Rap1, and Ras signaling pathway. There is crosstalk among PI3K/Akt, MAPK and Ras signaling pathways. AM intervenes in VCI with complex disease molecular mechanisms through a multi-ingredient, multi-target, and multi-pathway coordination mechanism. It is an important clinical treatment drug for VCI. It is worthy of further in-depth experimental and clinical research around its key targets and signaling pathways.

Declarations

Author's Contribution LL and WC designed the study schedule and made equal contributions to this work. XLL, YLL and YLC searched the relevant literature. LL and QY analyzed the data. XHC, SJZ and GFZ wrote the manuscript. CY and LW wrote the manuscript. All authors approved the final manuscript and declare that all data were generated in-house and that no paper mill was used.

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Data Availability All authors declare that the data are available.

Compliance with ethical standards

Ethical approval Does not apply

Consent to participate Does not apply

Consent to publish Does not apply

Conflict of interests All authors declare that there is no conflict of interest in this study

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Figures

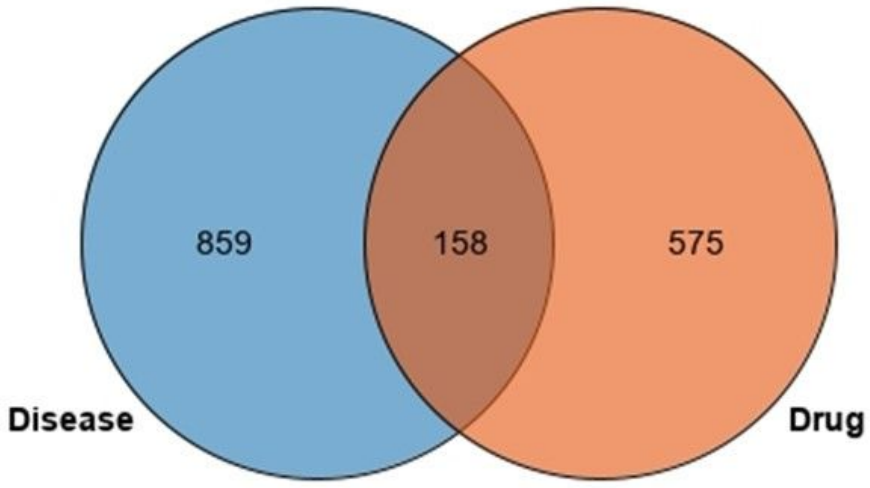

Figure 2

Target Wayne diagram of AM and VCI (blue represents VCI and red represents AM)

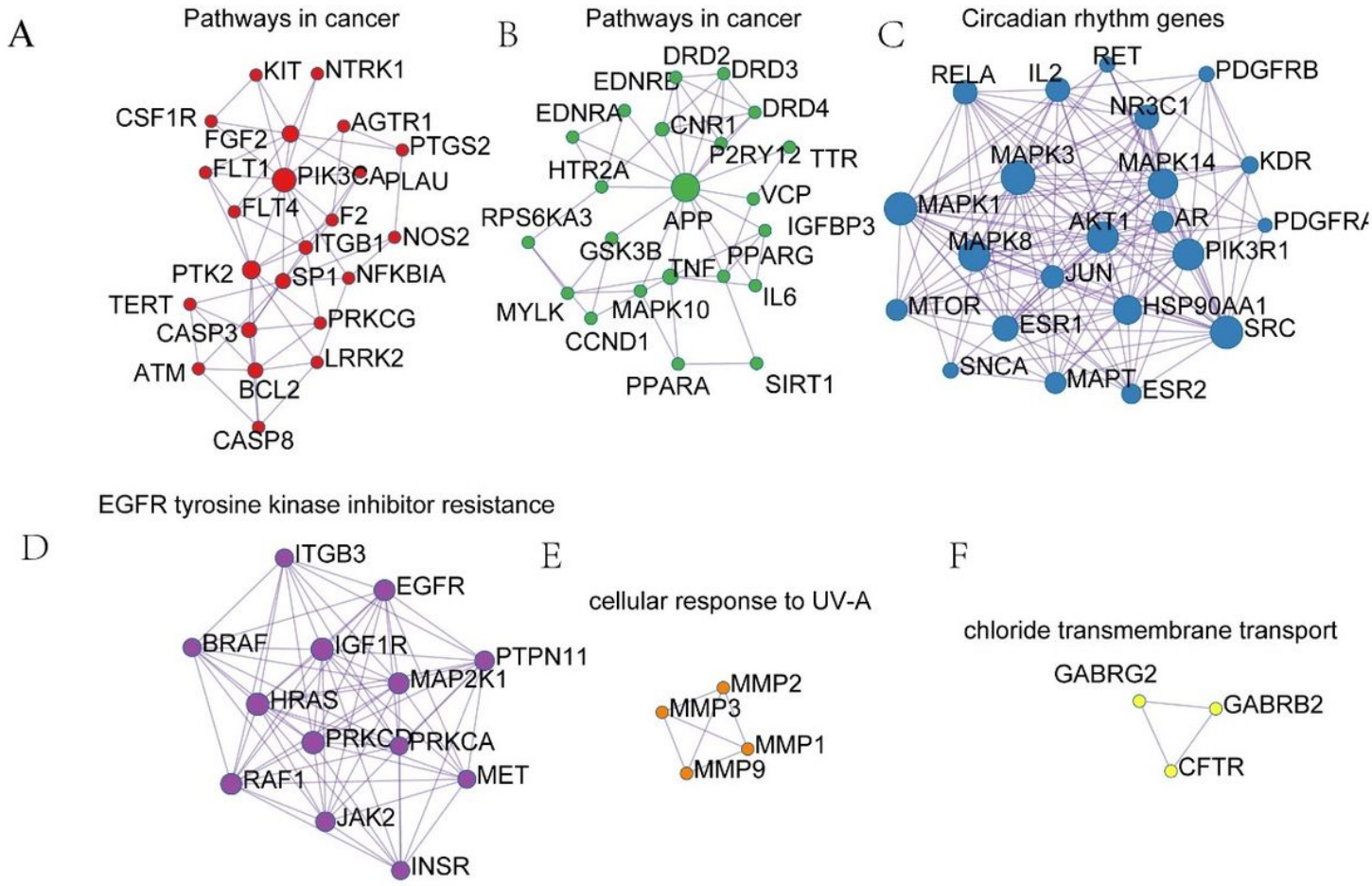

Figure 4

MCODE analysis of potential cotargets of AM on VCI. (A): Module 1; (B): Module 2; (C): Module 3; (D): Module 4; (E): Module 5; (F): Module 6.

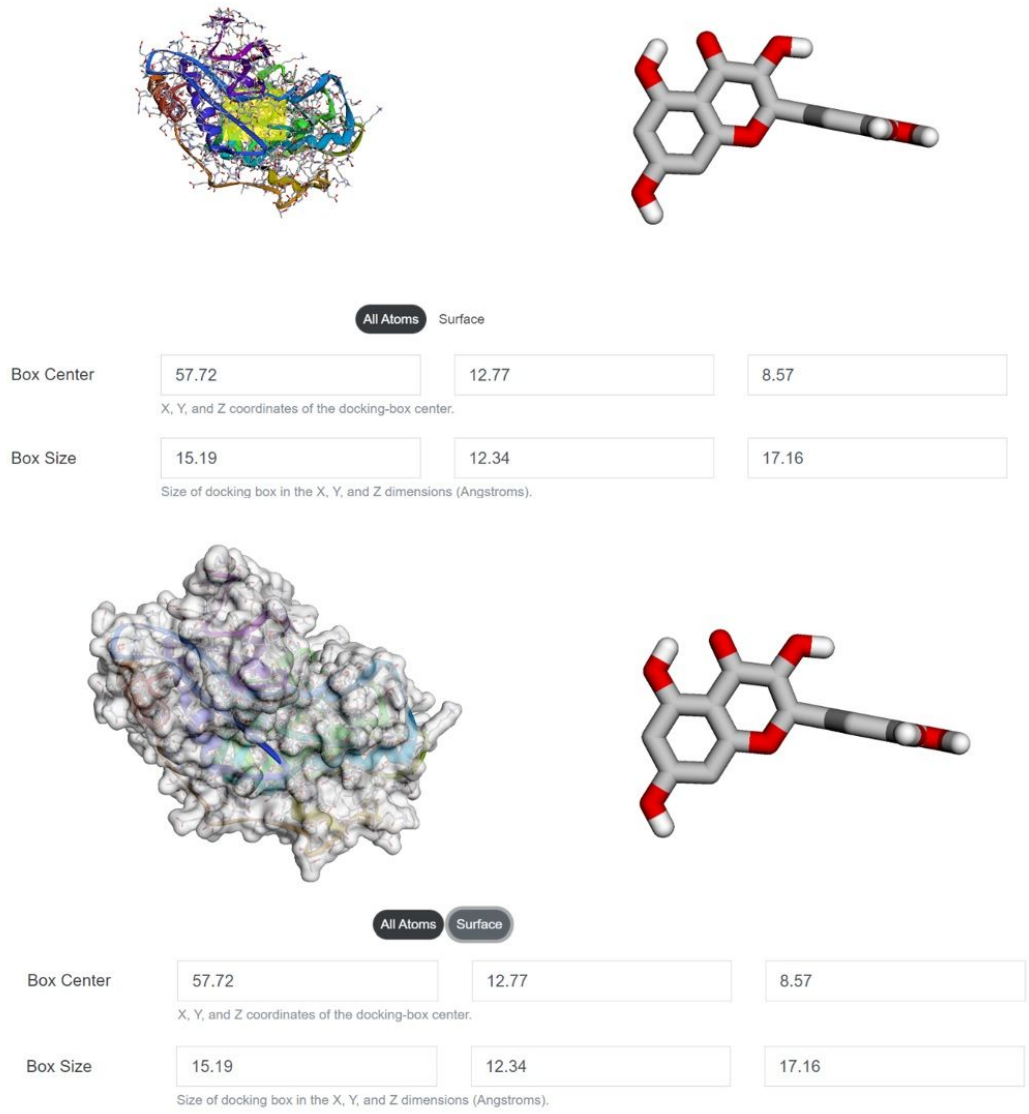

Figure 8

MMP9 and quercetin molecular docking diagram after adjustment

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- 11GO.csv
- 2GeneCardsVascularcognitiveimpairmentSearchResults.xlsx
- 5SwissTargetSTITCHVenndiagram.xlsx
- 8metascaperesult..xlsx