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Network pharmacology analysis of molecular targets and related mechanisms of Guizhi decoction in treating of menopausal syndrome

Qian Zhang, MD[a], Jingtao Liang, MD[a], Ying Zhou, PhD[b,*]

Abstract

Compared with hormone therapy, TCM had the advantages of overall adjustment and less side effects in the treatment of menopausal syndrome. But due to the complex pharmacodynamic composition of Guizhi decoction (GZD), the mechanism of TCM treating diseases was not clear. Network pharmacology could analyze drug action pathways through multi-pathway and multi-target, which provide a new direction for TCM mechanism research. The common targets of GZD and menopausal syndrome (MPS) were obtained by TCMSP and DisGeNET databases. And for the common targets, protein-protein interaction networks were established using the STRING database and analyzed by Gene Ontology and the Kyoto Encyclopedia of Genes and Genomes. (Our research does not require ethical approval). One hundred forty-six active ingredients with 283 targets were obtained from GZD by network pharmacological analysis. Besides, 230 target genes were found to have interactions with MPS, 52 of which were common targets between MPS and GZD and were predicted to be potential targets for MPS treatment of GZD. GO enrichment analysis revealed that GZD could affect 51 biological processes, 15 cellular components, and 13 molecular functions. Kyoto Encyclopedia of Genes and Genomes enrichment analysis yielded a total of 223. The pathways that are closely related to the pathogenesis of MPS are MAPK, PI3K-Akt. In this study, the relevant targets and mechanisms of GZD in the treatment of MPS were discussed from the perspective of network pharmacological analysis, reflecting the characteristics of multi-component, multi-target and multiple pathways, and it provides a good theoretical basis for the clinical application of GZD.

Abbreviations: DL = drug similarity, GZD = Guizhi decoction, KEGG = Kyoto Encyclopedia of Genes and Genomes, MPS = menopausal syndrome, OB = oral bioavailability.

Keywords: MPS, network pharmacology, GZD

1. Introduction

Menopausal syndrome (MPS) refers to a series of physical and psychosomatic states caused by fluctuations or decreases in sex hormones in women before and after menopause, which may even cause coronary heart disease, diabetes mellitus, and other highly lethal diseases without timely intervention.[1] In China, about 170,000 women were in perimenopause in 2018 as the aging society intensified,[2] accompanied by a gradually increasing incidence of menopausal syndrome. At this stage, the medical treatment for this disease is mainly hormone replacement therapy (estrogen and progestin supplementation, hormone replacement therapy). Although hormone replacement therapy can effectively relieve most postmenopausal symptoms,[3] there are adverse effects such as abnormal uterine bleeding, breast tenderness, and atherosclerosis. The search for non-hormonal therapies with low adverse effects has become a significance task in the current efforts to control and alleviate the symptoms of menopausal syndrome.

GZD (Guizhi decoction) is the first formula in the Treatise on Typhoid Fever by Zhang Zhongjing and consists of five herbs, namely Gui Zhi (Cmnamomi Mmulus, GZ), Bai Shao (Paeonia Radix Alba, BS), Zhi Gan Cao (Raxix Glycyrrhizae Preparata, ZGC), Da Zao (Ziziphus jujubaMill, DZ), and Sheng Jiang (Zingiber officinale Roscoe, SJ). GZD is commonly used clinically to treat internal, external, gynecological, and pediatric diseases caused by the imbalance of yin and

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The datasets generated during and/or analyzed during the current study are publicly available.

The data used to support the findings of this study are included within the article.

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yang and disharmony of Ying and Wei.[4] TCM believes that MPS is mainly caused by the imbalance between Yin and Yang of the kidney, which often involves other Zang-fu, especially the heart, liver, and spleen, and can be combined with complex pathogenesis such as qi depression, blood stasis, phlegm and dampness.[5] And GZD fits well with the pathomechanism of imbalance of kidney yin and yang in MPS. In a study, It was found that GZD can be the basis of body temperature, sweat derangement, and bidirectional regulation of heart rate, blood pressure, and large intestine conduction derangement.[6] Among them, the bidirectional regulation of body temperature may be achieved by affecting adenylate cyclase activity in the hypothalamus,[7] and some studies have shown that: the antipyretic effect of Gui Zhi Tang may be related to the reduction of serum IL-1, TNF-α, plasma and hypothalamic PGE2.[8] Besides, animal experiments have shown that GZD can inhibit inflammation and oxidative stress in rats with high-fat myocardial ischemia, exerting cardiovascular protective effects.[9] However, the mechanism of its therapeutic effect on MPS has not been clarified.

Due to the complex pharmacodynamic composition of GZD, the molecular mechanism of its therapeutic effect on MPS is still not well understood. In this study, the mechanism of action of GZD was analyzed by using network pharmacology to systematically elucidate and explore the mechanism of the therapeutic effect of GZD on MPS, which provides a theoretical basis for further research on the mechanism of the therapeutic effect of GZD on MPS.

2. Materials and Methods

2.1. Database building and active compound screening

The active ingredients of GZD (GZ, BS, ZGC, DZ, SJ) were collected from the TCMSP database (http://lsp.nwu.edu.cn/tcmsp.php). TCMSP is a Chinese medicine pharmacology database containing information about the herbs used in TCM, and absorption, distribution, metabolism and excretion (ADME) characteristics of the individual compounds, their targets, related diseases, and pathways. Pharmacokinetics parameters such as oral bioavailability (OB) and drug likeness (DL) were investigated. OB is commonly used to measure whether oral drugs can be through obstacles as well as be transported into the systemic blood circulation. DL is mainly used to predict exactly how "drug like" an ingredient is, which helps to assist pharmacokinetic and pharmaceutical properties, for example, solubility and chemical stability. OB≥30% and DL≥0.18 were used as screening criteria to screen out the possible active ingredients in GZD.

2.2. Identification of drug targets

After screening out the active ingredients in GZD, the target proteins retrieved from the TCMSP database were entered into Uniprot (https://www.uniprot.org/),[10] and the corresponding gene names were extracted by Uniprot to standardize the target proteins corresponding to each herbal ingredient. The species was selected as "Homo sapiens." A GZD active ingredient-disease-target network interplay map was constructed using Cytoscape 3.7.2 software, and the screened GZD active ingredients were correlated with MPS-related potential targets to clarify the interaction between their active ingredients and potential targets.

2.3. Screening of potential targets for MPS

The search was conducted from the DisGeNET database (https://www.disgenet.org/) and Genecard database (https://www.genecards) using "menopause syndrome" as the keyword (The results of the two databases were combined and duplicate targets were removed to obtain the disease targets of MPS. The specific target information corresponding to the active ingredient was entered into the Uniprot database to obtain the standard gene names of the targets of action.

2.4. PPI network of compound-disease targets PPI network

The GZD and MPS common targets were entered into the STRING database (https://string-db.org/),[11] the study species was selected as human (Homo sapiens), the minimum required interaction score was selected >0.9, and other The protein interaction network (PPI) was obtained by selecting human (Homo sapiens) as the study species, selecting the minimum required interaction score >0.9, and keeping other parameters as default settings.

2.5. GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis

To understand the relational role of GZD active ingredients and disease corresponding common targets in gene function and pathways, GO enrichment analysis was performed on the common targets of GZD active ingredients and diseases using David (HTTPS://David.ncifcrf.gov/) and KEGG databases (species set to human, $P \leq .05$ for effective pathway) and KEGG pathway enrichment analysis. The GO enrichment analysis included three parts: cellular component, biological process, and molecular function. The results of the obtained data were presented in the form of histograms.

2.6. Construction of active ingredient-target-pathway network

Cytoscape v3.7.2 was used to construct a network of common targets and signaling pathways of active ingredients and MPS in Chinese medicine. The "nodes" in different colors represent the targets and signaling pathways respectively. The "edges" refer to the interactions between common targets and signaling pathways.

2.7. Molecular docking

To further validate the interaction effect of GZD acting on MPS, molecular docking was applied for key compounds and kernel targets. The plug-in CytoHubba of Cytoscape software was used to screen top 10 hub genes (Table 1).Subsequently, Akt1 was moleculardocking with key active ingredients, and Autodock Vina was used to predict the accuracy of key ingredients and predicted targets.[12] Choose the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and protein data bank (http://www.rcsb.org/) to download MOL2 format of ligand and protein PDB format.[13] Protein crystals were introduced into the PyMol software (https://pymol.org/2/; Version 2.4.1) for the dehydration and separation of ligands.[14] The crystal is then introduced into AutoDockTools to build the target's docking grid framework.[15] Autodock Vina was used to realize molecular docking.[14] Low Vina score is one of the results of molecular docking, representing a more stable binding affinity between protein and ligand.[16] Finally, PyMol software was used to visualize the protein and compound complexes.

3. Results

3.1. Active ingredient and target screening for each herbal medicine in GZD

The chemical active ingredients of each herbal medicine in GZD were retrieved from TCMSP, and after the screening, 7 active

Table 1

Basic information of active compounds of GZD.

Herb	Mol ID	Molecule name	OB (%)	DL
GZ	MOL001736	(-)-Taxifolin	60.51	0.27
GZ	MOL000492	(+)-Catechin	54.83	0.24
GZ	MOL000358	Beta-sitosterol	36.91	0.75
GZ	MOL000073	Ent-Epicatechin	48.96	0.24
GZ	MOL011169	Peroxyergosterol	44.39	0.82
GZ	MOL000359	Sitosterol	36.91	0.75
GZ	MOL004576	Taxifolin	57.84	0.27
BS	MOL001910	11alpha,12alpha-epoxy-3beta-23-dihydroxy-30-norolean-20-en-28,12beta-olide	64.77	0.38
BS	MOL001918	Paeoniflorgenone	87.59	0.37
BS	MOL001919	(3S,5R,8R,9R,10S,14S)-3,17-dihydroxy-4,4,8,10,14-pentamethyl-2,3,5,6,7,9-hexahydro-1H-cyclopenta[a]phenanthrene-15,16-dione	43.56	0.53
BS	MOL001921	Lactiflorin	49.12	0.8
BS	MOL001924	Paeoniflorin	53.87	0.79
BS	MOL001925	Paeoniflorin_qt	68.18	0.4
BS	MOL001928	Albiflorin_qt	66.64	0.33
BS	MOL001930	Benzoyl paeoniflorin	31.27	0.75
BS	MOL000211	Mairin	55.38	0.78
BS	MOL000358	Beta-sitosterol	36.91	0.75
BS	MOL000359	Sitosterol	36.91	0.75
BS	MOL000422	Kaempferol	41.88	0.24
BS	MOL000492	(+)-Catechin	54.83	0.24
GC	MOL001484	Inermine	75.18	0.54
GC	MOL001792	DFV	32.76	0.18
GC	MOL000211	Mairin	55.38	0.78
GC	MOL002311	Glycyrol	90.78	0.67
GC	MOL000239	Jaranol	50.83	0.29
GC	MOL002565	Medicarpin	49.22	0.34
GC	MOL000354	Isorhamnetin	49.6	0.31
GC	MOL000359	Sitosterol	36.91	0.75
GC	MOL003656	Lupiwighteone	51.64	0.37
GC	MOL003896	7-Methoxy-2-methyl Isoflavone	42.56	0.2
GC	MOL000392	Formononetin	69.67	0.21
GC	MOL000417	Calycosin	47.75	0.24
GC	MOL000422	Kaempferol	41.88	0.24
GC	MOL004328	Naringenin	59.29	0.21
GC	MOL004805	(2S)-2-[4-hydroxy-3-(3-methylbut-2-enyl)phenyl]-8,8-dimethyl-2,3-dihydropyrano[2,3-f]Chromen-4-one	31.79	0.72
GC	MOL004806	Euchrenone	30.29	0.57
GC	MOL004808	Glyasperin B	65.22	0.44
GC	MOL004810	Glyasperin F	75.84	0.54
GC	MOL004811	Glyasperin C	45.56	0.4
GC	MOL004814	Isotrifoliol	31.94	0.42
GC	MOL004815	(E)-1-(2,4-dihydroxyphenyl)-3-(2,2-dimethylchromen-6-yl)prop-2-en-1-one	39.62	0.35
GC	MOL004820	Kanzonols W	50.48	0.52
GC	MOL004824	(2S)-6-(2,4-dihydroxyphenyl)-2-(2-hydroxypropan-2-yl)-4-methoxy-2,3-dihydrofuro[3,2-g]chromen-7-one	60.25	0.63
GC	MOL004827	Semilicoisoflavone B	48.78	0.55
GC	MOL004828	Glepidotin A	44.72	0.35
GC	MOL004829	Glepidotin B	64.46	0.34
GC	MOL004833	Phaseolinisoflavan	32.01	0.45
GC	MOL004835	Glypallichalcone	61.6	0.19
GC	MOL004838	8-(6-hydroxy-2-benzofuranyl)-2,2-dimethyl-5-chromenol	58.44	0.38
GC	MOL004841	Licochalcone B	76.76	0.19
GC	MOL004848	Licochalcone G	49.25	0.32
GC	MOL004849	3-(2,4-dihydroxyphenyl)-8-(1,1-dimethylprop-2-enyl)-7-hydroxy-5-methoxy-coumarin	59.62	0.43
GC	MOL004855	Licoricone	63.58	0.47
GC	MOL004856	Gancaonin A	51.08	0.4
GC	MOL004857	Gancaonin B	48.79	0.45

(*Continued*)

Table 1

(***Continued***)

Herb	Mol ID	Molecule name	OB (%)	DL
GC	MOL004860	licorice glycoside E	32.89	0.27
GC	MOL004863	3-(3,4-dihydroxyphenyl)-5,7-dihydroxy-8-(3-methylbut-2-enyl) chromone	66.37	0.41
GC	MOL004864	5,7-dihydroxy-3-(4-methoxyphenyl)-8-(3-methylbut-2-enyl) chromone	30.49	0.41
GC	MOL004866	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-6-(3-methylbut-2-enyl) chromone	44.15	0.41
GC	MOL004879	Glycyrin	52.61	0.47
GC	MOL004882	Licocoumarone	33.21	0.36
GC	MOL004883	Licoisoflavone	41.61	0.42
GC	MOL004884	Licoisoflavone B	38.93	0.55
GC	MOL004885	Licoisoflavanone	52.47	0.54
GC	MOL004891	Shinpterocarpin	80.3	0.73
GC	MOL004898	(E)-3-[3,4-dihydroxy-5-(3-methylbut-2-enyl)phenyl]-1-(2,4-dihydroxyphenyl)prop-2-en-1-one	46.27	0.31
GC	MOL004903	Liquiritin	65.69	0.74
GC	MOL004904	Licopyranocoumarin	80.36	0.65
GC	MOL004905	3,22-Dihydroxy-11-oxo-delta(12)-oleanene-27-alpha-methoxycarbonyl-29-oic acid	34.32	0.55
GC	MOL004907	Glyzaglabrin	61.07	0.35
GC	MOL004908	Glabridin	53.25	0.47
GC	MOL004910	Glabranin	52.9	0.31
GC	MOL004911	Glabrene	46.27	0.44
GC	MOL004912	Glabrone	52.51	0.5
GC	MOL004913	1,3-dihydroxy-9-methoxy-6-benzofurano[3,2-c]chromenone	48.14	0.43
GC	MOL004914	1,3-dihydroxy-8,9-dimethoxy-6-benzofurano[3,2-c] chromenone	62.9	0.53
GC	MOL004915	Eurycarpin A	43.28	0.37
GC	MOL004917	glycyroside	37.25	0.79
GC	MOL004924	(-)-Medicocarpin	40.99	0.95
GC	MOL004935	Sigmoidin-B	34.88	0.41
GC	MOL004941	(2R)-7-hydroxy-2-(4-hydroxyphenyl)chroman-4-one	71.12	0.18
GC	MOL004945	(2S)-7-hydroxy-2-(4-hydroxyphenyl)-8-(3-methylbut-2-enyl) chroman-4-one	36.57	0.32
GC	MOL004948	Isoglycyrol	44.7	0.84
GC	MOL004949	Isolicoflavonol	45.17	0.42
GC	MOL004957	HMO	38.37	0.21
GC	MOL004959	1-Methoxyphaseollidin	69.98	0.64
GC	MOL004961	Quercetin der.	46.45	0.33
GC	MOL004966	3'-Hydroxy-4'-O-Methylglabridin	43.71	0.57
GC	MOL000497	licochalcone a	40.79	0.29
GC	MOL004974	3'-Methoxyglabridin	46.16	0.57
GC	MOL004978	2-[(3R)-8,8-dimethyl-3,4-dihydro-2H-pyrano[6,5-f] chromen-3-yl]-5-methoxyphenol	36.21	0.52
GC	MOL004980	Inflacoumarin A	39.71	0.33
GC	MOL004985	Icos-5-enoic acid	30.7	0.2
GC	MOL004988	Kanzonol F	32.47	0.89
GC	MOL004989	6-prenylated eriodictyol	39.22	0.41
GC	MOL004990	7,2',4'-trihydroxy-5-methoxy-3-arylcoumarin	83.71	0.27
GC	MOL004991	7-Acetoxy-2-methylisoflavone	38.92	0.26
GC	MOL004993	8-prenylated eriodictyol	53.79	0.4
GC	MOL004996	Gadelaidic acid	30.7	0.2
GC	MOL000500	Vestitol	74.66	0.21
GC	MOL005000	Gancaonin G	60.44	0.39
GC	MOL005001	Gancaonin H	50.1	0.78
GC	MOL005003	Licoagrocarpin	58.81	0.58
GC	MOL005007	Glyasperins M	72.67	0.59
GC	MOL005008	Glycyrrhiza flavonol A	41.28	0.6
GC	MOL005012	Licoagroisoflavone	57.28	0.49
GC	MOL005013	18a-hydroxyglycyrrhetic acid	41.16	0.71
GC	MOL005016	Odoratin	49.95	0.3
GC	MOL005017	Phaseol	78.77	0.58

(*Continued*)

Table 1

(*Continued*)

Herb	Mol ID	Molecule name	OB (%)	DL
GC	MOL005018	Xambioona	54.85	0.87
GC	MOL005020	Dehydroglyasperins C	53.82	0.37
GC	MOL000098	Quercetin	46.43	0.28
SJ	MOL000358	Beta-sitosterol	36.91	0.75
SJ	MOL006129	6-methylgingediacetate2	48.73	0.32
SJ	MOL000449	Stigmasterol	43.83	0.76
SJ	MOL001771	Poriferast-5-en-3beta-ol	36.91	0.75
SJ	MOL008698	Dihydrocapsaicin	47.07	0.19
DZ	MOL012921	Stepharine	31.55	0.33
DZ	MOL012940	Spiradine A	113.52	0.61
DZ	MOL012946	Zizyphus saponin I_qt	32.69	0.62
DZ	MOL012961	Jujuboside A_qt	36.67	0.62
DZ	MOL012976	Coumestrol	32.49	0.34
DZ	MOL012980	Daechuine S6	46.48	0.79
DZ	MOL012981	Daechuine S7	44.82	0.83
DZ	MOL012986	Jujubasaponin V_qt	36.99	0.63
DZ	MOL012989	Jujuboside C_qt	40.26	0.62
DZ	MOL012992	Mauritine D	89.13	0.45
DZ	MOL001454	Berberine	36.86	0.78
DZ	MOL001522	(S)-Coclaurine	42.35	0.24
DZ	MOL000211	Mairin	55.38	0.78
DZ	MOL000449	Stigmasterol	43.83	0.76
DZ	MOL003410	Ziziphin_qt	66.95	0.62
DZ	MOL000358	Beta-sitosterol	36.91	0.75
DZ	MOL004350	Ruvoside_qt	36.12	0.76
DZ	MOL000492	(+)-catechin	54.83	0.24
DZ	MOL005360	Malkangunin	57.71	0.63
DZ	MOL000627	Stepholidine	33.11	0.54
DZ	MOL007213	Nuciferin	34.43	0.4
DZ	MOL000783	Protoporphyrin	30.86	0.56
DZ	MOL000787	Fumarine	59.26	0.83
DZ	MOL008034	21302-79-4	73.52	0.77
DZ	MOL008647	Moupinamide	86.71	0.26
DZ	MOL002773	Beta-carotene	37.18	0.58
DZ	MOL000096	(-)-catechin	49.68	0.24
DZ	MOL000098	quercetin	46.43	0.28
DZ	MOL013357	(3S,6R,8S,9S,10R,13R,14S,17R)-17-[(1R,4R)-4-ethyl-1,5-diMethylhexyl]-10,13-dimethyl-2,3,6,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthrene-3,6-diol	34.37	0.78

BS=Bai Shao, DL=drug similarity, DZ=Da Zao, GC=Gan Cao, GZ=Gui Zhi, OB=oral bioavailability, SJ=Sheng Jiang.

ingredients of GZ, 13 active ingredients of BS, 92 active ingredients of ZGC, 5 active ingredients of SJ, and 29 active ingredients of DZ were obtained, totaling 146 active ingredients. Information about the active ingredients of GZD is shown in Table 1. The active ingredient action targets of GZD were queried in the TCMSP database, and 45 potential targets of GZ, 111 potential targets of BS, 222 potential targets of ZGC, 53 potential targets of SJ, and 209 potential targets of DZ were obtained. After protein targets were entered into Uniprot and duplicate entries were removed, a total of 282 gene targets were obtained for further study.

3.2. GZD component-target network graph analysis

To better show the relationship between the active ingredient and MPS-related targets in GZD, a component-disease-target network diagram was constructed by Cytoscape 3.7.0 software (Fig. 1). The degree indicates the number of routers connected to the node by other nodes in the network, such as each oval node represents the number of targets owned by the active ingredient. The active ingredients with a high number of associated targets can be found as follows: quercetin (131), kaempferol (63), β-sitosterol (43),stepharine (27), naringenin (23), and beta-carotene (20).

3.3. Prediction of target genes associated with MPS in each component

The disease targets obtained from the DisGeNET and Genecard databases were 1412, and after the screening, the disease targets were uploaded to the UniProt database for correction, and 1148 were obtained after removing duplicates. Active ingredient and disease targets were obtained through the Veeny 2.1 website for 52 generic targets (Fig. 2). These targets are common targets for GZD and diseases.

3.4. Construction of PPI GZD and MPS protein interaction network

The 52 potential genes were recorded into the STRING database for analysis to obtain the protein-protein interaction relationships, and then the target protein PPI network graph was drawn using Cytoscape 3.7.0, as shown in Figure 3.

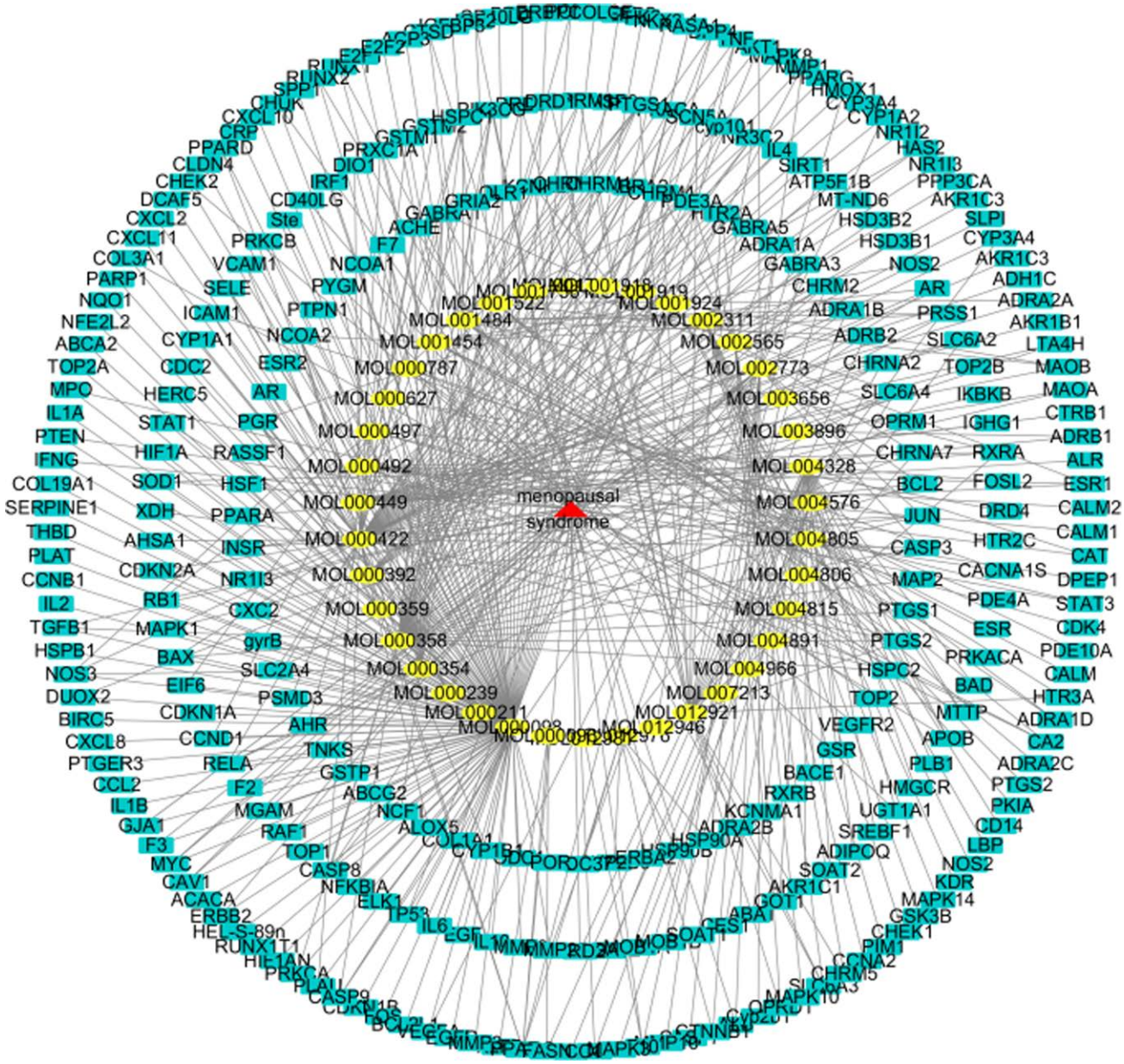

Figure 1. Compounds-disease-targets network. The red triangle represents disease, yellow ovals represent active compounds, and blue rectangles represent targets.

The network graph contains a total of 51 nodes and 334 edges with an average node degree value of 12.8 and a PPI enrichment P value of less than 1.0×10^{-16}, where the nodes represent proteins and each edge represents the protein-protein interaction relationship, with more lines indicating a greater association, as shown in Figure 3. By CytoNCA (an application in Cytoscape) after performing visual topology analysis, 10 core targets were obtained (Table 2). Among the 14 core targets, AKT1 had the highest degree (37), followed by ALB (36), TP53 (29), and MYC (27).

3.5. GO enrichment analysis

The 52 common targets obtained were analyzed by David for GO enrichment (Fig. 4). Cellular component terms mainly included nucleus (16%), cytoplasm (15%) and plasma membrane (12%); molecular function terms mainly included DNA binding (16%), transcription factor activity, sequence-specific DNA binding (8%), and identical protein binding (5%); biological process terms mainly included positive regulation of transcription from RNA polymerase II promoter (9%), positive regulation of transcription, DNA-templated (7%), transcription, DNA-templated (6%), negative regulation of apoptotic process (6%) and positive regulation of cell proliferation (6%).

3.6. KEGG pathway enrichment analysis

KEGG was a database that systematically analyzed the metabolic pathways of gene products in cells and the functions of these gene products. 52 common targets were input into the KEGG database, and 223 signaling pathways were obtained. The main pathways were: Pathways in cancer, Proteoglycans in cancer, Hepatitis C, MAPK signaling pathway, Network Human cytomegalovirus infection, PI3K-Akt signaling pathway, Hepatocellular carcinoma, Hepatitis B etc (Fig. 5).

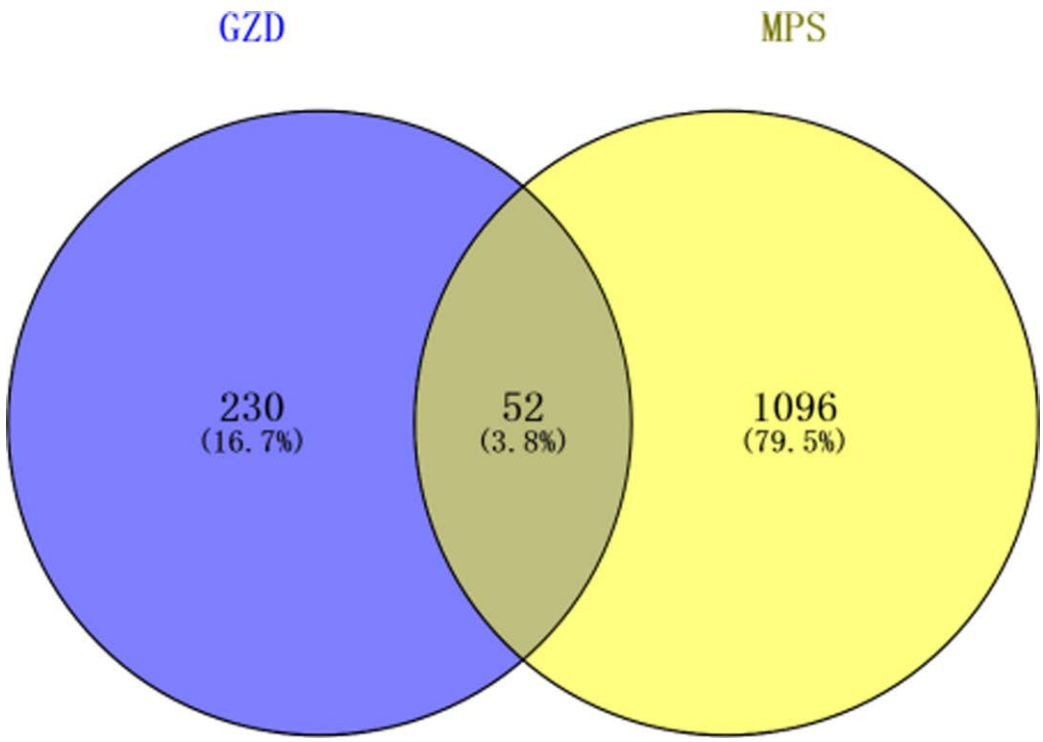

Figure 2. Venn diagram of GZD-related targets and MPS-related targets. GZD = Guizhi decoction, MPS = menopausal syndrome.

In these pathways, the ones most closely related to the treatment of MPs were: MAPK signaling pathway (Fig. 6), PI3K-Akt signaling pathway (Fig. 7). MAPK signaling pathway played an important role by regulating: EGF-EGFR-RAS-ERK/JNK, IL1-IL1R-JNK/p38, RAC/CDC42-PAK-ERetc; PI3K-Akt signaling pathway mainly regulatedEGF-EGFR-RAS-PI3K, PTEN-PIP3-AKT, EGF-EGFR-PI3K-NFKB.

3.7. Targets-pathways network

Targets-pathways network (Fig. 8), there were 8 main pathways and 27 related targets. As can be seen from the figure, Pathways in cancer, MAPK, and PI3K-Akt were highly enriched, which suggested that these Pathways may play an important role in the treatment of MPS. AKT1, TP53, RAF1, MYC, and other targets had more interaction lines with pathways, which suggested that they may play a major role in the process of various biological pathways.

3.8. Molecular docking results

We carried out molecular docking of the core target protein and active compound involved. Molecular docking between top 1 target proteins (AKT1) and key active compounds (quercetin, kaempferol, β-sitosterol, stepharine) was carried out using AutoDock Vina. The binding energy between AKT1 and the active compounds was approximately between 8.7 and 10.8kcalmol. AKT1, ALB, MAPK3, JUN, CASP3, and EGFR have stronger docking energy. Finally, the top four target protein macromolecules and small compound molecules with the best docking affinity were selected and visualized with PyMoL (Fig. 9).

4. Discussion

TCM herbs usually act synergistically on multiple diseases with multiple components, targets, and pathways. Network pharmacology has made great progress in exploring the application of active ingredients, targets, and systems in TCM. In this study, we applied a network pharmacology approach to investigate the relevant targets and pathways of GZD for the treatment of MPS, thus elucidating the synergistic mechanism of GZD on MPS. In menopausal syndrome, the decline in ovarian function, fluctuations, and decreases in estrogen, leading to menstrual irregularities, in addition to mediating the development of chronic diseases such as cardiovascular disease (CVD), obesity, hyperlipidemia, and fatty liver.[17]

In the study, after screening the OB and DL of drug components, 146 active ingredients were identified, the most potent of which was quercetin (131), kaempferol (63), β-sitosterol (43), stepharine (27), naringenin (23), and beta-carotene(20), and their potential value for the treatment of MPS was shown by network and functional analysis.

Quercetin is a naturally occurring flavonoid with strong biological activity that exerts antioxidant, antitumor, anti-inflammatory, antibacterial, and cardiovascular protective pharmacological effects by reducing oxidative stress, interfering with the renin-angiotensin-aldosterone system, and downregulating reactive oxygen species-mediated downstream signaling pathways.[18] The experimental results of Wang et al. indicated that quercetin increased the mRNA and protein expression levels of oxidative stress-related genes SOD-1, CAT, and GSS in postmenopausal rats, suggesting that quercetin improves the antioxidant capacity of the ovary by upregulating the expression of some oxidative stress-related genes.[19] An experiment by Huang et al. found that intake of quercetin significantly reduced blood pressure in humans, and besides, in a parallel design In a parallel design trial, participants who took quercetin for 8weeks or longer showed significant changes in HDL cholesterol and triglyceride levels.[20]

The dietary flavonoid kaempferol has antioxidant, anti-inflammatory, anti-apoptotic, anti-cancer, estrogenic, and anti-estrogenic activities.[21] The results of an experiment by Touillaud MS et al. showed that low intake of kaempferol, the trace element boron, and β-sitosterol reduced the risk of estrogen

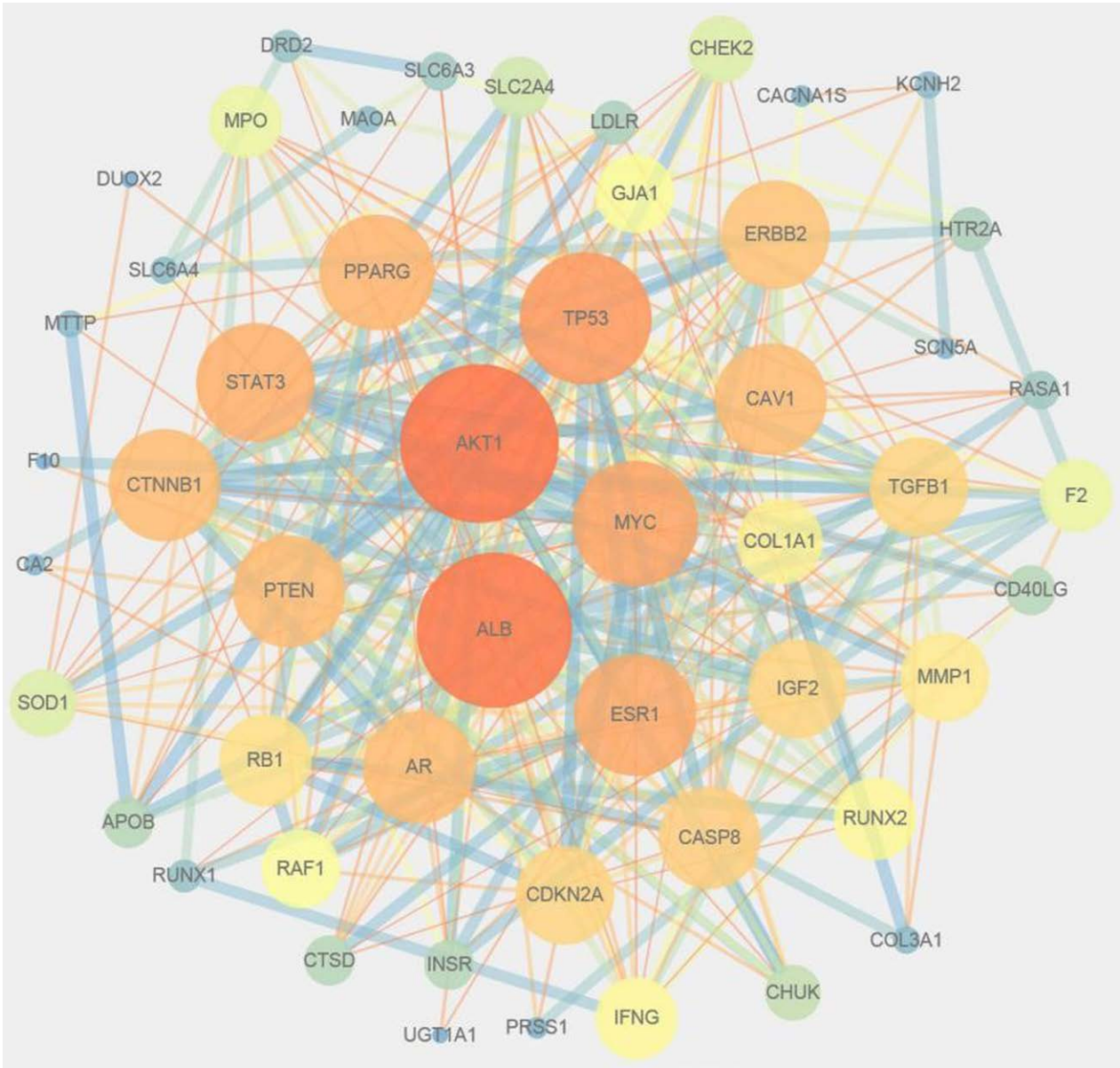

Figure 3. Protein-protein interaction (PPI) network of the active components of GZD for the treatment of MPS. Each node represents the gene of interest and the thickness of the line indicated by the edge indicates the strength of the interaction. GZD = Guizhi decoction, MPS = menopausal syndrome.

receptor-negative tumors. Other phytoestrogens were not significantly correlated with estrogen receptor status.[22]

A trial by Sriraman Sandhiya et al. found that β-sitosterol had similar effects on cell proliferation rates as standard 17βestradiol, suggesting that β-sitosterol could be a safe alternative to estrogen replacement therapy.[23] Babu Shyamaladevi et al. showed that β-sitosterol attenuates insulin resistance through its modulatory effects on the insulin signaling pathway IRS-1/Akt.[24]

Table 2

Core targets of GZD-MPS.

Gene symbol	Degree	Gene symbol	Gene symbol
AKT1	37	STAT3	25
ALB	36	PPARG	24
TP53	29	AR	23
MYC	27	PTEN	23
ESR1	26	CTNNB1	23

Naringenin is a dietary flavonoid found in a variety of products including citrus fruits. The substance has several pharmacological activities such as anti-inflammatory and antioxidant.[25] A trial by Yu et al. showed that long-term supplementation with 3% naringenin resulted in significant accumulation of naringenin in the plasma and tissues of obese de-ovulated mice and reduced metabolic disorders and muscle loss.[26] A randomized, double-blind trial showed that regular administration of pentenyl naringin affected alleviating mild vasodilatory symptoms in menopausal women.[27]

The results of GO and KEGG pathways indicated that the MAPK and PI3K-Akt were most closely related to the pathogenesis of MPS. These pathways were mainly linked to the core genes including AKT1, TP53, RAF1, MYC e in our research. A lots of studies could confirm these results.

MAPK was a group of serine/threonine protein kinases. As one of the main signaling pathways for intracellular information transmission, MAPK played a key role in many biological processes and could regulate cell growth, differentiation, apoptosis, inflammation, and other biological processes.

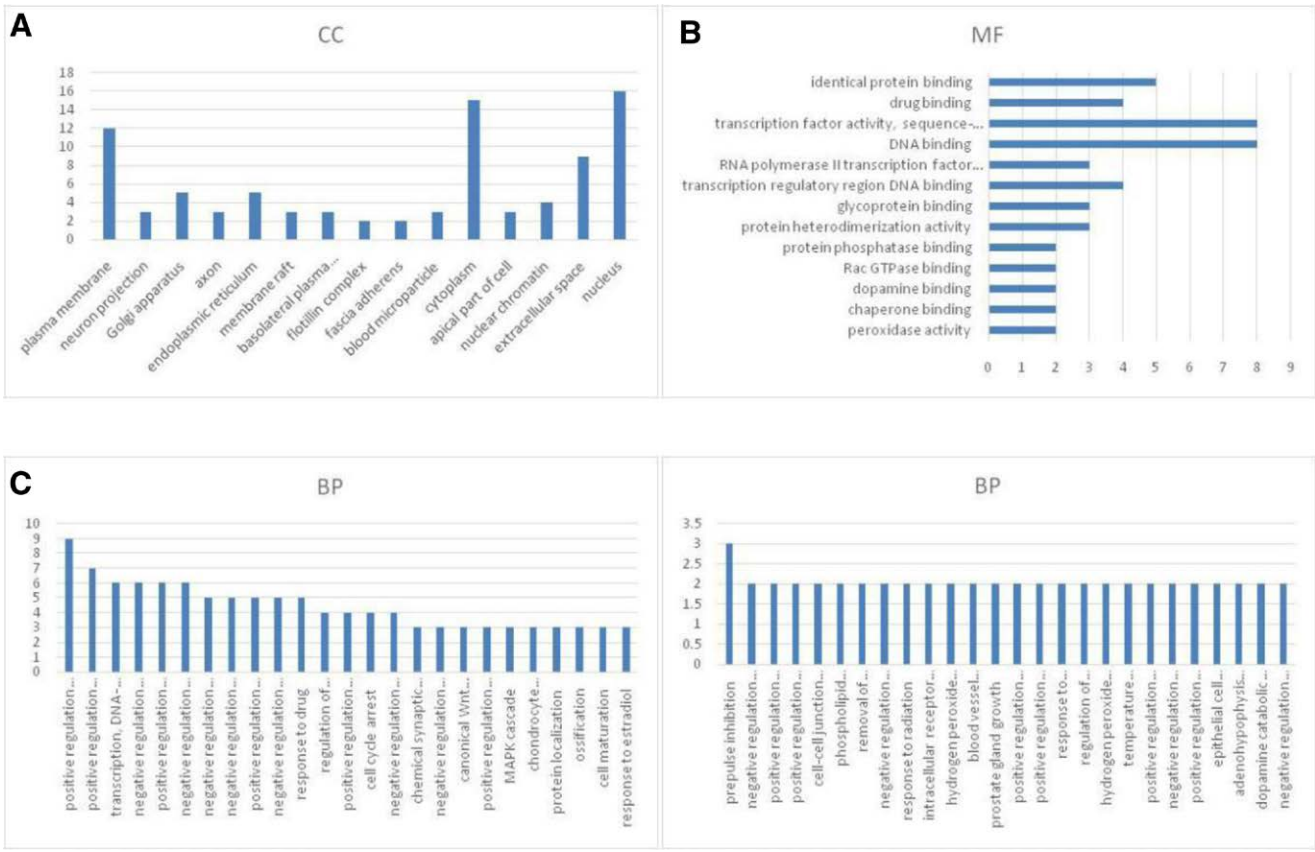

Figure 4. Functional analysis. (A) Cellular component (CC). (B) Molecular function (MF). (C) Biological process (BP).

Over-activation or reduced expression of MAPK would lead to the occurrence and development of various diseases.[28–30] In menopausal women, due to the decrease of estrogen levels, some women began to experience rapid bone loss, mainly manifested as bone loss, and in severe cases, further development of osteoporosis. This pathway could affect the differentiation and proliferation of osteoblasts and osteoclasts by regulating downstream factors.[31] In our study, MAPK mainly included ERK, JNK, p38 kinase and ERK5 activated kinase 4 subfamilies. Two ERK subtypes and three JNK subtypes were associated with proliferation of osteoclast precursor cells and apoptosis of osteoclasts.[32] In addition, four subtypes of p38 was highly expressed in both osteoclast precursor cells and mature osteoclasts, and played a key role in osteoclast differentiation and bone resorption.[33] In vitro experiments showed that osteoclast formation could be inhibited by regulating MAPK signaling pathway to prevent bone loss in rats.[34] Meanwhile, MAPK was also a positive

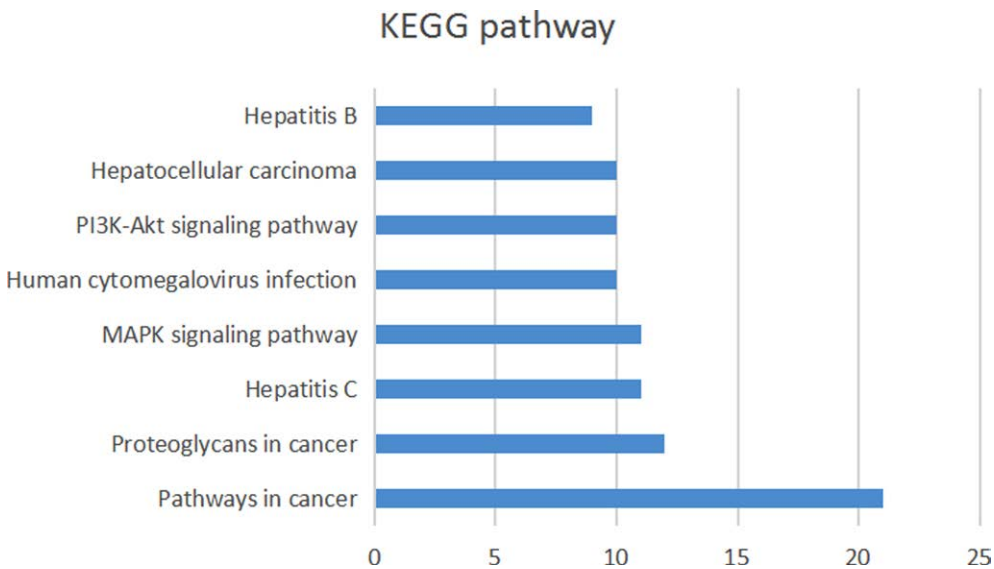

Figure 5. KEGG pathway. The length of each bar represents the number of enriched genes. KEGG = Kyoto Encyclopedia of Genes and Genomes.

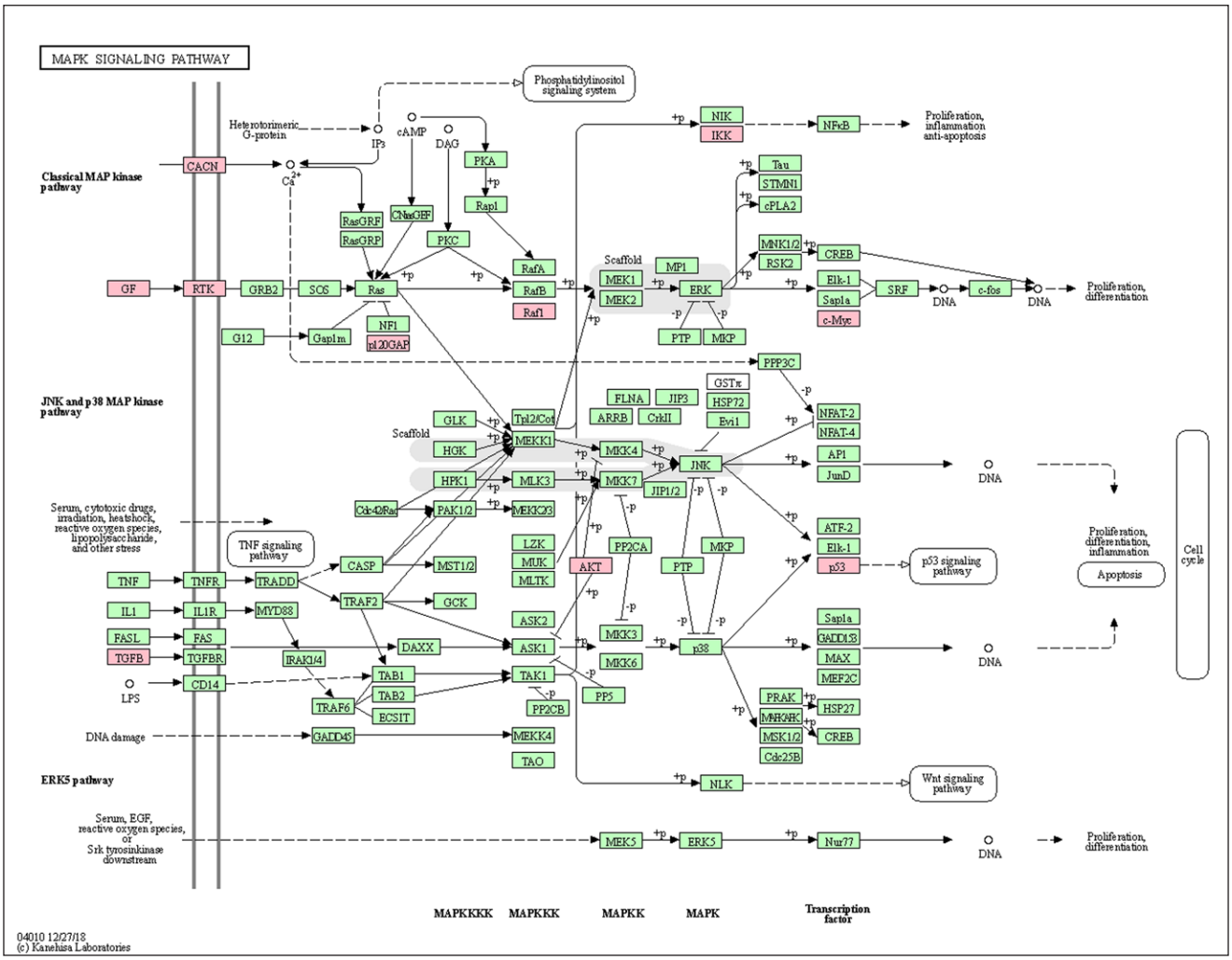

Figure 6. MAPK signaling pathway. Core targets are marked in red.

regulator of osteoblastic differentiation and bone formation. Research has shown that collagen peptide promoted osteogenesis by activating the p38MAPK and the levels of osteogenic biomarkers such as collagen, alkaline phosphatase and osteocalcin were significantly increased.[35] And, a clinical study had shown that polymorphism of MAPK-1 signaling pathway protein gene was associated with the severity of coronary heart disease and coronary artery disease in menopausal women.[36]

Women with MPS began to lose their ovarian function and menstrual cycle due to hormonal fluctuations.PI3K-Akt was a pathway closely related to cell proliferation and differentiation, and the PI3K-Akt signaling pathway existed in oocytes and granulocytes, which regulated the growth, development, maturation, and periodic ovulation of follicles through mutual coordination.[37] AKT, also known as protein kinase B, was an important downstream molecule of PI3K.[38] AKT activation was an important prerequisite for its function. Activated AKT became Phosphorylated AKT(PAKT) through phosphorylation, and activated or inhibited its downstream target protein to inhibit the apoptosis of granulosa cells, thereby inhibiting the atresia of follicles to some extent.[39] Animal experiments have found that compared with the control group, the fertility of the gene knockout mice decreased, and the number of mature follicles in the ovary decreased, and the number of apoptotic follicles increased. At the same time, the ovarian response to E2 was also very low, which did not cause the growth of follicles and granulosa cell proliferation.[40] Besides, women during this time due to low estrogen caused by hot flashes, night sweats, joint pain or life stress, and many other factors, the quality of sleep significantly decreased. Studies have shown that the PI3K-Akt signaling pathway played a certain role in sleep regulation by regulating its downstream effector molecules, such as immunoregulatory factors such as interleukin 1-β (IL-1β) and tumor necrosis factor-α (TNF-α).[41] In conclusion, MAPK and PI3K-Akt could improve the symptoms of MPS in multiple ways.

5. Conclusion

Based on network pharmacological analysis, this study demonstrated that GZD treated MPS through multi-compounds, multi-targets, and multi-pathways, and preliminarily clarified the related potential mechanism of GZD in the treatment of MPs. Through KEGG pathway enrichment analysis, it was found that GZD played an important role in the treatment of MPS, including MAPK and PI3K-Akt, which might play a role in regulating cell growth, differentiation, and apoptosis. This study provided a potential biological basis for the further study of GZD in the treatment of MPs, but further experimental studies were needed.

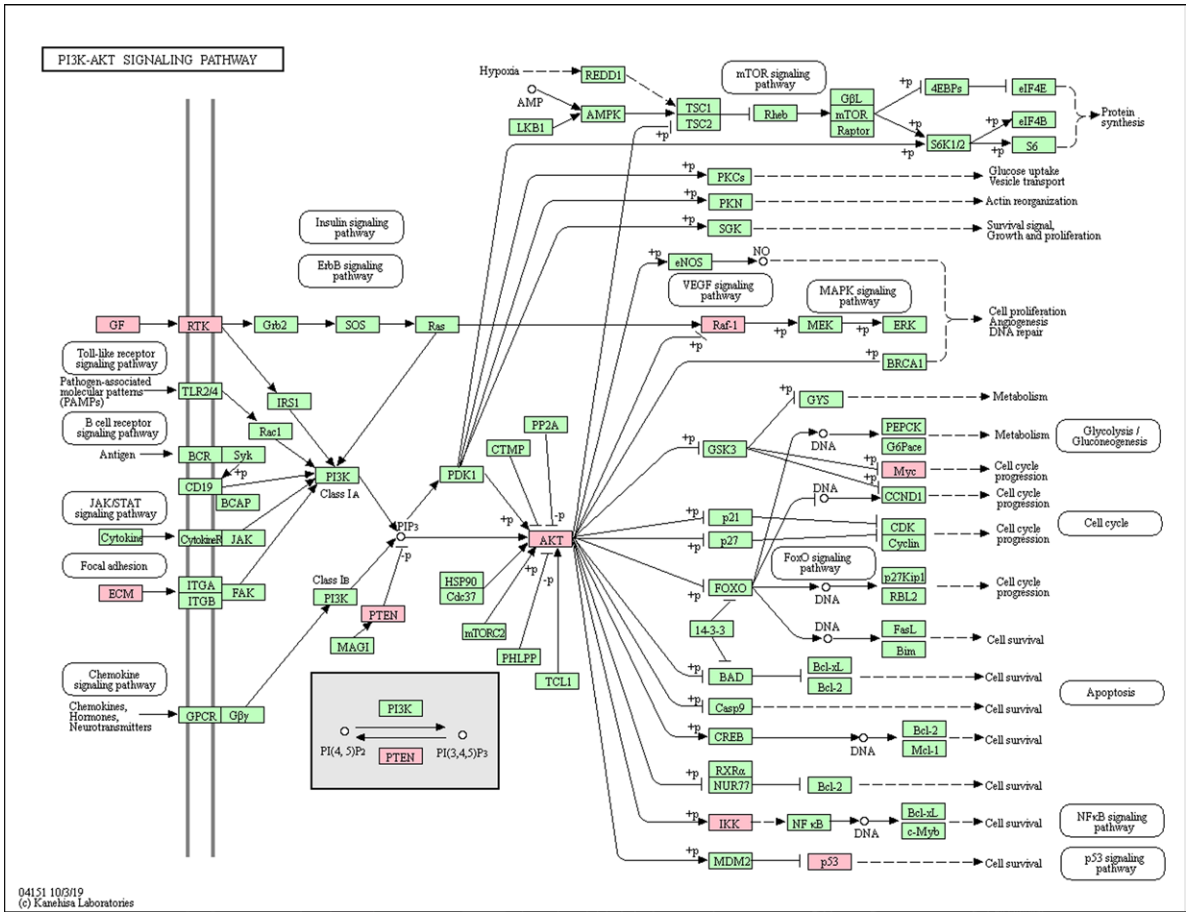

Figure 7. PI3K-Akt signaling pathway. Core targets are marked in red.

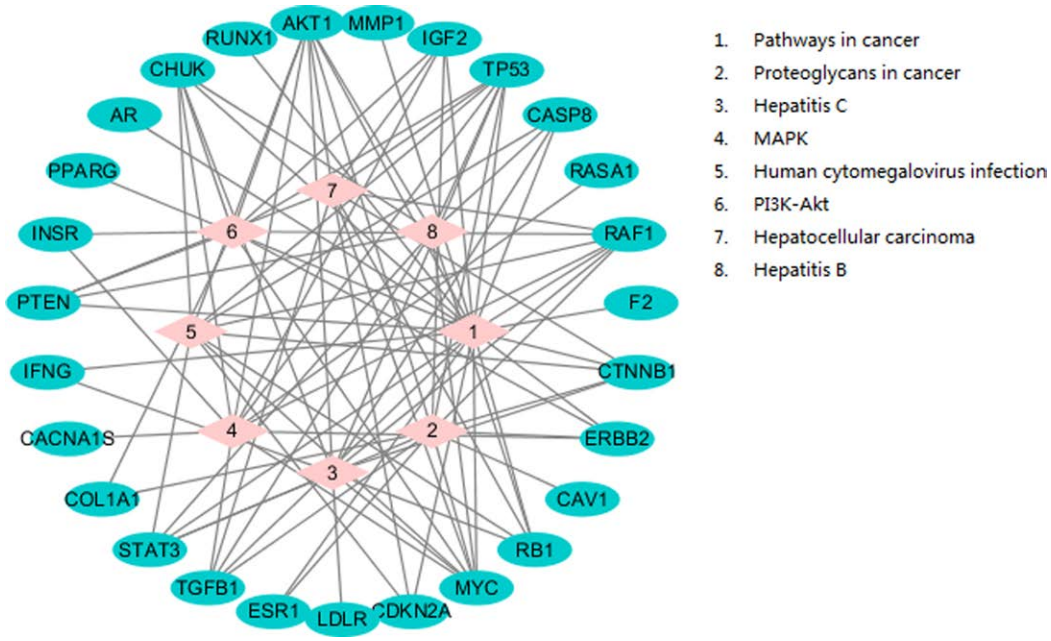

Figure 8. Targets-pathways network. The pink diamond nodes stand for major biological pathways; The blue ellipse nodes stand for the active component of GZD and the common target of MPS. The gray lines stand for the relationship between the target and the pathway.

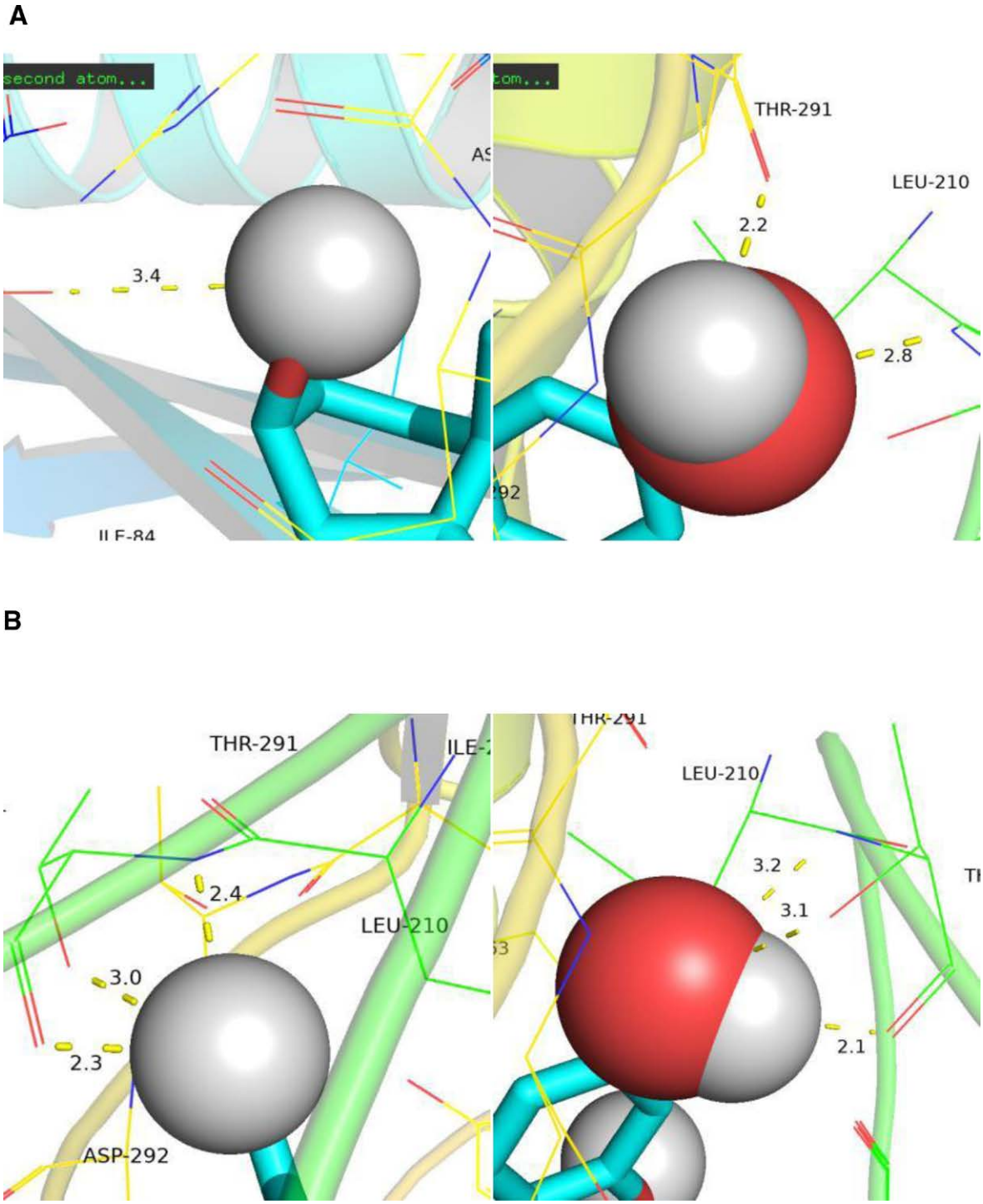

Figure 9. Molecular docking results of "bioactive compound-hub gene." (A) Quercetin to AKT1; (B) Kaempferol to AKT1; (C) β-sitosterolto AKT1; (D) Stepharine to AKT1.

Author contributions

Data curation: Jingtao Liang, Qian Zhang
Formal analysis: Jingtao Liang
Methodology: Qian Zhang, Ying Zhou
Supervision: Ying Zhou
Writing – original draft: Qian Zhang

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