

Network pharmacology-based screening of the active ingredients and mechanisms of evodiae fructus anti-glioblastoma multiforme

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Background: Evodiae fructus has been shown to have anti-glioblastoma multiforme (GBM) effects. However, its anti-GBM active components and mechanism remain unclear. In this study, the active components of evodiae fructus were screened by network pharmacology to explore the possible molecular mechanism of resistance to GBM.

Materials and methods: The main active ingredients of evodiae fructus were derived from Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) and Batch-traditional Chinese medicine (TCM). TCMSP and Swiss absorption, distribution, metabolism and elimination (ADME) predict genetic targets for ingredients that meet pharmacological criteria. GBM-related targets were obtained from DisGeNet, GeneCards, Online Mendelian Inheritance in Man (OMIM), Therapeutic Target Database (TTD), and TCGA. A Venn diagram was used to obtain the common targets of evodiae fructus and GBM. Protein-protein interaction (PPI) networks and component-disease target networks were constructed using Cytoscape 3.8.1 software for visualization. GBM gene differential expression was visualized by VolcanoR, and potential targets were enriched by Gene Ontology (GO) function and annotated by the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway by SRplot. Molecular docking verification was conducted using AutoDock Vina software.

Results: According to the screening conditions, 24 active components and 80 drug targets were obtained. The PPI network contains 80 proteins. The molecular docking verification showed the molecular docking affinity of the core active compounds in evodiae fructus with CASP3, JUN, EGFR, and AKT1.

Conclusions: This study preliminarily identified the various molecular targets and multiple pathways of evodiae fructus against GBM.

Abbreviations: BATMAN-TCM = Bioinformatics Analysis Tool for Molecular mechanism of Traditional Chinese Medicine, BP = biological process, CC = cellular component, DEGs = differentially expressed genes, EMT = epithelial-mesenchymal transition, GBM = Glioblastoma multiforme, GEPIA = Gene Expression Profiling Interactive Analysis, GO = Gene Ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes, MF = molecular function, OMIM = Online Mendelian Inheritance in Man, PPI = protein-protein interaction, TCM = Traditional Chinese Medicine, TCMSP = Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform, TTD = Therapeutic Target Database.

1. Introduction

Glioblastoma multiforme (GBM) is the most malignant brain tumor in adults and originates from glial cells. GBM is a highly invasive and lethal tumor of the central nervous system. It not only easily relapses but also has a high mortality rate.^[1,2] The 5-year survival rate for GBM is only 4 to 5%.^[3] Despite surgical reduction of the tumor and postoperative radiotherapy and chemotherapy, the median survival time of GBM is only 15 months. Residual tumor cells can be exempted and relapse

under traditional therapy.^[3] Corresponding to the complex immunosuppression and immune tolerance in patients with GBM, tumor cells have adapted to the immune environment and can reignite in a short time.^[4] Gene mutations, tumor stem cells and the tumor microenvironment are considered to be important factors leading to GBM diversity and chemoradiotherapy resistance.^[5] This is also a pivotal invalid reason for the current therapy system. Chinese medicine has been used for thousands of years, and its effects and side effects have been widely recognized.^[6] The extraction of active

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

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components from natural herbs is an important strategy in antitumor studies. Evodiae fructus is one of the traditional Chinese medicines and is used in the treatment of diseases of the nervous, digestive and blood systems.^[7,8] Rutaecarpine, evodiamine, and other chemical components of evodiae fructus have antitumor activity.^[8,9] Rutaecarpine can induce breast cancer cell apoptosis by affecting the cell signal transduction pathway^[10] and inhibit prostate cancer cell growth in mice.^[11] Evodiamine induces glioma cell apoptosis by inhibiting PI3K/AKT signaling and inducing MAPK phosphorylation to regulate apoptotic proteins.^[12] Rutaecarpine can also reverse multidrug resistance of tumors by inhibiting overexpression of (ATP)-binding cassette (ABC) subfamily B member 1 (ABCB1), thus achieving an adjuvant chemotherapy effect on tumors.^[13] Although the antitumor mechanism of evodiae fructus may have the characteristics of multiple targets^[9], the mechanism of action in glioblastoma multiforme has not been reported.

Molecular docking is an effective strategy for virtual drug screening of known ligands and molecular target structures.^[14] AutoDock software, a docking program based on free energy fields and fast Lamarckian genetic algorithms, can predict ligand-macromolecule interactions. The software's process involves removing water and ligands from the receptor structure, then adding hydrogen atoms and estimating the free energy of ligand binding to the target.^[15]

Based on network pharmacology and molecular docking, this study sought to identify the therapeutic target and possible mechanism of evodiae fructus in the treatment of GBM.

2. Materials and Methods

2.1. Active components and corresponding target collection

Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) <http://lsp.nwu.edu.cn/tcmsp.php>^[16] and the Bioinformatics Analysis Tool for Molecular Mechanism of Traditional Chinese Medicine (BATMAN-TCM) platform (<http://bionet.ncpsb.org/batman-tcm/>)^[17] integrated Chinese medicine-related compounds and protein targets. The potential evodiae fructus active ingredients of TCMSP were evaluated at oral bioavailability (OB) $\geq 30\%$, drug-likeness (DL) ≥ 0.18 and blood brain barrier (BBB) ≥ 0.3 . The score cutoff of BATMAN-TCM was ≥ 20 , and the *P* value was < 0.05 . The study was approved by the Ethics Committee of Peking University People's Hospital.

2.2. Further evaluating the drug activity of the compounds

The obtained compounds Canonical SMILES format were screened from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) then transported to Swiss absorption, distribution, metabolism and elimination (ADME) (<http://www.swissadme.ch/>).^[18] The information of compounds formula, molecular weight, GI absorption, BBB permeant, druglikeness were retrieved from SwissADME.

Druglikeness was no less than three “yes” for accept.

2.3. Screening target and candidate genes associated with GBM

Glioblastoma multiforme, as the Keyword, was screened from DisGeNet (<https://www.disgenet.org/>),^[19] GeneCards database (<https://www.genecards.org/>),^[20] Online Mendelian Inheritance in Man (OMIM) database (<https://omim.org/>),^[21] and Therapeutic Target Database (TTD) database (<http://db.idrblab.net/ttd/>)^[22] in the search related target protein. Repeated protein targets were deduplication processes. To

standardize the protein target information, the protein targets related to disease and evodiae fructus officinalis compound were imported into the UniProt database (<https://www.UniProt.org/>) to obtain a unified gene name. Differential expression gene information of GBM in the TCGA database was analyzed by Gene Expression Profiling Interactive Analysis (GEPIA).^[23] The intersection genes obtained were alternative targets shown by Venn diagram (<http://bioinformatics.psb.ugent.be/webtools/Venn/>).^[24] (see Table, Supplementary Content, <http://links.lww.com/MD/H437> which lists the related genes).

2.4. Construction of the protein-protein interaction (PPI) network between evodiae fructus and GBM

The candidate protein genes were input into the Search Tool for the Retrieval of the Interacting Genes (STRING) database (Search Tool for the Retrieval of the Interacting Genes, <https://string-db.org/>)^[25] for construction of the PPI network with species as “Homo sapiens” and confidence score > 0.4 . The data were also input into the Metascape platform (<http://metascape.org>) to calculate the three topological features of the network “degree”, “interdegree” and “betweenness”, and identify key genes.^[26] Cytoscape software (Version 3.8.1) was used for visualization of the PPI network.^[27] In the network, nodes of different colors and shapes represent components, and target genes and “edges” are the associations between nodes.

2.5. Differential analysis of GBM targets and key genes

Differentially expressed genes (DEGs) that were significantly different from the normal group in the GBM group. DEGs for GBM were obtained from GEPIA and analyzed by VolcanoR (<https://huygens.science.uva.nl/VolcanoR/>)^[28] based on two criteria (| Fold Change(log2) | > 1.5 and adj. *P* value < 0.05).

2.6. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

GO and KEGG enrichment were completed by the SRplot platform (<http://www.bioinformatics.com.cn/>). This is a freely accessible web server for data visualization and graphing based on the R-platform.^[29,30] Functionally, GO annotates key genes into three main terms, including cellular component (CCs), molecular function (MF), and biological process (BPs). KEGG enrichment analysis revealed the possible biological processes of key genes.

2.7. Molecular docking simulation

Pivotal target genes were included in the molecular docking simulation, which had good correlation with other genes and active components. The structure maps of these target genes were obtained from the Protein Data Bank (PDB). Molecular docking was processed with the free open source software Autodock_Vina (Version 1.2.0).^[31] The main processes include pretreatment of active components (ligands), removal of excess ligands from target proteins (macromolecules), crystal water molecules, and added hydrogenation atoms. Set the coordinates of the macromolecule docking box and then run AutoDock Vina to simulate molecular docking and calculate the docking energy of molecules with different conformations. The conformation with the highest hydrogen bond energy was used as the active component of the protein interaction. PYMOL software (version 2.5.2) was introduced for visualization.

Table 1**Main components of evodiae fructus.**

MOL	DataBases	Molecule ID	Molecule Name	OB%	BBB	DL	Chemical structure (2D)
MOL01	TCMSP	MOL003960	1-(5,7,8-Trimethoxy-2,2-dimethylchromen-6-yl)ethanone	30.39	0.75	0.18	
MOL02	TCMSP &BATMAN-TCM	MOL003950	1-Methyl-2-[(Z)-6-undecenyl]-4(1H)-quinolone	48.48	1.14	0.27	
MOL03	TCMSP	MOL003947	1-Methyl-2-[(Z)-pentadec-10-enyl]-4-quinolone	48.45	1.11	0.46	
MOL04	TCMSP &BATMAN-TCM	MOL004345	1-Methyl-2-Nonyl-4(1H)-Quinolone	31.54	0.82	0.5	
MOL05	TCMSP	MOL003972	1-Methyl-2-nonyl-4-quinolone	48.42	1.21	0.2	
MOL06	TCMSP &BATMAN-TCM	MOL003957	1-Methyl-2-pentadecyl-4-quinolone	44.52	1.05	0.46	
MOL07	TCMSP &BATMAN-TCM	MOL003964	1-Methyl-2-undecyl-4-quinolone	47.59	1.19	0.27	
MOL08	TCMSP	MOL003994	24-Methyl-31-norlanost-9(11)-enol	38	1	0.75	
MOL09	TCMSP	MOL001454	Berberine	36.86	0.57	0.78	
MOL10	TCMSP	MOL000359	Beta-sitosterol	36.91	0.87	0.75	
MOL11	TCMSP	MOL003956	Dihydrotutaecarpine	42.27	0.7	0.6	
MOL12	BATMAN-TCM	-	Echinopsine	-	-	-	
MOL13	TCMSP	MOL003974	Evocarpine	48.66	1.17	0.36	
MOL14	TCMSP &BATMAN-TCM	MOL004014	Evodiamide	73.77	0.81	0.28	
MOL15	TCMSP &BATMAN-TCM	MOL003958	Evodiamine	86.02	0.85	0.64	
MOL16	TCMSP	MOL004017	Fordimine	55.11	0.75	0.26	
MOL17	TCMSP	MOL004018	Goshuyamide I	83.19	0.64	0.39	
MOL18	BATMAN-TCM	-	Graveoline	-	-	-	
MOL19	BATMAN-TCM	-	Hydroxyevodiamine	-	-	-	
MOL20	BATMAN-TCM	-	Isoevodiamine	-	-	-	
MOL21	TCMSP	MOL004025	N-(2-Methylaminobenzoyl)tryptamine	56.96	0.8	0.26	
MOL22	BATMAN-TCM	-	N,N-Dimethyl-5-Methoxy Tryptamine	-	-	-	
MOL23	BATMAN-TCM	-	Rutaecarpine	-	-	-	
MOL24	TCMSP	MOL000359	Sitosterol	36.91	0.87	0.75	

BATMAN-TCM = Bioinformatics Analysis Tool for Molecular mechANism of Traditional Chinese Medicine, BBB = blood brain barrier, DL = drug-likeness, OB = oral bioavailability, TCMSP = Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform. "-" refers to data absent in the TCMSP, exist in SwissADME in Supplementary Data, <http://links.lww.com/MD/H437>.

This study was approved by the ethics committee of Peking University People's Hospital.

3. Results

3.1. Evodiae fructus components and candidate genes associated with GBM

After searching, filtering and deduplication processes, 24 presumed components were screened from the TCMSP database and BATMAN-TCM database (see Table 1). A total of 383 target genes interacting with these predicted components were collected. In addition, 2941 GBM-related genes were obtained from the DisGeNet, GeneCards, OMIM, and TTD databases, and 7648 DEGs were obtained from the TCGA database. To further study the mechanism of evodiae fructus treatment of GBM, a total of 80 crossover genes were collected and named candidate genes (Fig. 1.; see Table, Supplementary Content, <http://links.lww.com/MD/H437> which lists the related genes). The network of evodiae fructus components and GBM candidate target genes was created by Cytoscape software 3.8.2 (Fig. 2).

3.2. PPI network construction of evodiae fructus GBM targets

The PPI network of evodiae fructus targets was obtained by inputting the targets to the STRING11.0 platform and weighted by Cytoscape 3.8.2, as shown in Figure 3a.

Genes with high "degree", "betweenness", and "closeness" (above median) values were selected as evodiae fructus's key targets for GBM. Finally, AKT1, CASP3, JUN, and EGFR were screened as the four pivotal target genes (Fig. 3b).

3.3. Pivotal target genes in DEGs

DEGs for GBM were obtained from GEPIA and analyzed by VolcanoR. The 4 pivotal target genes were also marked in

a volcano plot (Fig. 4). CASP3, JUN, EGFR, and AKT1 were significantly upregulated in the GBM group of TCGA.

3.4. GO enrichment analysis

To illustrate the biological mechanism of E. fructus in glioblastoma multiforme, candidate genes were imported into

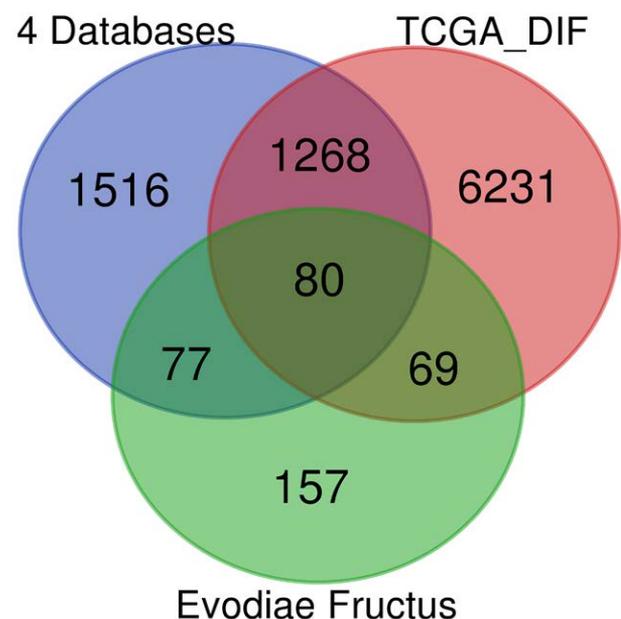


Figure 1. Venn map of intersection targets in evodiae fructus and glioblastoma multiforme. The blue circle indicates GBM targets screened from 4 disease databases (DisGeNet, GeneCards, OMIM, and TTD), the red circle indicates differentially expressed genes of GBM from TCGA database, and the green circle indicates the targets of evodiae fructus. GBM = Glioblastoma multiforme.

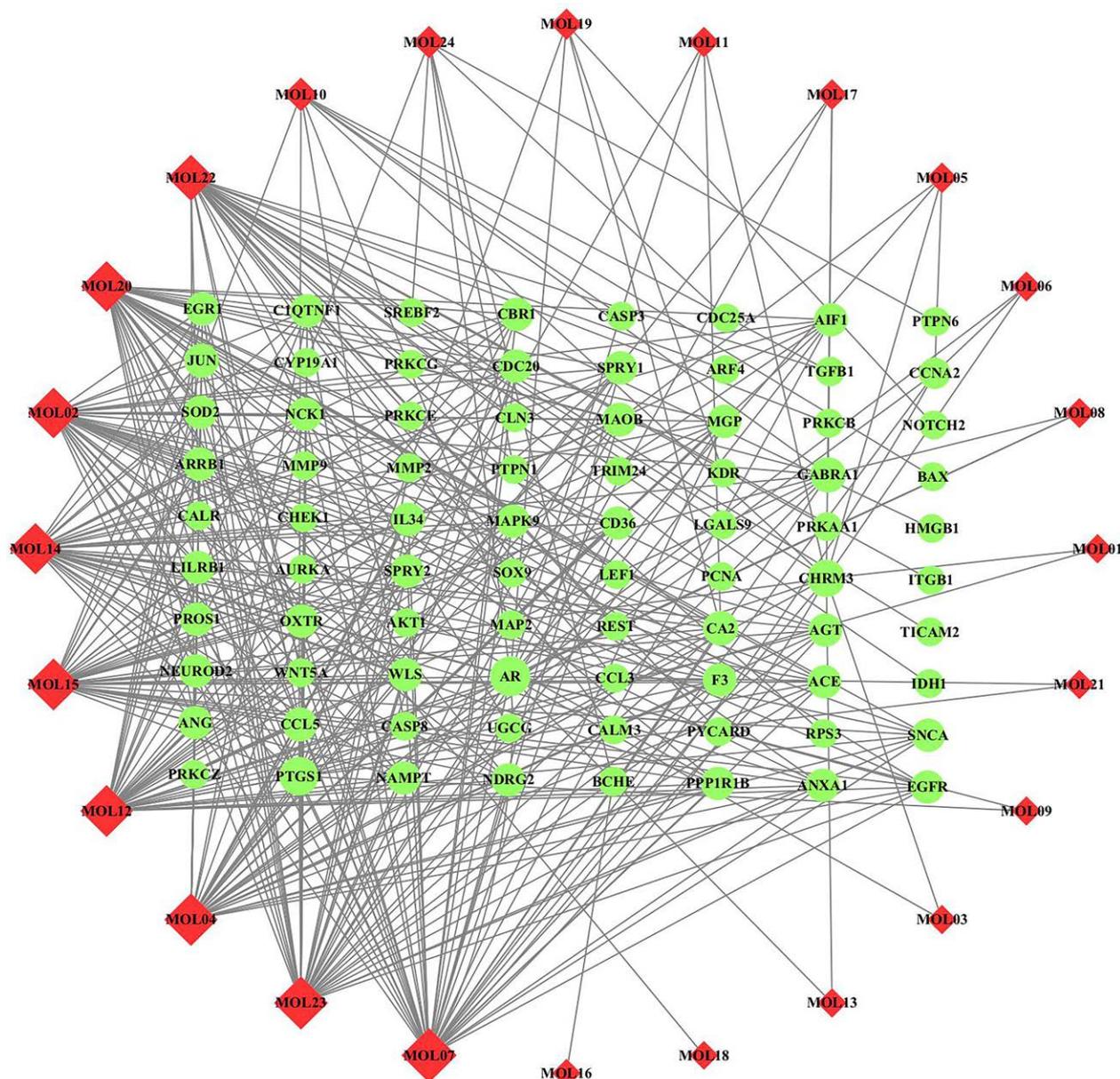


Figure 2. Evodia fructus components and GBM target network created by Cytoscape 3.8.2 (<https://cytoscape.org/>). The red diamond node represents evodia fructus components, and the green round node represents GBM targets. GBM = Glioblastoma multiforme.

the SRplot platform, and the results are shown (Fig. 5a). BPs, CCs, and MFs showed 10 significant enrichments ($P < .01$). The significantly enriched BPs were ERK1 and ERK2 cascade GO:0070371, Regulation of ERK1 and ERK2 cascade GO:0070372, and cellular response to chemical stress GO:0062197. The significant enrichments in CCs were collagen-containing extracellular matrix GO:0062023, leading edge membrane GO:0031256 and distal axon GO:0150034. The significant enrichments in MFs were protein kinase C activity GO:0004697, calcium-dependent protein kinase C activity GO:0004698, and calcium-dependent protein serine/threonine kinase activity GO:0009931.

3.5 KEGG pathway enrichment analysis

The enrichment of KEGG revealed the possible signaling pathway by which *E. fructus* played an anti-GBM role. A total of 10 significant signaling pathways ($P < .01$) were screened out

by KEGG analysis (Fig. 5b), including the age-rage signaling pathway in diabetic complications HSA04933, hepatitis B HSA05161, lipid and atherosclerosis HSA05417, and proteoglycans in cancer HSA05205. Hsa04933 and HSA05205 had more target enrichment and lower P values in GBM, which were key pathways highly correlated with tumor growth.

3.6. Molecular docking results and analysis

The 4 selected core targets were combined with five presumed components of *E. fructus* (Table 2). It is generally believed that the structural stability of small molecule ligand compounds and large molecule receptor proteins is determined by the binding energy between them. The lower the binding energy is, the greater the possibility of free binding. There were strong interactions between MOL10 and Casp3, MOL22, and AKT1, MOL04, MOL07, MOL23, and EGFR, and JUN, and the binding energy was less than 0. These results

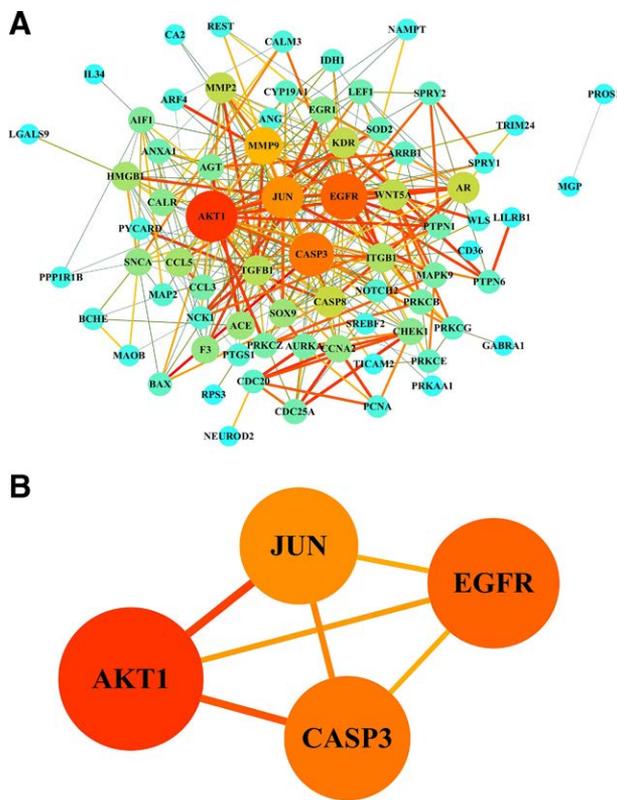


Figure 3. The PPI network exported from the STRING (<https://string-db.org/>) database and weighted by Cytoscape 3.8.2 (A). The pivotal target genes were screened by the network parameters “degree”, “betweenness”, and “closeness” (B). The size and color of nodes were weighted by degree, and the width and color of edges were weighted by betweenness. PPI = protein-protein interaction, STRING = Search Tool for the Retrieval of the Interacting Genes.

also indicated that the five compounds of *E. fructus* might be the main components that inhibit GBM and act through key target proteins (Fig. 6).

4. Discussion

A total of 24 components of *E. fructus* were screened out by the network pharmacology method, with good oral bioavailability, drug-likeness and blood-brain barrier permeability. After the combined screening of DisGeNet, GeneCards, OMIM, TTD, TCGA database, and evodiae fructus component-targeted genes, a total of 80 candidate genes were found to be related to 24 drug components. The key target genes AKT1, EGFR, CASP3, and JUN were identified based on PPI network analysis. Key target genes guide the drug components 1-methyl-2-NonYL-4 (1H)-quinolone (MOL04), 1-methyl-2-undecyl-4-quinolone (MOL07), beta-sitosterol (MOL10), N,N-dimethyl-5-methoxy tryptamine (MOL22), and rautacarpine (MOL23).

EGFR is significantly overexpressed among differentially expressed genes in GBM in the TCGA database. EGFR is a transmembrane glycoprotein, a member of the protein kinase superfamily that promotes GBM proliferation, invasion, and drug resistance^[32] and regulates multiple GBM signaling pathways with a global model.^[33] EGFR expression upregulation could further activate the downstream ERK1/2 system, resulting in cell proliferation, activating the PI3K/Akt/mTOR system to maintain cell survival and develop drug resistance,^[34] and reducing the damage to tumor cells by oxidative stress of radiotherapy and chemotherapy.^[35]

AKT1 is a serine/threonine kinase that is an important protein in the PI3K/Akt/mTOR signaling pathway and maintains tumor survival.^[36] Although there was no more than 2 times higher expression of AKT1 in the GBM group than in the control group, the difference in expression between the 2 groups was still significant. Growth factor receptor tyrosine kinase (RTK) and G protein-coupled receptor activation leads to phosphorylation of phosphatidylinositol 4,5-diphosphate (PIP 2) to produce phosphatidylinositol 3,4,5-triphosphate (PIP 3).^[37]

Gene Difference Expression of GBM (TCGA)

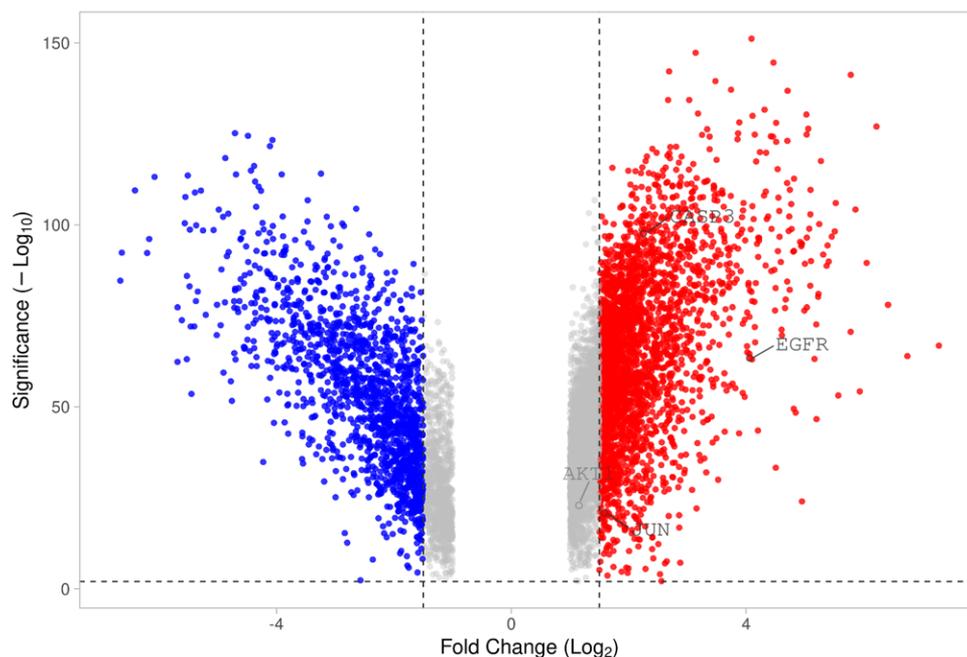


Figure 4. Differentially expressed genes of GBM (TCGA), CASP3, JUN, EGFR and AKT1 were upregulated in GBM. GBM = Glioblastoma multiforme.

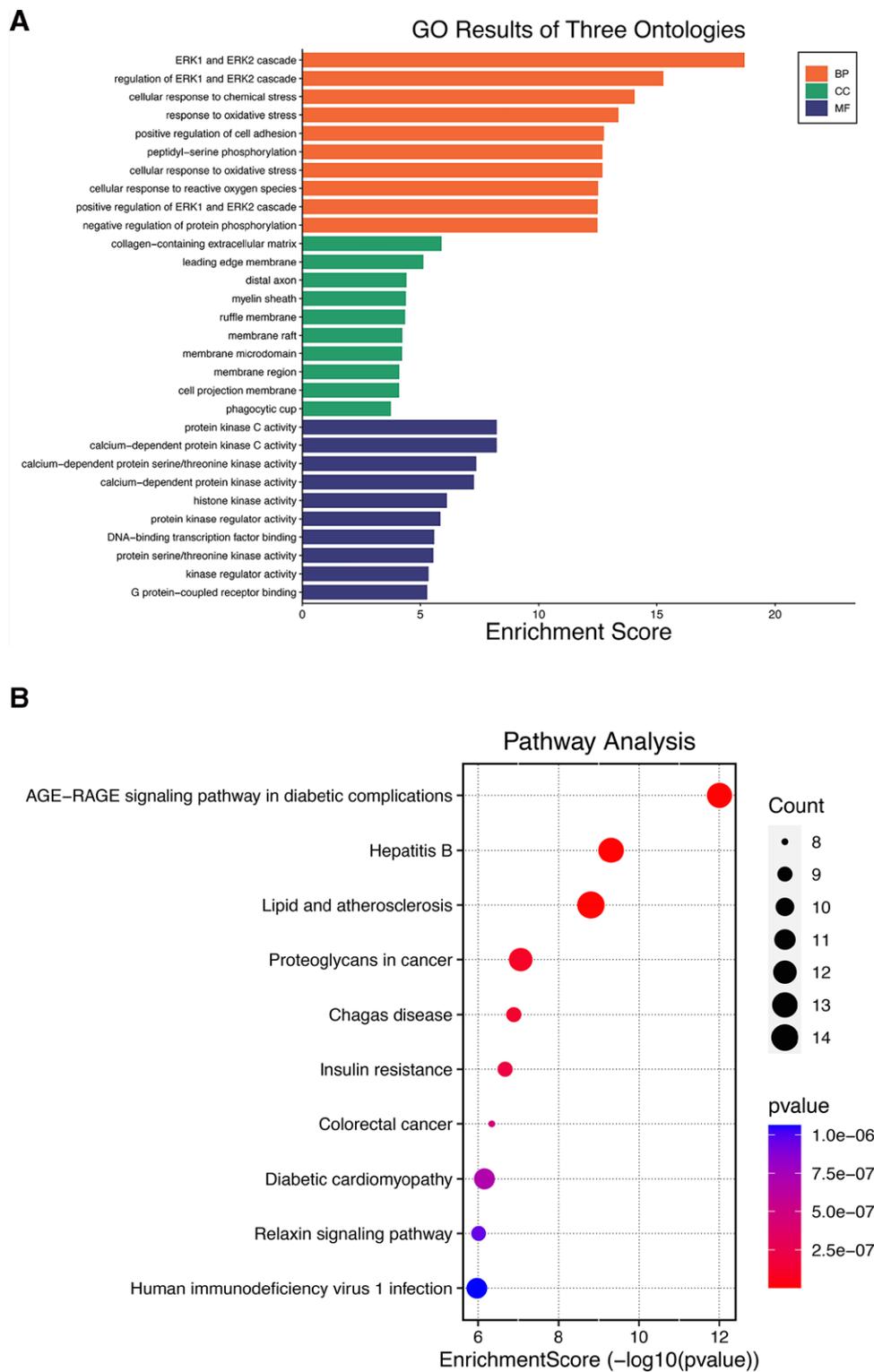


Figure 5. GO and KEGG enrichment. GO results of three ontologies 5A. KEGG pathway analysis 5B. GO = Gene Ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes.

PIP3 phosphorylates AKT amino acids Thr308 and Ser473 to activate this enzyme, which leads to phosphorylation of downstream host cell proteins, including GSK3 α , GSK3 β , FoxO transcription factor, MDM2, BAD, and p27KIP1. These proteins promote cell survival and activate the cell cycle.^[36] Inhibition of Akt/mTOR phosphorylation can result in autophagy and apoptosis in U251 and C6 glioma cell lines.^[38] Activation of the PI3K/Akt/mTOR pathway promotes GBM drug resistance

and weakens the efficacy of chemotherapy, such as temozolomide (TMZ).^[34] AKT is also an important molecule of the AGE-RAGE signaling pathway, and inhibition of AKT can reduce cell glucose uptake and further activate the Ins/IGF-IR pathway to maintain tumor cell survival.^[36,39]

CASP3 is a member of the cysteine-aspartate protease (Caspase) family. CASP3 is generally believed to mediate apoptosis induced by radiotherapy or immunotherapy. However, the

Table 2**The binding energy of molecular docking in evodiae fructus compounds.**

MOL	Compound	Molecular formula	Molecular weight (g/mol)	CAS	Target protein	PBDID	Amino acid residue and deoxynucleoside	Binding energy (KJ/mol)	
MOL10	Beta-sitosterol	C29H50O	414.7	83-46-5	Casp3	1QX3	GLU248	-28.451	a
MOL22	N,N-Dimethyl-5-Methoxy Tryptamine	C13H18N2O	218.3	1019-45-0	AKT1	3O5S	ASN296	-27.196	b
MOL04	1-Methyl-2-Nonyl-4(1H)-Quinolone	C19H27NO	285.4	68353-24-2	EGFR	3W32	GLY721	-26.359	c
MOL04	1-Methyl-2-Nonyl-4(1H)-Quinolone	C19H27NO	285.4	68353-24-2	JUN	5T01	DC3	-17.154	d
MOL07	1-methyl-2-undecyl-4-quinolone	C21H31NO	313.5	59443-02-6	EGFR	3W32	GLY719	-28.033	e
MOL07	1-Methyl-2-undecyl-4-quinolone	C21H31NO	313.5	59443-02-6	JUN	5T01	DA28	-23.849	f
MOL23	Rutaecarpine	C18H13N3O	287.3	84-26-4	EGFR	3W32	ALA743	-48.585	g
MOL23	Rutaecarpine	C18H13N3O	287.3	84-26-4	JUN	5T01	DA37	-39.330	h

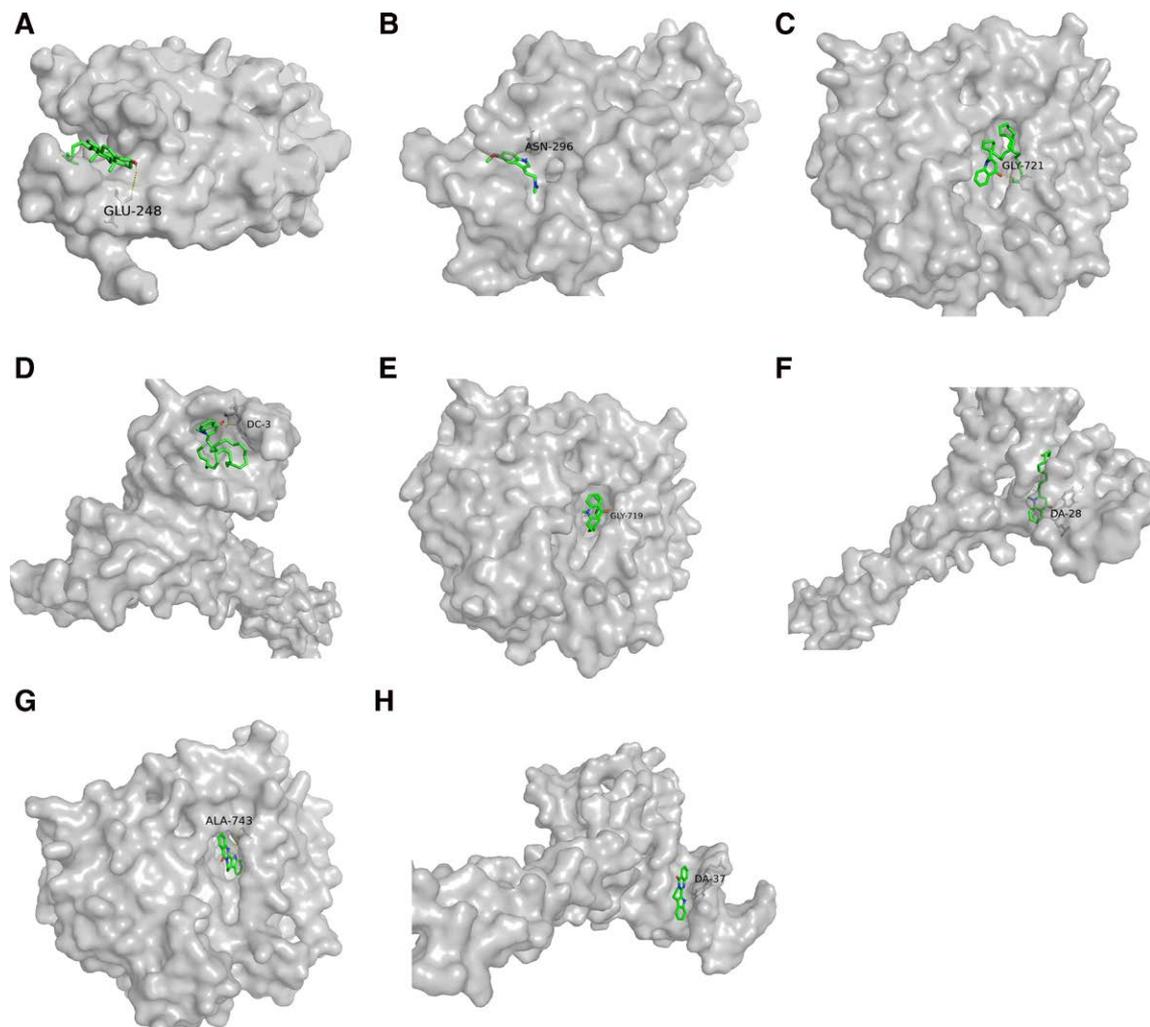


Figure 6 . The results of molecular docking between evodiae fructus main components and genes Casp3, AKT1, EGFR and JUN. The green molecule is evodiae fructus main component which docked with the gray molecule-amino acid residue of target protein, the yellow dotted line is the hydrogen bond. (A) The molecular docking between MOL10 and Casp3, and the bonding amino acid residue is GLU248; (B) the molecular docking between MOL22 and AKT1, and the bonding amino acid residue is ASN296; The molecule MOL04 docking with EGFR (C) and JUN (D), and the bonding amino acid residues are GLY721 and DC3, respectively; The molecule MOL07 docking with EGFR (E) and JUN (F) and the bonding amino acid residues are GLY719 and DA28, respectively; The molecule MOL23 docking with EGFR (G) and JUN (H), the bonding amino acid residues are ALA743 and DA37, respectively. Append chart: The important data processing for the results. GBM-4database: GBM target genes retrieved from disease databases DisGeNet, GeneCards, OMIM, and TTD databases. All the target genes were unformed by uniprot database. GBM-TCGA: GBM difference expression genes screened from TCGA database. Batmantcm-compounds&genes: The components of evodiae fructus and related target gene screened from TCMSp database. The absent data in Table 1: The compounds datas of "Molecule ID", "OB%", "BBB" and "DL" absent in the TCMSp, while exist in BATMAN-TCM, these parameters were matched and verified by SwissADME database. go-bp: The biological process of the 80 candidate genes were annotation in Gene Ontology. go-cc: The cellular component of the 80 candidate genes were annotation in Gene Ontology. go-mf: The molecular function of the 80 candidate genes were annotation in Gene Ontology. kegg: The 80 candidate genes were enrichment analysis by KEGG.

high expression of CASP3 in some tumors has also attracted attention.^[40-43] Upregulation of CASP3 expression in GBM in this study is not a rare event. Recent studies suggest that CASP3 protein has a nonapoptotic effect and promotes tumor recurrence and angiogenesis.^[40,41] Tumor cells with CASP3 knockout were more sensitive to chemoradiotherapy, and epithelial-mesenchymal transition (EMT) transformation was inhibited.^[42] Although the significance of upregulation of CASP3 expression in GBM is not clear in the literature, it needs further study as a potential target.

JUN is a proto-oncogene encoding the product c-Jun, which is elevated by growth factors, cytokines, cellular stress, and ultraviolet radiation stimulation. JUN plays a key role in cell proliferation, cell death and malignant transformation in the ERK pathway,^[44] and MAPK pathway.^[45] The c-Jun protein is an important activating factor of GBM, which is consistent with its role in cell proliferation, apoptosis, and tumor promotion.^[46]

In conclusion, the above molecules are located in the ERK1/2 cascade or are related to its regulation, participating in the cellular response to chemical stress, the response to oxidative stress, tumor cell proliferation, angiogenesis, tumor invasion, drug resistance, and EMT.

1-Methyl-2-Nonyl-4(1H)-Quinolone and 1-methyl-2-undecyl-4-quinolone are quinolone alkaloids isolated from *evodia fructus*^[47] and have cytotoxic effects against hL-60, N-87, H-460 and HepG tumor cells.^[48] Quinolones and their derivatives have both antibacterial activity and antitumor activity.^[49] In this study, we found that they have strong binding energy with Jun and EGFR, which may be a new anti-GBM target. N,N-Dimethyl-5-methoxy tryptamine has tumor cytotoxicity.^[50] We deduced that the binding of the compound and AKT1 residue ASN²⁹⁶ may alter the local conformation and reduce the phosphorylation of Thr308, thereby affecting the activation of AKT1. Beta-sitosterol is a plant active ingredient that has been proven to inhibit tumor growth and induce apoptosis in glioma, liver cancer, colon cancer and pancreatic cancer.^[51,52] Through molecular docking, it was found that beta-sitosterol binds CASP3, which may inhibit GBM drug resistance and EMT. Rutaecarpine has a biological inhibitory effect on JUN in the central nervous system.^[53] Rutaecarpine can bind aryl hydrocarbon receptor (AhR) and affect the migration of glioblastoma U87 cells.^[54] The binding of rutaecarpine with EGFR and JUN may directly inhibit the activity of GBM cells and inhibit EMT.

However, due to the limitations of network pharmacology and the complexity of traditional Chinese medicine components, this study could only preliminarily explain the *Evodia ruevodia* inhibition of GBM by bioinformatics and mass data calculation results. Based on the results, the main regulatory targets of *Evodia ruevodia* should be screened and verified in vivo or in vitro.

5. Conclusions

Evodia fructus may inhibit GBM growth, resist drug resistance, assist radiotherapy and chemotherapy, and inhibit EMT through the AKT1, EGFR, CASP3, and JUN tumor-related signaling pathways. As a traditional Chinese medicine, *evodia fructus* has the characteristics of multiple components and multiple regulatory targets, and there is a complex regulatory network between components and targets. The whole network can be adjusted by regulating single or multiple important targets in the network, which is also consistent with the holistic organic concept of traditional Chinese medicine (TCM). This study provides a new idea for the development of new anti-GBM active components from *evodia fructus*.

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