



Exploring the Action Mechanism of Yadanzi (*Brucea javanica*) in the Treatment of Glioblastoma Based on Bioinformatics and Network Pharmacology

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Abstract

Objective The aim of the study is to explore the molecular mechanism of Yadanzi (*Brucea javanica*) in the treatment of glioblastoma (GBM) by using the methods of bioinformatics and network pharmacology.

Methods The Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) and literature retrieval method were applied to obtain the active ingredients of Yadanzi (*Brucea javanica*), and to predict the relevant targets of the active ingredients. The GBM-related targets were retrieved and screened through the Gene Expression Profiling Interactive Analysis (GEPIA) database, and mapped to each other with the targets of the components of Yadanzi (*Brucea javanica*) to obtain the intersection targets. The GBM differentially expressed gene targets were imported into the String database to obtain the protein interaction relationship, the Cytoscape software was used to draw the protein interaction network, the Cytobba and MCODE plug-ins were used to screen the core genes and important protein interaction modules, and the GEPIA database was applied to make survival analysis of the core genes. The network map of “active ingredients-targets” was constructed through the Cytoscape 3.6.1 software. Gene Ontology (GO) biological function enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway enrichment analysis for GBM differentially expressed genes were performed through the DAVID database.

Results Through TCMSP and literature retrieval, 23 potential active ingredients and 129 related targets were obtained from Yadanzi (*Brucea javanica*). In the GEPIA database, 247 GBM differentially expressed genes were screened, including 113 up-regulated genes and 134 downregulated genes. After mapping with the targets related to the active ingredients of Yadanzi (*Brucea javanica*), six intersection targets were obtained, that is, the potential action targets of Yadanzi (*Brucea javanica*) in treating GBM, including *MMP2*, *HMOX1*, *BIRC5*, *EGFR*, *CCNB2*, and *TOP2A*. Cytoscape software was applied to build an “active ingredient-action target” network. Two active ingredients

Keywords

- ▶ Yadanzi (*Brucea javanica*)
- ▶ glioblastoma
- ▶ bioinformatics
- ▶ network pharmacology
- ▶ action mechanism

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and five action targets of β -sitosterol (BS) and luteolin were found, and the targets were mainly concentrated in BS. It was found by KEGG pathway enrichment analysis that GBM differentially expressed genes were mainly involved in signaling pathways related to *Staphylococcus aureus* infection, phagosome formation, tuberculosis and systemic lupus erythematosus and other infectious and autoimmune diseases. It was found by GO enrichment analysis that the GBM differentially expressed genes mainly involved such biological processes (BP) as the processing and presentation of exogenous antigenic peptides and polysaccharide antigens through MHC II molecules, γ -interferon-mediated signaling pathways, extracellular matrix composition, and chemical synapses transmission; it involved cellular components such as cell junctions, axon terminal buttons, extracellular space, vesicle membranes for endocytosis, and MHC II protein complexes; molecular functions such as calcium-mediated ionic protein binding, MHC II molecular receptor activity, immunoglobulin binding, and phospholipase inhibitor activity were also involved. Survival analysis was conducted by GEPIA on the top 37 core targets in degree value, and a total of five genes related to GBM prognosis were obtained. Among them, *FN1* and *MMP2* were highly expressed while *GABRD* (γ -aminobutyric acid A receptor delta subunit), *RBFOX1*, and *SLC6A7* were expressed at a low level in cancer patients.

Conclusion The pathogenesis of GBM is closely related to the human immune system, and BS and luteolin may be the main material basis of Yadanzi (*Brucea javanica*) for the treatment of GBM and the improvement of prognosis. The molecular mechanism may be related to the physical barrier formed by destroying the tumor cell stromal molecules and its involvement in tumor immune response.

Introduction

Glioblastoma (GBM) is a kind of malignant tumor with the highest incidence in the central nervous system. Due to its low degree of differentiation, rapid proliferation, and strong invasiveness, comprehensive treatment such as surgical resection combined with chemoradiotherapy is difficult to change its malignant progression. However, temozolomide is the only first-line chemotherapy drug that can pass the blood–cerebrospinal fluid barrier and has been used so far,¹ so, the prognosis of GBM is extremely poor. The median survival time of high-grade GBM after standardized treatment is only 14.6 months,² and the 5-year survival rate is still less than 10%. Along with the pancreatic cancer, it is a kind of malignant tumor which is most difficult to cure.³ The diagnosis of GBM relies on neuroimaging and tissue biopsy. Although some recent studies have proposed some diagnostic markers for the diagnosis and judgment of prognosis of GBM, such as tenascin C, nestin, vascular endothelial growth factor, etc.,^{4–6} the current status of the difficulty in treatment and poor prognosis is still gloomy.

The Chinese herb Yadanzi (*Brucea javanica*) is the dried and mature fruit of *Brassica chinensis*, which is bitter and cold in nature, with the effects of killing parasites and relieving dysentery, fighting against malaria and tumor.^{7,8} In 1978, China began to develop Yadanzi (*Brucea javanica*) oil intravenous emulsion to treat various malignant tumors.⁹ At present, Yadanzi (*Brucea javanica*) oil emulsion injection

has been widely used in the adjuvant treatment of GBM,^{10,11} which has the effect of enhancing efficacy and reducing toxicity. Although the curative effect of Yadanzi (*Brucea javanica*) oil emulsion in the treatment of GBM is relatively definite, the composition of Yadanzi (*Brucea javanica*) is complex, and the molecular mechanism of anti-GBM needs further exploration. Now data mining and integration can quickly and effectively screen differentially expressed genes between tumor tissue and normal tissue, and at the same time, clinical data can be used to obtain the relationship between target gene expression levels and patient's survival prognosis, which will help provide important clinical evidence for finding reliable therapeutic targets and prognostic indicators and will open up new horizons for studying the progression and recurrence of GBM after the treatment.

Due to the complex composition of Chinese herb, multiple pathways, and multiple targets of its action, it is relatively difficult to clarify the material basis and action mechanism. In this study, the methods of bioinformatics and network pharmacology were used to screen out the biomarkers that may be involved in the malignant progression of GBM from the Gene Expression Profiling Interactive Analysis (GEPIA) database.¹² Survival analysis was performed on the core genes. Meanwhile, the active ingredients and targets of Yadanzi (*Brucea javanica*) were obtained by screening with the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP), and they mapped

to the GBM disease targets, aiming to explore the key targets of Yadanzi (*Brucea javanica*) in the treatment of GBM and provide a theoretical basis for in-depth exploration of the molecular mechanism of GBM.

Materials and Methods

Screening and Target Prediction of Active Ingredients of Yadanzi (*Brucea javanica*)

The TCMSP database was used to obtain the chemical constituents of Yadanzi (*Brucea javanica*). Taking oral bioavailability (OB) and drug likeness (DL)¹³ as parameters, the active ingredients that met the condition of both OB $\geq 30\%$ and DL ≥ 0.18 and the active ingredients reported in the literature were screened out. The TCMSP target prediction model was used to predict the targets of the active ingredients.

Select the cancer type “GBM” in the GEPIA database, set “|Log2FC|” > 4 , “*q*-value” < 0.05 , select the calculation method “ANOVA,” set the gene distribution as “Both,” and search for reported genes associated with GBM. The genes and the components’ targets of Yadanzi (*Brucea javanica*) were mapped to each other, and the intersection targets were obtained, which were the main targets of Yadanzi (*Brucea javanica*) in the treatment of GBM. The names of the main targets of Yadanzi (*Brucea javanica*) in the treatment of GBM were corrected and unified by using the UniProt (<http://www.uniprot.org/>) database.

Construction of Protein Interaction Network

Import the GBM differentially expressed gene targets into the String database ([https://string-db.org/Version 10.5](https://string-db.org/Version%2010.5)),¹⁴ limit the study species to human, obtain the protein interaction relationship, save it in TSV format, and import the file into Cytoscape software to draw the protein interaction network, further use Cytobba and MCODE plug-ins to screen core genes, and obtain important modules of protein interaction network and protein interaction relationship.

Gene Ontology (GO) Biological Function Enrichment Analysis and KEGG Signaling Pathway Enrichment Analysis

The retrieved GBM differentially expressed genes were imported into the DAVID database for functional annotation, and the species was limited to human. The differentially expressed genes were subjected to GO biological function enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway enrichment analysis. OmicShare (<http://www.omicshare.com>) online software was used to draw the advanced bubble chart of KEGG signaling pathway. Set the threshold $p < 0.05$, and screen the top 15 pathways for enrichment; the results of GO biological function enrichment analysis were sorted using Cytoscape according to the number of targets involved, and the bar graphs of BP, cellular component (CC), and molecular function (MF) were drawn.

Survival Analysis

Survival analysis was performed on the core genes using GEPIA database, and the log value and risk ratio of 95%

confidence interval were calculated and displayed in a prognostic map. $p < 0.05$ was considered statistically significant. The GEPIA database was used to verify the expression levels of prognosis-related genes, and at least one “***” ($p < 0.05$) was considered statistically significant.

Construction of “Active Ingredient-Action Target” Network

Cytoscape 3.6.1 software was used to build the relationship network model of “active ingredient-action target.” The nodes represented the potential active ingredients and potential targets of Yadanzi (*Brucea javanica*). The edge was used to connect the active ingredients and the action targets, showing the connection between the active ingredients and the action targets. Based on the constructed network, the main constituents and key action targets of Yadanzi (*Brucea javanica*) in the treatment of GBM were discussed.

Results

Screening of Active Ingredients of Yadanzi (*Brucea javanica*)

Through database retrieval and literature review, a total of 67 chemical constituents of Yadanzi (*Brucea javanica*) were collected, and 23 potential active constituents were obtained through further screening by OB $\geq 30\%$ and DL ≥ 0.18 ¹⁵ and literature research¹⁵⁻¹⁸ (► Table 1).

Screening of Action Targets of Yadanzi (*Brucea javanica*) in the Treatment of GBM

In the GEPIA database, 247 GBM differentially expressed genes were screened, including 113 upregulated genes (Log2FC > 0) and 134 downregulated genes (Log2FC < 0). Through the TCMSP database, 129 targets related to the potential active ingredients of Yadanzi (*Brucea javanica*) were obtained. After mapping the 247 differentially expressed genes with 129 targets related to active ingredients of Yadanzi (*Brucea javanica*), six potential targets for the treatment of GBM were finally obtained, namely MMP2, HMOX1, BIRC5, EGFR, CCNB2, and TOP2A (► Fig. 1).

“Active Ingredient-Action Target” Network Construction and Analysis

An “active ingredient-action target” network was constructed by using Cytoscape software. The network graph has eight nodes and 13 edges, of which the green diamonds represent two active ingredients, namely β -sitosterol (BS) and luteolin; the red circles represent six targets, which are mainly concentrated on BS, indicating that β -sitosterol may be the main material basis for the treatment of GBM by Yadanzi (*Brucea javanica*) (► Fig. 2).

Construction and Analysis of Protein Interaction Network

The screened 247 GBM differentially expressed genes were entered into the String database, the specie was selected as “homo sapiens,” the TSV file was exported, and the Cytoscape software was used to obtain the interaction network

Table 1 Information table of potential active ingredients of Yadanzi (*Brucea javanica*)

No.	Chemical constituents	Relative molecular weight	OB/%	DL
1	β -sitosterol	414.79	36.91	0.75
2	Luteolin	286.25	36.16	0.25
3	Brusatol	520.58	45.69	0.75
4	BruceosideA_qt	520.58	31.05	0.75
5	Yadanzioside B	700.76	46.16	0.31
6	Yadanzioside H	686.78	62.77	0.32
7	Yadanzioside I	642.67	61.13	0.38
8	Yadanzioside J	700.76	38.7	0.3
9	Yadanzioside L	726.8	31.37	0.27
10	Yadanzioside M	704.74	45.04	0.23
11	Yadanzioside P	710.8	58.76	0.29
12	Bruceoside B	682.74	56.54	0.32
13	Yadanziolide C_qt	564.64	31.8	0.66
14	Yadanziolide D	396.43	55.76	0.65
15	Bruceine C	564.64	31.38	0.66
16	Bruceaketolic acid	482.53	11.45	0.79
17	Olein	885.61	27.27	0.13
18	Bruceantin	548.64	13.97	0.7
19	Bruceantinol	606.68	11.13	0.57
20	Bruceine A	522.6	19.88	0.75
21	Bruceine B	480.51	16.46	0.81
22	Bruceine D	410.46	15.99	0.75
23	Brucine	394.51	7.61	0.41

between the targets. A total of 207 valid nodes and 1,477 edges were obtained. Cytoscape plugin was used to draw nodes with different shades of color according to the degree value of the nodes. The redder the color is, the greater the degree value and the more complex the protein interaction are. Then the MCODE plugin was applied to further analyze and obtain three core modules (►Figs. 3 and 4).

GO Biological Function Enrichment Analysis and KEGG Signaling Pathway Enrichment Analysis

GO enrichment analysis and KEGG pathway enrichment analysis were performed on 247 effectively differentially expressed genes in GBM through the DAVID website. Set the threshold value $p < 0.05$, screen the top 15 pathways, and use the OmicShare (<http://www.omicshare.com>) online software to draw a high-level bubble chart of the KEGG pathway. The larger the point is, the more enriched the genes are. The smaller the p -value is, the redder the color is, indicating the closer the relationship with the incidence of GBM. The results of pathway analysis indicated that the differentially expressed genes in GBM mainly involved sig-

naling pathways such as *Staphylococcus aureus* infection, phagosome formation, tuberculosis, systemic lupus erythematosus, and other infectious and autoimmune disease-related signaling pathways. Bar graphs of GO enrichment analysis results were drawn by using Cytoscape. GO enrichment analysis showed that GBM differentially expressed genes mainly involved the biological processes such as processing and presentation of exogenous antigenic peptides and polysaccharide antigens via MHC II molecules, interferon- γ -mediated signaling pathways, extracellular matrix composition, and chemical synapses transmission; it involved the CCs such as cell junctions, axon terminal buttons, extracellular space, vesicle membranes for endocytosis, and MHC II protein complexes; it involved the MFs such as calcium-mediated ionic protein binding, MHC II molecular receptor activity, immunoglobulin binding, and phospholipase inhibitor activity (►Figs. 5 and 6).

Correlation Analysis of Core Target Prognosis

Survival analysis was conducted by using GEPIA on the top 37 core targets in degree value, a total of five genes related to GBM prognosis were obtained ($p < 0.05$), namely fibronectin 1 (*FN1*), *GABRD* (γ -aminobutyric acid A receptor delta subunit), *MMP2*, *RBFOX1*, *SLC6A7*, and the gene expression levels were negatively correlated with prognosis. The GEPIA database gene expression box plot was further used to verify that compared with healthy people. *FN1* and *MMP2* were highly expressed while the *GABRD*, *RBFOX1* and *SLC6A7* were expressed at a low level in cancer patients (►Figs. 7 and 8).

Discussion

GBM is the most common primary central nervous system tumor. Due to the existence of tumor stem cells with self-renewal ability in the brain,¹⁶ glioma has its own special microenvironment.¹⁷ The invasive growth is strong, and the existence of the blood-brain barrier affects the tumor's response to external apoptosis signals. Based on this, to prevent the recurrence of GBM and prolong the survival period of patients, a comprehensive treatment of surgery combined with radiotherapy and chemotherapy is generally advocated. Although immunological methods such as immune checkpoint inhibitors, individualized peptide vaccines, and viral therapy have emerged in recent years for the treatment of GBM,^{1,18} the action mechanism, inoculation doses, and inoculation methods have not yet been unified, and thus it still cannot benefit the majority of glioma patients. Therefore, patients with GBM have a poor prognosis. At present, it is believed that changing the tumor microenvironment and anti-tumor immunotherapy are still the "ultimate nemesis" of malignant GBM.¹

The inhibition of the immune system by malignant GBM is not only localized to the tumor, but also spreads throughout the system.¹ This study found that GBM differentially expressed genes mainly involved *Staphylococcus aureus* infection, phagosome formation, tuberculosis and systemic lupus erythematosus and other infectious and autoimmune disease-related signaling pathways, and were also associated

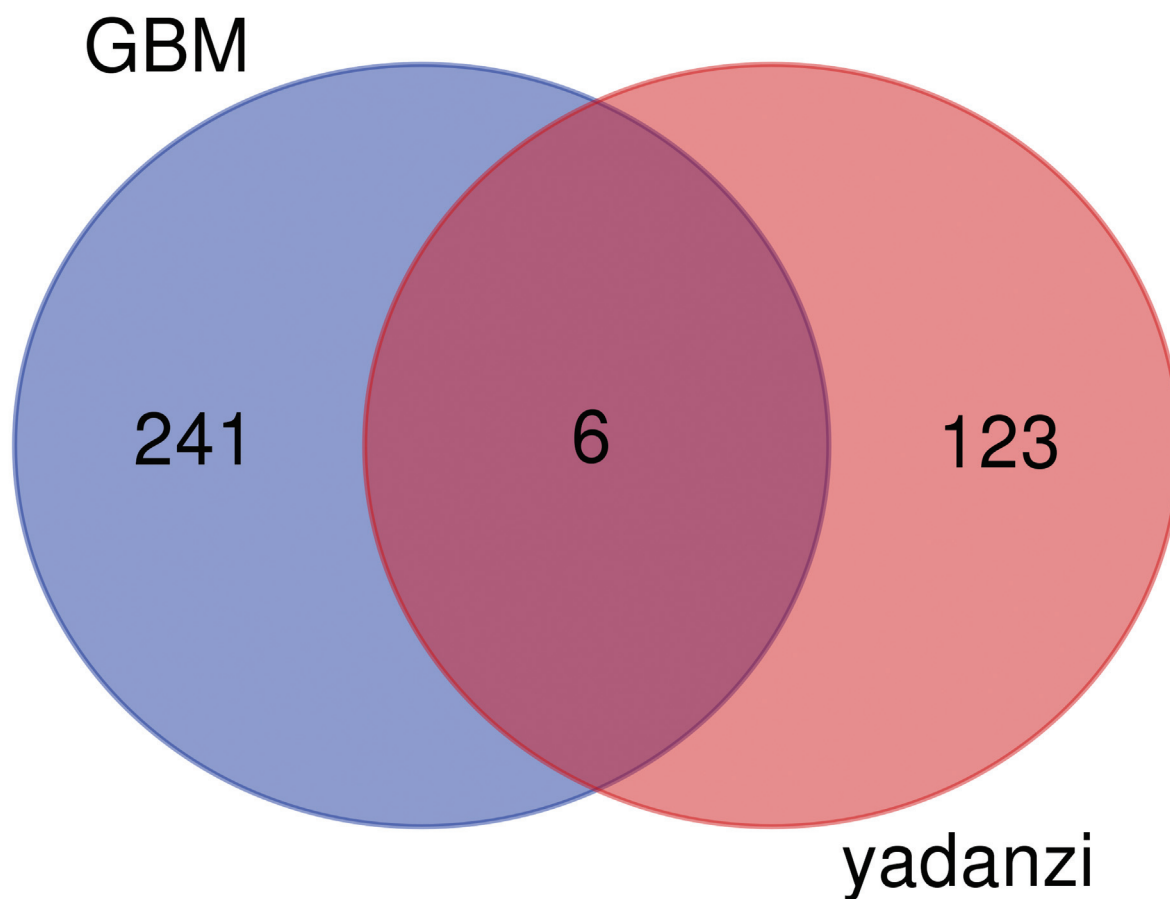


Fig. 1 Potential targets of Yadanzi (*Brucea javanica*) in the treatment of GBM. GBM, glioblastoma.

with the processing and presentation of exogenous antigenic peptides and polysaccharide antigens. It also involved the CCs and MFs of the human body involved in the immune process, such as the MHC II protein complex and its molecular receptor activity. The results of the study further illus-

trated that the occurrence and development of GBM were closely related to the immune system. Survival analysis of differentially expressed core genes found that *FN1*, *MMP2*, *RBFOX1*, *GABRD*, and *SLC6A7* were associated with GBM prognosis. As glycoprotein, *FN1* are involved in cell adhesion and migration of various tumor cells, they are considered as the important regulators for the formation, development, and signal transduction of the cancer cells in the lung cancer, ovarian cancer, breast cancer, and colorectal cancer.^{19–27} Currently, *FN1* has been used as a marker to detect epithelial–mesenchymal transition. Recent studies have found that gliomas can form vascular morphology rich in tumor extracellular matrix. This structure, called vasculogenic mimicry, enables gliomas to obtain blood supply and promotes glioma invasion and migration.²⁸ Several studies suggested that matrix metalloproteinases (MMPs) might destroy the physical barrier formed by tumor cell matrix molecules, stimulate potential biological activities in the human body, and participate in the immune response of tumors, and the expressions of *MMP1*, *MMP2*, and *MMP9* in the intracranial tumor were especially more pronounced.^{29,30} The protein encoded by *RBFOX1* can participate in cell architecture, cell deformation, and cell migration by regulating actin, kinesin, and microtubule-binding proteins.³¹ Studies showed that *RBFOX1* was also a tissue-specific selective splicing gene in malignant glioma, which can control cell migration by affecting clathrin

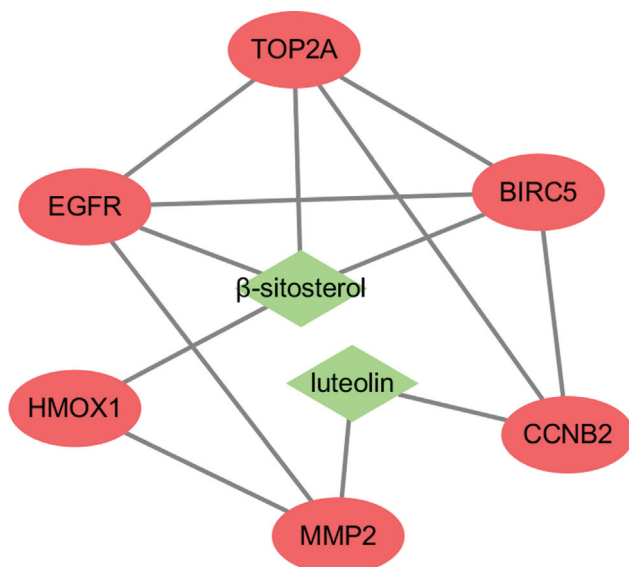


Fig. 2 The network diagram of active ingredient-action target of Yadanzi (*Brucea javanica*).

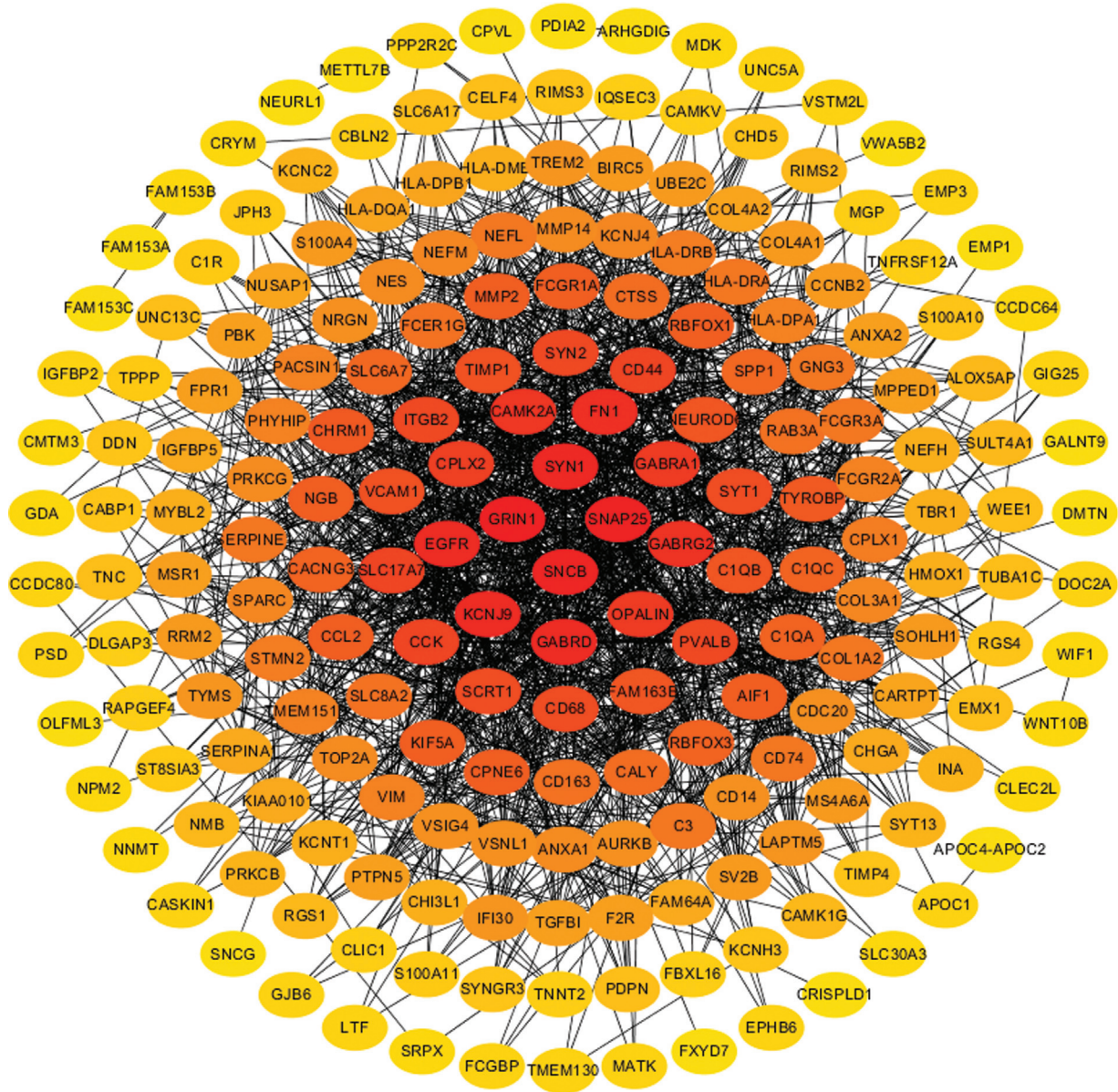


Fig. 3 GBM differentially expressed protein interaction network. GBM, glioblastoma.

light chain B.³² γ -aminobutyric acid (GABA) is an important inhibitory neurotransmitter, and the GABRD is involved in the process of chronic stress injury. The expression of a gene can regulate a series of neurological and mental disorders

caused by chronic stress injury.³³ The expression of GABRD is moderately negatively correlated with the expression of tumor infiltrating macrophages and colony stimulating factor 1, so it can be used as a potential independent prognostic

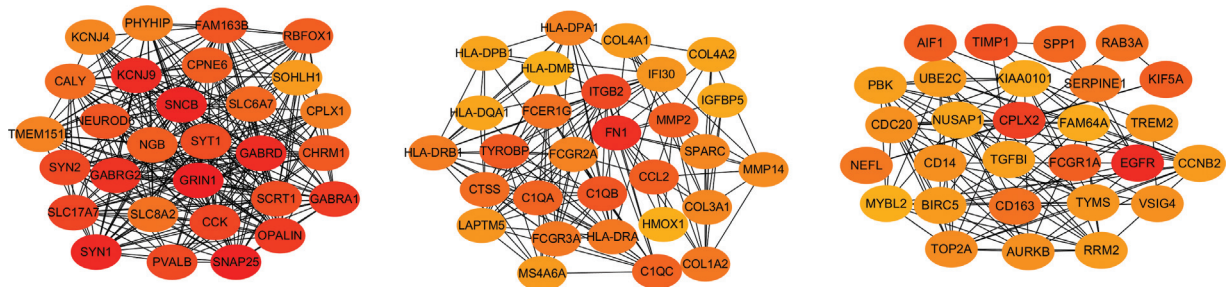


Fig. 4 Core modules of GBM differentially expressed protein interaction network. GBM, glioblastoma. Note: The darker the color, the more important the target is.

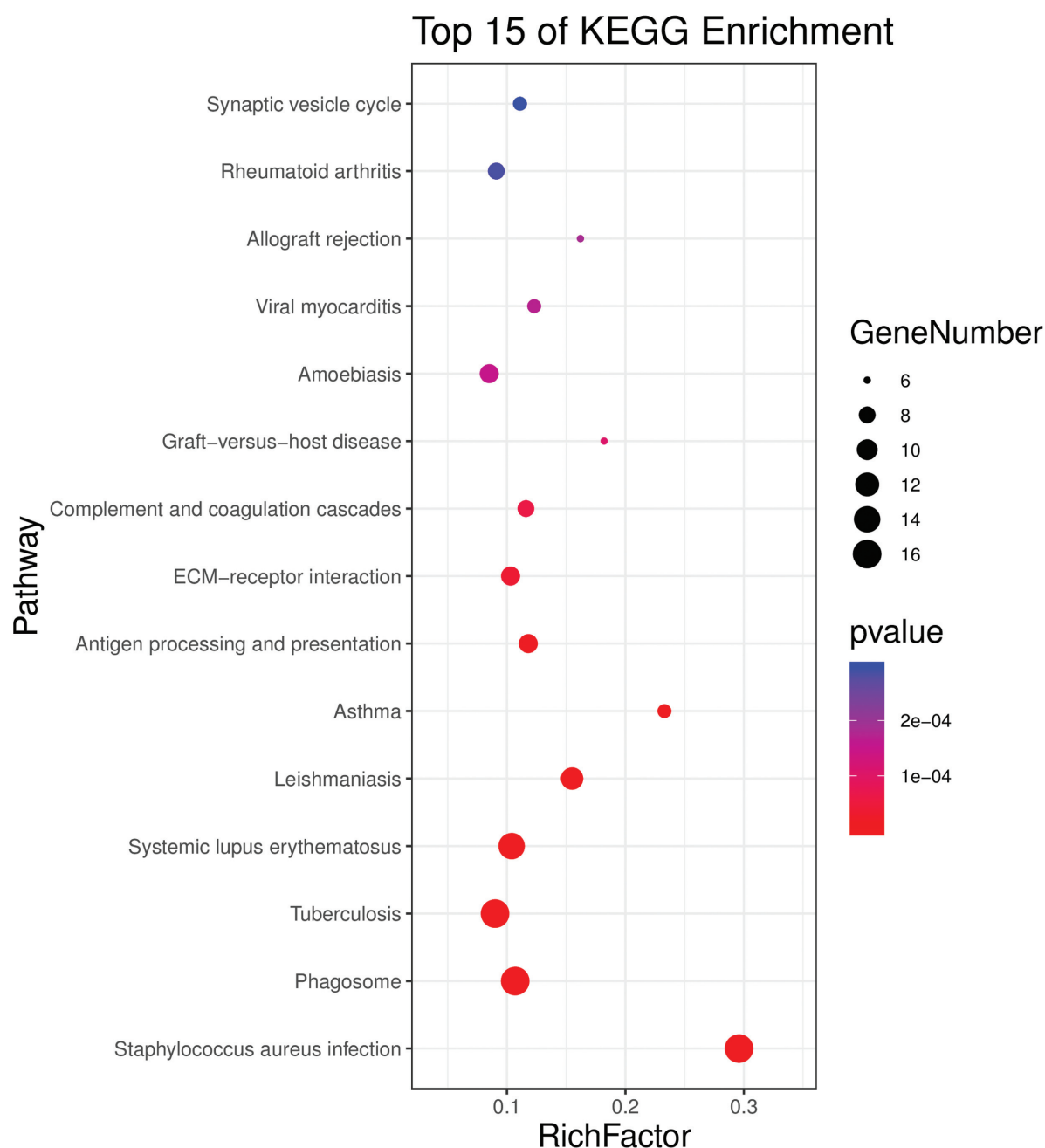


Fig. 5 KEGG pathway enrichment analysis of differentially expressed genes in GBM. GBM, glioblastoma; KEGG, Kyoto Encyclopedia of Genes and Genomes.

marker in patients with low-grade neoplasia.³⁴ *SLC6A7* is also a member of the GABA neurotransmitter gene family, which encodes a high-affinity mammalian brain 1-proline transporter. Studies have shown that this gene may be a susceptibility gene for asthma.³⁵ Prognosis-related genes suggested that regulating immunity and changing the tumor microenvironment were essential entry points to improve the prognosis of GBM.

In this study, it was found that BS and luteolin were the main components in the treatment of GBM by constructing an “active ingredient-action target” network. BS is widely present in various drugs and plants, and is the most abundant phytosterol. It has strong anticancer activity and can

inhibit multiple cell signaling pathways such as tumor cell proliferation, metastasis, invasion, peripheral angiogenesis and inflammation, and can be used to treat various tumors such as lung cancer, breast cancer, prostate cancer, and colon cancer.^{1,36–38} Luteolin is a flavonoid present in natural plants. Studies reported that luteolin had inhibitory effects on the proliferation of various tumor cells such as liver cancer,³⁹ prostate cancer,⁴⁰ lung cancer,⁴¹ and gastric cancer.⁴² Although Yadanzi (*Brucea javanica*) oil emulsion has been widely used in the postoperative adjuvant treatment of neuroglioma, the main anti-tumor component of Yadanzi (*Brucea javanica*) oil emulsion has been reported to be fatty acid.⁴³ There are few studies on the anti-neuroglioma BS and

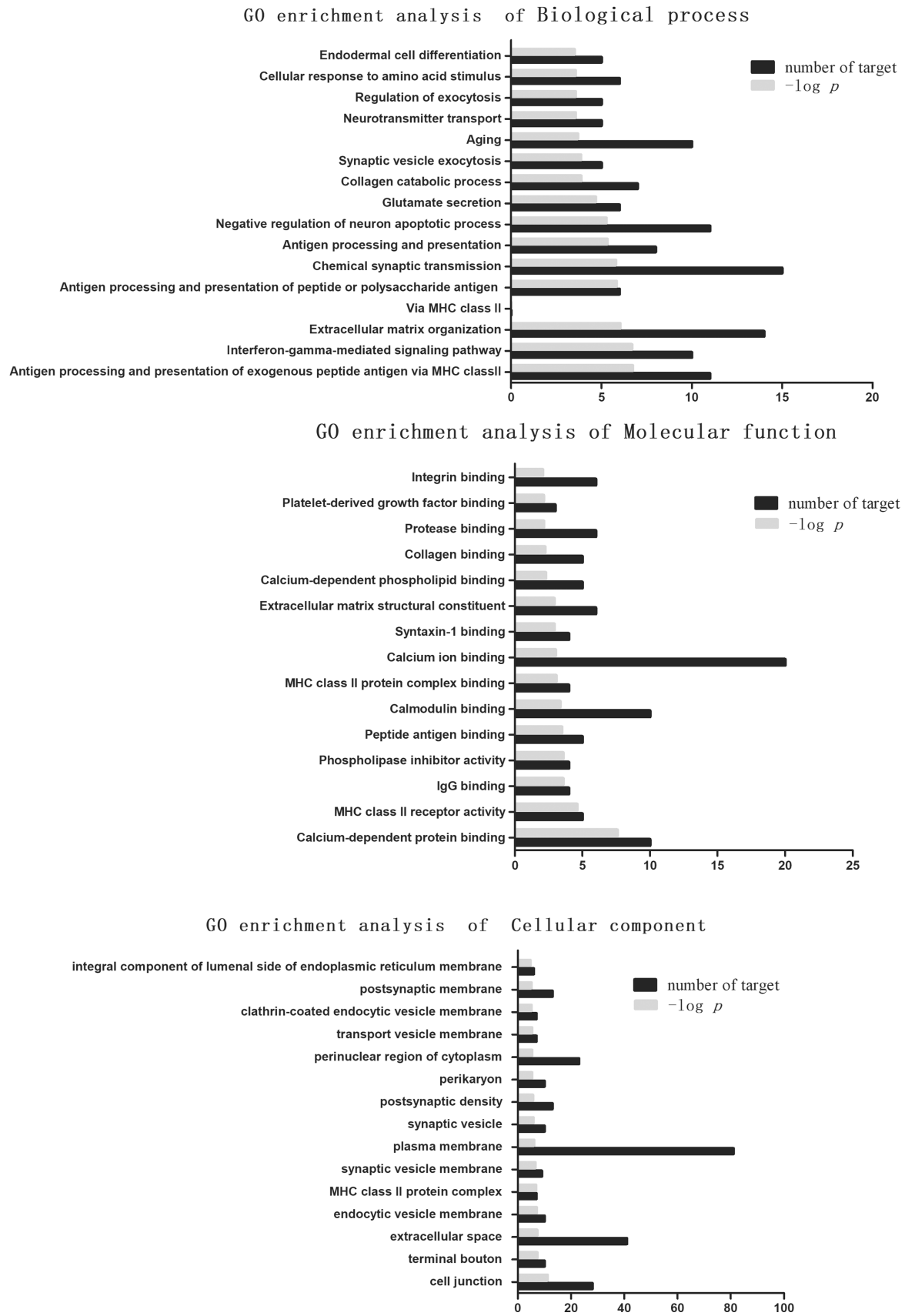


Fig. 6 GO enrichment analysis of differentially expressed genes in GBM. GBM, glioblastoma; GO, gene ontology.

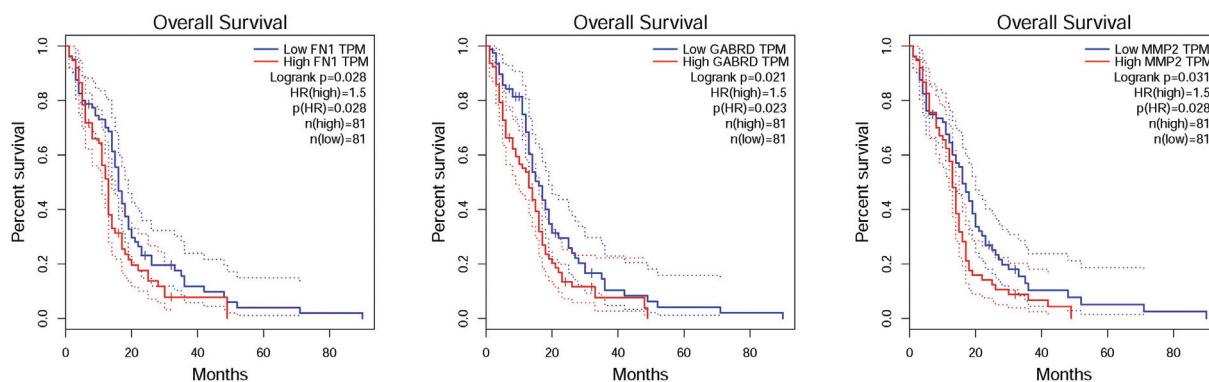


Fig. 7 Screening of GBM prognosis-related genes by GEPIA website. GBM, glioblastoma; GEPIA, Gene Expression Profiling Interactive Analysis.

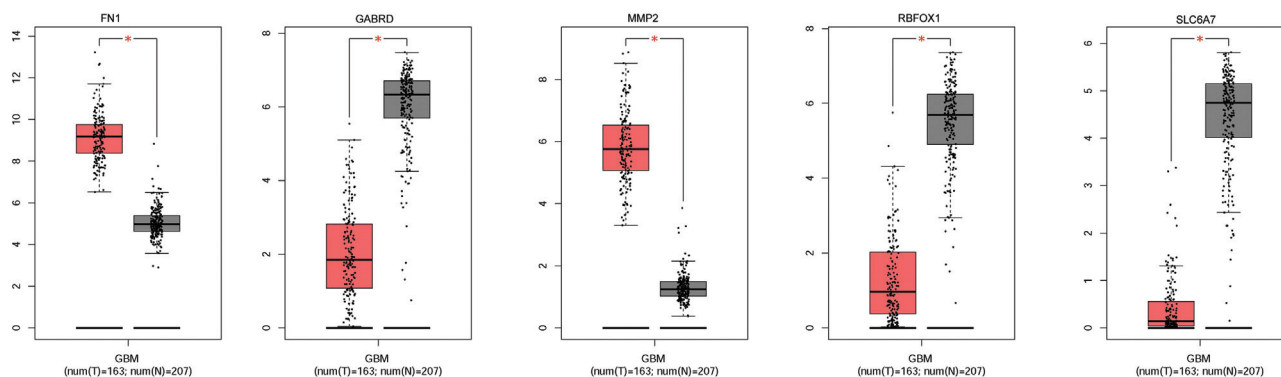


Fig. 8 Expression levels of GBM prognosis-related genes. GBM, glioblastoma.

luteolin in *Yadanza (Brucea javanica)*. In this study, the main targets of *Yadanza (Brucea javanica)* in the treatment of GBM are *MMP2*, *HMOX1*, *BIRC5*, *EGFR*, *CCNB2*, and *TOP2A*, among which *MMP2* is associated with the prognosis of GBM, suggesting that *Yadanza (Brucea javanica)* may reduce the expression of *MMP2* by regulating related pathways, affect the blood supply of gliomas, inhibit the invasion and migration of gliomas, destroy the physical barrier formed by tumor cell matrix molecules, and participate in the immune response of tumors, thereby improving the prognosis of GBM patients.

In this study, the key differentially expressed genes, molecular mechanism, and prognosis-related genes of GBM were screened and analyzed based on bioinformatics methods. At the same time, the active ingredients and key targets of *Yadanza (Brucea javanica)* were studied in the treatment of GBM by applying network pharmacology. The results of the study suggested that the pathogenesis of GBM was closely related to the human immune system. Meanwhile, it was found that BS and luteolin may be the main material basis for the treatment of GBM and the improvement of the prognosis in the *Yadanza (Brucea javanica)* oil emulsion besides fatty acid. This study provides a basis for the molecular mechanism study of the etiology and prognosis of GBM, and also provides a theoretical basis for further exploring the pharmacological effects of *Yadanza (Brucea javanica)* against neuroglioma.

Credit Authorship Contribution Statement

Wenyu Zhao: Data collection and curation, formal analysis, software, and writing original draft. **Fuchun Si:** Conceptualization, methodology, and writing—review & editing.

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Conflict of Interest

The authors declare no conflict of interest.

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