

A study on the potential mechanism of key components of panax notoginseng in treating brain injury by network pharmacology combined with molecular docking technique

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Abstract

Objective To investigate the effect and molecular mechanism of panax notoginseng on brain injury by network pharmacology, molecular docking and biological experiments.

Method TCMSP database was used to analyze the major active ingredients of panax notoginseng. LC-MS was employed for quantitative analysis of quercetin content in panax notoginseng. The potential targets of panax notoginseng components interfering with craniocranial injury were analyzed by network pharmacology, and the function and signal pathway of potential targets were enriched and analyzed. Protein interaction networks of potential targets were constructed, core targets were screened, active components corresponding to core targets were analyzed in reverse, and key active components and their targets were screened for vina software molecular docking and subsequent experimental verification. The TBI cell model was constructed, and the effect of quercetin on the activity of the TBI cell model was detected by CCK-8 method, the effect of quercetin on the apoptosis of the TBI cell model was detected by flow cytometry, and the effect of quercetin on the mRNA and protein expression levels of the key targets in the TBI cell model was detected by QPCR and western blot. Finally, the signaling pathway of quercetin improving traumatic brain injury was constructed.

Results Panax notoginseng mainly contained 9 components, a total of 333 potential targets were obtained, and 290 targets were combined with craniocerebral injury. In enrichment analysis, 10 potential targets were found in GO and KEGG signaling pathways respectively. The TOP10 core targets in the protein interaction network were CASR, APP, PIK3CA, PIK3R1, F2, S1PR1, ADORA1, ADORA3, DRD2 and CNR1. According to the number and order of corresponding core targets, quercetin was selected for molecular docking and subsequent experimental verification. Molecular docking showed that the binding energies of quercetin and corresponding targets APP, F2 and PIK3R1 were -7.7 kcal/mol, -7.3 kcal/mol and -8.4 kcal/mol, forming 2, 1 and 5 hydrogen bonds, respectively. It can be seen that quercetin and APP, F2, PIK3R1 all showed good binding activity. Quercetin experiment results showed that 0, 0.1, 0.3, 1, 3, 10 μ M quercetin treated TBI cells, the cell activity increased significantly ($65.1\% \pm 2.2\%$, $77.7\% \pm 5.3\%$, $87.4\% \pm 1.5\%$, $96.4\% \pm 0.4\%$, $93.6\% \pm 3.5\%$), and showed concentration dependence. Flow cytometry was used to detect the apoptosis of TBI cells in control group, TBI group, low concentration and high concentration groups. The results showed that the apoptosis rates of the four groups were 11.85%, 30.64%, 19.76% and 14.21%, respectively. The expression level of APP (mRNA and protein) in the TBI model cells treated with quercetin was significantly decreased, and the expression level in the high-dose group was significantly lower than that in the low-dose group, while the expression of PIK3R and F2 was the opposite.

Conclusion Quercetin, the component of pantoginseng, has a certain protective effect on brain injury cells, and it may regulate the related signaling pathways by interfering with APP, PIK3R and F2, and play a role in the protection and improvement of brain injury.

Introduction

The occurrence of craniocerebral injury is mainly due to the direct or indirect violence on the patient's head, resulting in mechanical degeneration of the skull, meninges, cerebrovascular and brain tissues [1, 2]. The brain tissue of patients with this disease can be ischemia, hypoxia, and the brain tissue can produce a large number of oxygen free radicals due to the obvious lack of nutrient supply, which can lead to the function of the brain nerve cells appear different degrees of damage. Severe craniocerebral injury has a high disability rate and fatality rate [3, 4]. In recent years, traditional Chinese medicine therapy has been widely used in the clinical treatment of craniocerebral injury. TCM classifies craniocerebral injury into the category of "dizziness" [5], and acupuncture and traditional Chinese medicine are commonly used to treat patients with this disease, so as to improve the blood circulation in the brain and promote the recovery of brain nerve cell function [6–7].

The pathological basis of severe craniocerebral injury is blood stasis, and the intracranial pressure increases sharply when extremely severe craniocerebral injury is combined with widespread brain contusion and laceration, intracranial hematoma and brain edema, so it is necessary to reduce the intracranial pressure in time and effectively to save the patient's life to the greatest extent at the fastest speed [8]. The traditional Chinese medicine panax notoginseng temperature, entering the liver and stomach canal, can stop the bleeding and remove blood stasis, reduce swelling and pain, reduce blood pressure and improve blood circulation. However, the potential mechanism and target of panax notoginseng in the treatment of craniocerebral injury are not clear at present [9–10].

Due to the complex composition of traditional Chinese medicine, many pharmacological effects, and the long period of animal experiments limiting its research and development, there are few studies on the mechanism of the pharmacological effects of traditional Chinese medicine at present. At present, the continuous development of information science and technology and the cross of multiple disciplines provide efficient and rapid methods for the pharmacological research of TCM. Network pharmacology has become an indispensable part of the pharmacodynamic evaluation system by using computer simulation analysis, bioinformatics, molecular network data, etc., to reveal the mechanism of drug efficacy from different perspectives [11].

Therefore, in this study, we adopted network pharmacologic methods and molecular docking technology to clarify the potential molecular mechanism of panax notoginseng in the treatment of craniocerebral injury. In addition, we combined biological experiments to verify this mechanism, and finally constructed the signaling pathway of the key active ingredient of panax notoginseng to improve traumatic brain injury, providing theoretical support for the application of traditional Chinese medicine in the treatment of craniocerebral injury.

Materials and Methods

1.1 Screening of active ingredients contained in Panax Notoginseng

The bioactive compounds of panax notoginseng were downloaded from the TCMSP database. Based on the absorption, distribution, metabolism and excretion models, oral bioavailability (OB) and drug similarity (DL) were used for screening candidate active ingredients. We set $DL \geq 0.18$ and $OB \geq 30\%$ as the thresholds for screening active ingredients, and then downloaded the chemical structure formula of each active ingredient compound for subsequent prediction of target genes.

1.2 Determination of Quercetin Content in panax notoginseng

Extraction Method: Weigh 0.6g of panax notoginseng powder, add 50mL of methanol, soak, weigh to determine the initial weight, let it sit overnight, maintain a slight boiling using an 80°C water bath for 2 hours, allow to cool, weigh again, make up for the weight loss with methanol, shake well, filter, collect the filtrate, and filter it through a 0.22µm microporous membrane as the test solution.

Chromatographic Conditions: **Column:** ACQUITY UPLC® BEH C18 (1.7µm, 2.1×50 mm), mobile phase of 65% water/35% acetonitrile, isocratic elution, injection volume of 1uL, column temperature of 40°C.

Mass Spectrometry Conditions: Instrument: SCIEX QTRAO 5500 + Triple Quadrupole Mass Spectrometer, negative ion mode with MRM, Turbo V source.

1.3 Prediction of the target of the active ingredient in Panax notoginseng

Using the compound structure of the active ingredient of pantoginseng downloaded in the previous step, upload it to Batman-TCM and SwissTargetPrediction database, and set the parameter to the default value. Obtain potential targets for the active ingredient of panax notoginseng.

1.4 Screening of craniocerebral injury-related target genes

The target information related to craniocerebral injury was obtained from OMIM, NCBI-GENE and GeneCards databases. The associated genetic information was retrieved by inadding "Traumatic brain injury" to obtain the associated targets of craniocerebral injury.

The intersection of potential targets of panax notoginseng active ingredient and craniocerebral injury related targets was obtained by Venn diagram analysis, which was used as the target of panax notoginseng active ingredient intervention on craniocerebral injury.

1.5 Enrichment analysis and network construction of main targets of panax notoginseng active ingredient intervention in craniocranial injury

R language was used to extract the target gene information related to craniocerebral injury, and GO enrichment analysis and KEGG pathway analysis were conducted.

Cytoscape 3.6.1 software was used to establish the protein interaction network between the active components of *Panax notoginseng* and the targets associated with craniocerebral injury, and the intersection network was integrated and extracted for topological analysis. TOP10 key targets were selected based on the topological structure characteristic value "degree" in the network nodes. The corresponding drug components of the TOP10 targets were deduced backwards, and the key drug components were selected for subsequent experimental verification.

1.6 Molecular docking of quercetin, the active ingredient of *panax notoginseng*, and its targets

The chemical structure of WOG was obtained by TCMSP database, and the PDB files of the above targets were obtained by PDB database. Then PyMOL software was used to remove water molecules and small molecule ligands from the protein receptor structure, Autodock Tool software was used to hydrogenate the protein structure, and Autodock software was used to format the protein receptor file and small molecule ligand file, and the range of active pockets was determined. Finally, vina was used for molecular docking. And the visualization results were viewed with PyMOL software.

1.7 Experimental Verification

1.7.1 Cell Culture

After recovery, culture and amplification, HT22 cells were induced to differentiate into sympathetic neuron-like cells: HT22 cells were taken for more than five generations, digested and dispersed, and made into 5×10^5 cells/mL cell suspension was inoculated in plastic petri dishes and placed in incubators for culture. The liquid was changed twice a week, half of the fresh culture medium was changed each time, and 3 ~ 5 generations of cells were selected for the experiment.

1.7.2 Toxicological test and drug protection analysis

Toxicological test and drug protection analysis

Toxicological test: HT22 cells in 8×10^3 The cells were seeded into 96-well plates with a density of 100 μ L/well; After 24h, the medium in the 96-well plate was discarded, and the prepared concentrations of Chryseriol (0, 0.64, 3.2, 16, 80, 400, 2000, 10,000, 50,000 nm) were added at 100 μ L/well. Three re wells were set for each drug concentration, and the CON group was changed. The toxicity of Chryseriol to HT22 cells was detected by CCK-8 assay after 24h of drug action.

Drug protection: HT22 cells were measured by 2.5×10^5 Per/well density was seeded into 6-well plates, 2ml/well; The next day, model group and dosing group were molded with 6 lines across and down with gun ends. Liquid was changed for each group, and the dosing group was added with the drug of corresponding concentration (0, 0.03, 0.1, 0.3, 1.0, 2.0 μ M), and incubated in the incubator for 24h. Cells of each group were collected and counted to 8×10^3 Each cell density was implanted into 96-well plates with 3 multiple Wells in each group. CCK8 measurements were performed 24h later to detect the protective effect of Chryseriol on TBI cell models.

CCK-8 detection: Discard the medium in the 96-well plate (do not touch the bottom of the hole with pipette tip), add 100µl D-PBS to each well, and then gently sucked out and repeated; 100µl of complete medium was added to each well, and then 10µl of CCK-8 solution was added to each well, and placed in a CO₂ incubator at 37°C, 5%CO₂, and incubated for 2h. OD value was determined with a wavelength of 450nm using an enzymometer.

1.7.3 Experimental grouping and drug administration

A 6-well plate was prepared, and the suspension of HT22 cells was evenly planted in the well plate, and the well plate was placed in a 5%CO₂ incubator at 37°C for 24 h. The well plate was randomly divided into blank control group (CON group), cell model group (TBI group) and Chryseriol preconditioning group (Chryseriol group). Chryseriol group was divided into two groups: low dose (0.1µM) and high dose (1.0µM). After 6 days of administration and culture, the holes were scratched with 10 µL sterilized gun head for 6 uniform transverse and vertical channels (12 channels in total), and then placed in 37°C, 5% CO₂ incubator for 2 h to extract cells. Chryseriol preconditioning group maintained Chryseriol 0.1µM / 1.0µM for 72 h on day 2, and Con group was given equal dose of DMED on day 2.

1.7.4 Cell apoptosis was detected by flow cytometry

Cells were induced by apoptosis according to the experimental protocol, centrifuged at 300g for 5min, supernatant was discarded, cells were collected, washed once with PBS, and cells were gently suspended and counted. Take 1–5×10⁵ cells⁵ For the resuspended cells, centrifuge 300g for 5min and discard the supernatant. The cells were washed with PBS once, the supernatant was abandoned after centrifugation, and the cells were re-suspended with 500µL diluted 1×Annexin V Binding Buffer working solution. 5µl Annexin V-FITC and 5µl propyl iodide (PI) staining solution were added into the cell suspension; After gentle vortex mixing, incubate at room temperature and away from light for 15-20min; After the reaction is complete, test on the machine immediately.

1.7.5 QPCR was used to detect mRNA levels of key targets

RNA was extracted from cells by Trizol-chloroform-isoamyl alcohol method (Tianjin Zhiyuan, China), and the RNA was converted into cDNA by reverse transcription kit (Servicebio, Wuhan, China). The relative expression levels of TNF-α, IL-1β, MMP9 and PTGS2 were detected by real-time fluorescence quantitative PCR (RT-PCR). Reaction system: 2×NovoStart® SYBR High-Sensitivity qPCR SuperMix 10µl, Forward primer 1µl, Reverse primer 1µl, template 1µl, RNase Free Water 7µl. PCR amplification: predenaturation: 95°C, 5min; Cycle (40 times) : 95°C, 10s, 60°C, 10s, 72°C, 10s; Melting curve: 60°C→95°C, temperature rise of 0.5°C every 15s. Using β-actin as the internal reference, the 2^{-ΔΔCt} method was used to calculate the relative expression of mRNA.

1.7.6 Detection of key target protein levels by Western blot

The treated cells were collected and the total protein of the cells was extracted. The protein concentration was determined by Coomassie bright blue method. The protein was isolated and transferred to the nitrate cellulose membrane by polyacrylamide gel electrophoresis, which was bound to the antibodies of APP,

ADORA1, DRD4, OPRD1 (1:500) and β -actin (1:2000), respectively. And then combined with horseradish peroxidase labeled secondary antibody, electrochemiluminescence method after color photography. Finally, gel imaging system (Image master VDS) was used for photography, and Image analysis software (Image J) was used for gray scanning analysis. The relative quantity of protein level of β -actin was taken as the ratio of the gray value of the protein product band of target protein to that of β -actin. The expression of protein bands was calculated by scanning image analyzer.

1.8 Quercetin intervention protects nerve cells and improves signal pathway construction of brain injury

Based on the above bioinformatics analysis and experimental verification, the KEGG pathway was used to query the relevant regulatory network, and the signaling pathway of the active ingredient quercetin of pantoginseng was finally constructed.

1.9 Statistical analysis

SPSS23.0 and GraphPad Prism 9 were used to analyze and plot the experimental data. Variance analysis was used to compare data between groups. $P < 0.05$ indicated statistical significance.

Results

2.1 Target screening of the active components of Panax notoginseng and quantification of quercetin by LC-MS

TCMSP database was used to identify the known active components of turmeric, and 8 active components were obtained according to the screening conditions. Mandenol, DFV, Diop, beta-sitosterol, Stigmasterol, ginsenoside rh2, ginsenoside f2, quercetin, as shown in Table 1. Subsequently, we performed qualitative and quantitative analysis of quercetin in panax notoginseng using LC-MS. The results are shown in Figs. 1, Figs. 2 and Table 2. Quercetin, with a relative molecular mass of 302.2, was analyzed in negative ion mode using the quantification ion pair of 301.0/151.0. After analysis and calculation, the content of Quercetin was determined to be 326.67ng/g .

Table 1
Active components of *Panax notoginseng*

Molecule Name	MW	AlogP	Hdon	Hacc	OB (%)	Caco-2	BBB	DL	FASA-	HL
Mandenol	308.56	6.99	0	2	42	1.46	1.14	0.19	0.25	5.39
DFV	256.27	2.57	2	4	32.76	0.51	0.29	0.18	0.42	17.89
Diop	390.62	7.44	0	4	43.59	0.79	0.26	0.39	0.28	3.6
beta-sitosterol	414.79	8.08	1	1	36.91	1.32	0.99	0.75	0.23	5.36
Stigmasterol	412.77	7.64	1	1	43.83	1.44	1	0.76	0.22	5.57
ginsenoside rh2	622.98	4.04	6	8	36.32	0.51	1.38	0.56	0.24	11.08
ginsenoside f2	785.14	2.3	9	13	36.43	1.8	3.03	0.25	0.22	13.11
quercetin	302.25	1.5	5	7	46.43	0.05	0.77	0.28	0.38	14.4

Table 2
Concentrations and peak areas of Quercetin series.

	Concentration(ng/mL)	Peak area
Reference standard solution	5	152400
	50	1795000
	500	21920000
Sample	X	113600
Mixture of reference standard and sample in a 1:1 ratio (v/v).	5 + Sample	412600
	50 + Sample	1197000
	500 + Sample	13070000

Linear equation obtained by standard addition method:

$$y = 51801x + 101611$$

$$x = 1.96\text{ng/mL}$$

$$\text{Content: } W = 1.96\text{ng/mL} \times 2 \times 50\text{mL} \div 0.6\text{g} = 326.67\text{ng/g}$$

We used BATMAN-TCM combined with Swiss Target Prediction to analyze the potential targets of nine components, including Mandenol, DFV, Diop, beta-sitosterol, Stigmasterol, ginsenoside rh2, ginsenoside f2, quercetin, and Eicosapentaenoic acid. A total of 333 targets were obtained after merging.

2.2 Results of network pharmacological analysis

We searched 7963 targets related to craniocerebral injury through the GeneCards database, OMIM database and NCBI-GENE database. BATMAN-TCM combined with Swiss Target Prediction was used to predict the target gene of the active ingredient of Panax notoginseng to obtain 333 targets. The combined targets of craniocerebral injury and Panax notoginseng active ingredients were 290, and the results are shown in Fig. 3A.

We used the clusterProfiler package of R language to conduct GO annotation and KEGG pathway analysis for 290 target genes of active components of panax notoginseng acting on craniocerebral injury. Among them, the results of GO functional annotation analysis showed that the above target genes involved the following aspects: steroid hormone response, steroid metabolism process, antibiotic response, positive regulation of protein serine and threonine kinase activation, positive regulation of MAP kinase activation, receptor response to steroid hormone stimulation, synaptic membrane, protein serine/threonine kinase activity, carboxylic acid binding organic acid binding, steroid hormone receptor activity, protein tyrosine kinase activity, nuclear receptor activity. See Fig. 3B for details.

According to KEGG analysis results, the regulatory pathways involved in the stimulation of neural tissue interaction; PI3K-Akt signaling pathway; Ras signaling pathway; Rap1 signaling pathway; Proteoglycan in cancer; EGFR tyrosine kinase inhibitor resistance to prostate cancer; Inflammatory mediators regulate TRP channels; Non-small cell lung cancer; And arachidonic acid metabolism. See Fig. 3C for details.

In the above study, we have obtained a total of 290 target genes of effective active components of panax notoginseng acting on craniocerebral injury. The above gene information was analyzed using the String database tool, combined with Cytoscape tool to obtain the protein interaction network of the above genes (Fig. 3D). Cytoscape Hubba plug-in was utilized to screen the core targets of the top 10 in the scoring (Fig. 3E). The scoring sequence was CASR, APP, PIK3CA, PIK3R1, F2, S1PR1, ADORA1, ADORA3, DRD2, CNR1.

We used the TOP10 gene to derive its corresponding drug composition in reverse, and the results were as follows: Mandenol corresponds to CNR1, CASR; DFV corresponds to ADORA1, ADORA3; beta-sitosterol corresponds to ADORA3, DRD2; Stigmasterol corresponds to CNR1, DRD2; ginsenoside rh2 corresponds to ADORA1, S1PR1, PIK3R1, PIK3CA; quercetin corresponds to APP, PIK3R1, F2; Eicosapentaenoic acid corresponds to DRD2, CNR1. Among them, ginsenoside rh2 corresponds to 4 targets, quercetin corresponds to 3 targets, and quercetin corresponds to the top 5 of the core target, so the active ingredient is selected for subsequent molecular docking and experimental verification.

2.3 Molecular Docking

Vina was used for molecular docking to verify the combination of quercetin and corresponding targets APP, F2 and PIK3R1. It is generally believed that the minimum binding energy between small molecules and proteins is less than -5 kcal/mol, indicating good binding activity between them. The results showed that the binding energy of quercetin and APP was -7.7 kcal/mol, and two hydrogen bonds were formed between Quercetin and APP. The hydrogen bond residues were PRO-32 and THR-26. The binding capacity of quercetin and protein F2 was -7.3 kcal/mol, and they formed a hydrogen bond, and the amino acid residue producing hydrogen bond was PRJ-3. The binding capacity of quercetin to PIK3R1 was -8.4 kcal/mol, and they formed 5 hydrogen bonds. The amino acid residues producing hydrogen bonds were PRO-222 (2 hydrogen bonds), TYR-109, SER-95 and TRP-100, respectively. The docking results are shown in FIG. 4.

2.4 Analysis of experimental results

CCK-8 was used to detect the effect of quercetin treated with different concentrations (0, 0.1, 0.3, 0.5, 1, 3, 5, 10 μ M) on the cell activity of normal HT22 cells. The results showed that the cell activity of 6 groups was $100.0\% \pm 5.2\%$, $97.5\% \pm 1.3\%$, $97.9\% \pm 4.5\%$, $93.7\% \pm 1.7\%$, $95.1\% \pm 3.3\%$, $95.8\% \pm 2.2\%$, $97.4\% \pm 6.6\%$, $102.7\% \pm 4.0\%$, respectively. There was no significant difference between the drug treatment group and the control group, indicating that 0.1–10 μ M concentration of quercetin had little effect on the activity of HT22 cells. The toxicity of quercetin was low, as shown in Fig. 5A. The TBI cell model was constructed and treated with 0, 0.1, 0.3, 1, 3 and 10 μ M quercetin, respectively. The results of CCK-8 test showed that compared with normal HT22 cells, the cell activity of the TBI cell model was significantly decreased ($58.5\% \pm 2.4\%$). After different concentrations of quercetin were treated with TBI cell model, Cell activity significantly increased ($65.1\% \pm 2.2\%$, $77.7\% \pm 5.3\%$, $87.4\% \pm 1.5\%$, $96.4 \pm 0.4\%$, $93.6\% \pm 3.5\%$), and presents the concentration dependence, results as shown in the Fig. 5B. Further, 0.3 μ M and 3 μ M were selected as low concentration group and high concentration group, respectively, for follow-up experiments.

Flow cytometry was used to detect apoptosis in control group, TBI group, low concentration group and high concentration group. The results showed that the apoptosis rates of the four groups were 11.85%, 30.64%, 19.76% and 14.21%, respectively. The death rate of the TBI cell model group was significantly higher than that of normal HT22 cells. After quercetin treatment, the apoptosis rate of TBI cells decreased significantly, and the apoptosis rate of high concentration group was lower than that of low concentration group. The results were shown in Fig. 5C.

The mRNA and protein expression levels of three potential targets of quercetin for brain injury intervention were detected by QPCR and western blot assay, respectively. The results showed that the mRNA and protein expression levels of APP in TBI model cells group were significantly higher than those in CON group. The expression level of APP in the TBI model cells treated with quercetin was significantly decreased, and the expression level in the high-dose group was significantly lower than that in the low-dose group.

The expression levels of PIK3R and F2 in TBI model cells (mRNA and protein levels) were significantly lower than those in CON group, and the expression levels of PIK3R and F2 in TBI model cells (mRNA and

protein levels) were significantly increased after quercetin was added, while the expression levels in high-dose group were significantly higher than those in low-dose group. The results are shown in Fig. 5D-I.

2.5 Quercetin improves signal pathway construction in traumatic brain injury

Based on the above bioinformatics analysis and experimental verification, quercetin, the active ingredient of panax notoginseng, was finally constructed to improve the traumatic signaling pathway, as shown in Fig. 6. As can be seen from the figure, quercetin regulates F2 in the Neuroactive ligand-receptor interaction signaling pathway, while regulates PIK3R in the PI3K-Akt signaling pathway, and at the same time inhibits APP, thus playing a role in protecting nerve cells. Ameliorate brain injury.

Discussion

At present, there are numerous literatures on the pharmacological effects of panax notoginseng compounds, but the composition of panax notoginseng is complex, and the existing studies are difficult to systematically elaborate the network mechanism of action of promoting blood circulation, stopping bleeding, detumescence and relieving pain [12–13]. Network pharmacology is a discipline that uses network visualization and other technologies to reveal the complex biological network relationships among drugs, genes and targets. It can analyze the behavior of drugs acting on different targets, cells and organs from the molecular and gene levels, and predict and reveal the mechanism of action of drugs [14–15]. Network pharmacology is used to construct a "drug-target" network according to the structure and efficacy of drugs, which can effectively predict the pharmacodynamic components and mechanism of action of traditional Chinese medicine.

In this study, the main active substances of the components of Panax notoginseng were analyzed by GC-MS combined with TCMSP database, and 8 active ingredients were obtained according to the screening conditions. Mandenol, DFV, Diop, beta-sitosterol, Stigmasterol, ginsenoside rh2, ginsenoside f2, quercetin, the OB values of these components are greater than or equal to 30 and DL values are greater than or equal to 0.18. In line with the oral availability and drug-like requirements of conventional drugs. Network pharmacology was used to analyze the molecular mechanism of the active substances of panax notoginseng in the treatment of brain injury. The results showed that there were 290 common potential targets of the active substances and brain injury. Enrichment analysis showed that the above targets are involved in GO functions such as steroid hormone response, steroid metabolism, antibiotic response, positive regulation of protein serine and threonine kinase activation, interaction in stimulating neural tissue, PI3K-Akt signaling pathway, Ras signaling pathway, Rap1 signaling pathway and other signaling pathways. PI3K/Akt signaling pathway is a signaling pathway that promotes the survival of nerve cells, and mTOR is also one of the main downstream effector factors of this pathway [16]. PI3K is a member of the lipid kinase family and is activated by phosphorylation of the 3-hydroxyl group on the plasma membrane phosphatidylinositol, but it can be directly activated by cell surface receptors [17]. Akt is a key molecule in the PI3K signal transduction pathway and plays an important role in cell survival and

apoptosis. There are few studies on the role of PI3K/Akt in brain injury, and its role needs to be further confirmed [18]. Studies have shown that activation of PI3K/Akt pathway can inhibit inflammatory response and nerve cell apoptosis, reduce the area of brain injury in rats, and improve the brain histopathologic morphology and neurological symptoms of rats [19]. Rap1 is a small GTPase protein that is highly homologous to Ras proteins. It uses small Gase enzymes as a molecular switch to circulate between active GTP-binding states and inactive GDP-binding states. Previous studies have shown that the Rap1 signaling pathway is mainly mediated and involved in cell adhesion, cell junction formation, and endothelial barrier protection, and that Afadin plays an important role in the connection between cells as a downstream effector of Rap1. Therefore, the effect of the Rap1 signaling pathway on the blood-brain barrier after traumatic brain injury has been analyzed, and the results show that Rap1 activation can improve the permeability of the damaged blood-brain barrier and reduce cerebral edema. It is concluded that the main components of panax notoginseng may play a therapeutic role in brain injury by regulating the above signaling pathways.

PPI protein interaction network analysis showed that the TOP10 targets were CASR, APP, PIK3CA, PIK3R1, F2, S1PR1, ADORA1, ADORA3, DRD2 and CNR1. These indicated that the above targets played a significant role in the whole protein interaction network. Based on the number of corresponding targets and the sequencing of targets in the network, quercetin and its corresponding three targets APP, PIK3R1 and F2 were finally selected for subsequent molecular docking and experimental verification. Quercetin is a natural flavonoid widely distributed in nature, known as quercetin and quercetin. Quercetin is widely found in flowers, leaves and fruits of plants, and has pharmacological effects such as antioxidant, anti-inflammatory, anti-tumor, antiviral, anti-apoptosis and neuroprotective effects [20–22]. In the treatment of spinal cord injury [23] and ischemia-reperfusion animals [24], quercetin can effectively block the pathological link caused by secondary changes and antagonize neuronal apoptosis, and the ischemic hypoxia of brain tissue is also the main manifestation of the secondary pathological injury of traumatic brain injury, so it is of great significance to apply quercetin in the treatment of traumatic brain injury [25]. In recent years, domestic and foreign scholars have gradually begun to apply quercetin in the treatment of traumatic brain injury animal models. In this study, a TBI cell model was established. The results showed that quercetin with different concentrations increased the activity of TBI model cells significantly ($65.1\% \pm 2.2\%$, $77.7\% \pm 5.3\%$, $87.4\% \pm 1.5\%$, $96.4\% \pm 0.4\%$, $93.6\% \pm 3.5\%$) in a concentration-dependent manner. The results of apoptosis detection showed that the apoptosis rate of quercetin treated TBI cells decreased significantly, and the apoptosis rate of high concentration group was lower than that of low concentration group. In conclusion, quercetin can improve the activity of TBI model cells, and inhibit the occurrence of apoptosis, which has a certain protective effect on brain injury cells. An animal study showed that quercetin therapy significantly reduced nerve damage and improved cognitive function caused by TBI. Dual immunolabeling showed that quercetin significantly reduced LC3-positive NeuN co-labeled cells and significantly enhanced p-akt positive NeuN co-labeled cells. In addition, quercetin treatment decreased the expression of TBI-induced LC3, caspase-3, and Bax levels, and increased the expression of p-akt and Bcl-2 at 48 h [26]. Another experimental animal study showed that quercetin reduced brain edema and microglial cell proliferation in rats with traumatic brain injury. Quercetin alleviates cortical inflammatory

response and oxidative stress caused by TBI injury. Quercetin can activate the Nrf2/HO-1 pathway in the cortex of rats with TBI [27]. Molecular docking results showed that quercetin had good binding ability with APP, PIK3R1 and F2. The experimental results also showed that quercetin had regulatory ability on the above three targets, among which the expression level of APP in TBI model cells treated with quercetin was significantly decreased, and the expression level in high-dose group was significantly lower than that in low-dose group. The expression levels of PIK3R and F2 (mRNA and protein levels) in high-dose group were significantly increased, and the expression levels in high-dose group were significantly higher than those in low-dose group. As a member of the PI3K/Akt signaling pathway, studies have shown that PIK3R is involved in neurobehavioral function, pathological changes of brain tissue, oxidative stress and inflammatory response in mice [28]. The up-regulated expression of APP under conditions of metabolic stress, ischemia, brain injury and inflammation suggests that APP, as an acute phase protein, has a protective function in cell survival under metabolic challenges [29]. This confirmed the improvement effect of quercetin on brain injury cell models in the results of this study.

In summary, the molecular mechanism of panax notoginseng component's intervention in brain injury was analyzed by network pharmacology and molecular docking technology in this study, and the protective effects of key component quercetin on brain injury cells and the regulatory effects of key targets were analyzed experimentally. The results showed that the component quercetin of panax notoginseng had certain protective effects on brain injury cells. It is possible to regulate related signaling pathways including Neuroactive ligand-receptor interaction and PI3K-Akt signaling pathway through the intervention of APP, PIK3R and F2 and other key targets to play a role in protecting and improving brain injury. In addition, the experimental results confirmed the prediction of network pharmacology, confirmed the accuracy of network pharmacology research, and also provided a theoretical basis for the treatment of brain injury of panax notoginseng and its active components.

Declarations

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AUTHOR CONTRIBUTIONS

G.Z., J.Z. and Z.C. performed the experiments, analyzed the data, and wrote the manuscript; Y.L. and J.Z. performed the experiments and contributed to the study design and overall supervision. P.S., G.L. and Y.L. participated in the data collation and analysis. All authors reviewed the manuscript.

Competing interests

The author(s) declare no competing interests.

Data availability statement

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

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Figures

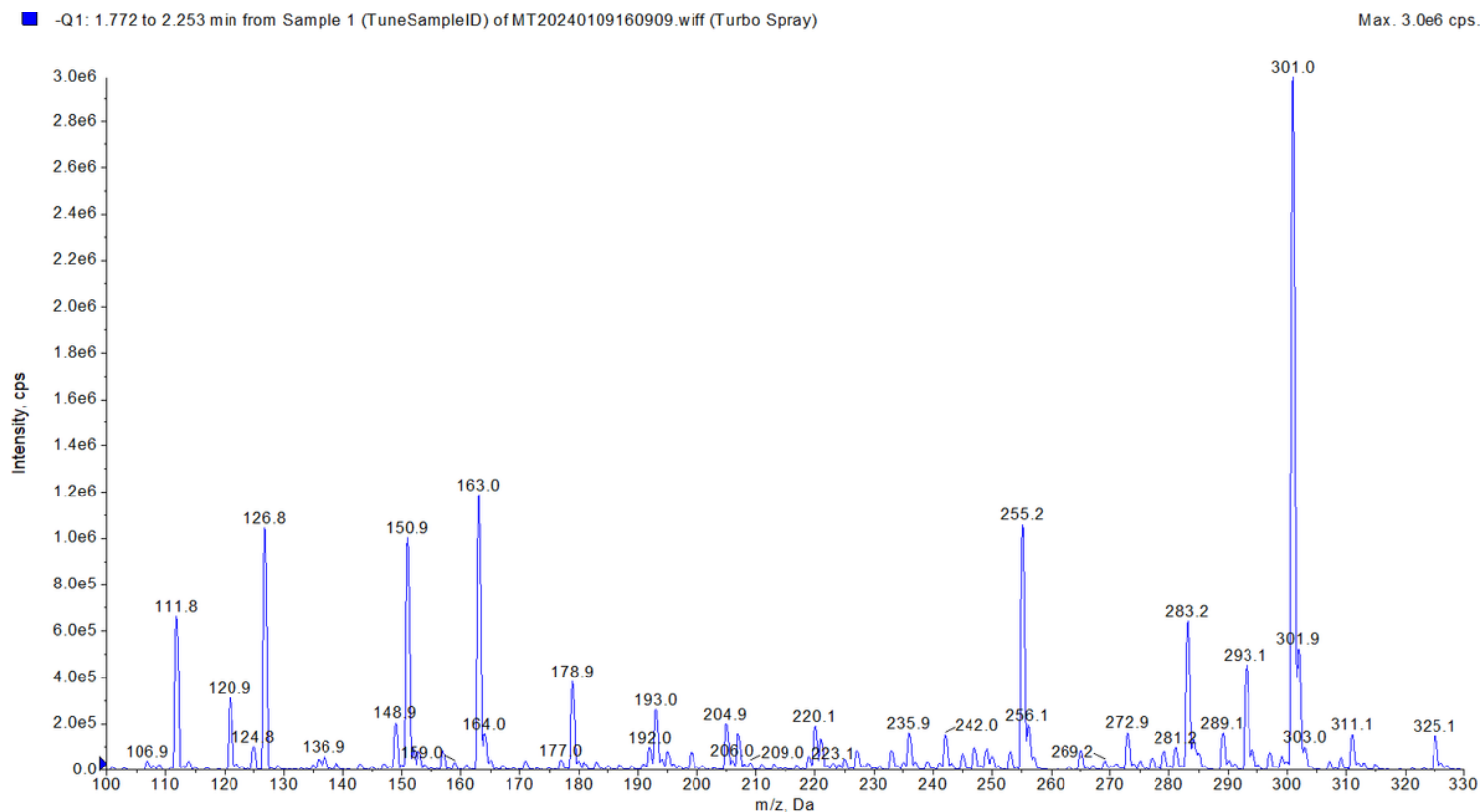


Figure 1

First-level mass spectrum of Quercetin.

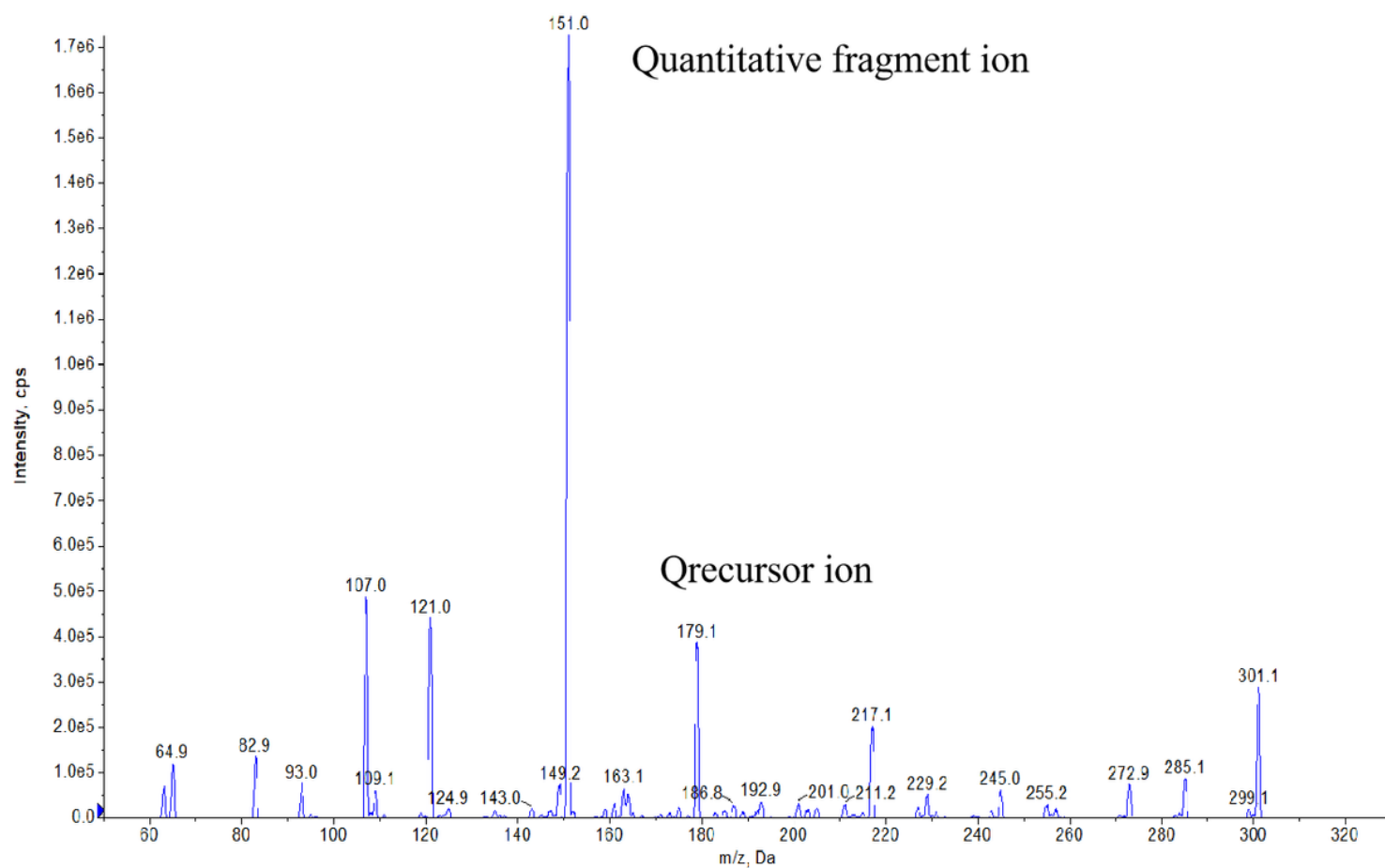


Figure 2

Second-level mass spectrum of Quercetin.

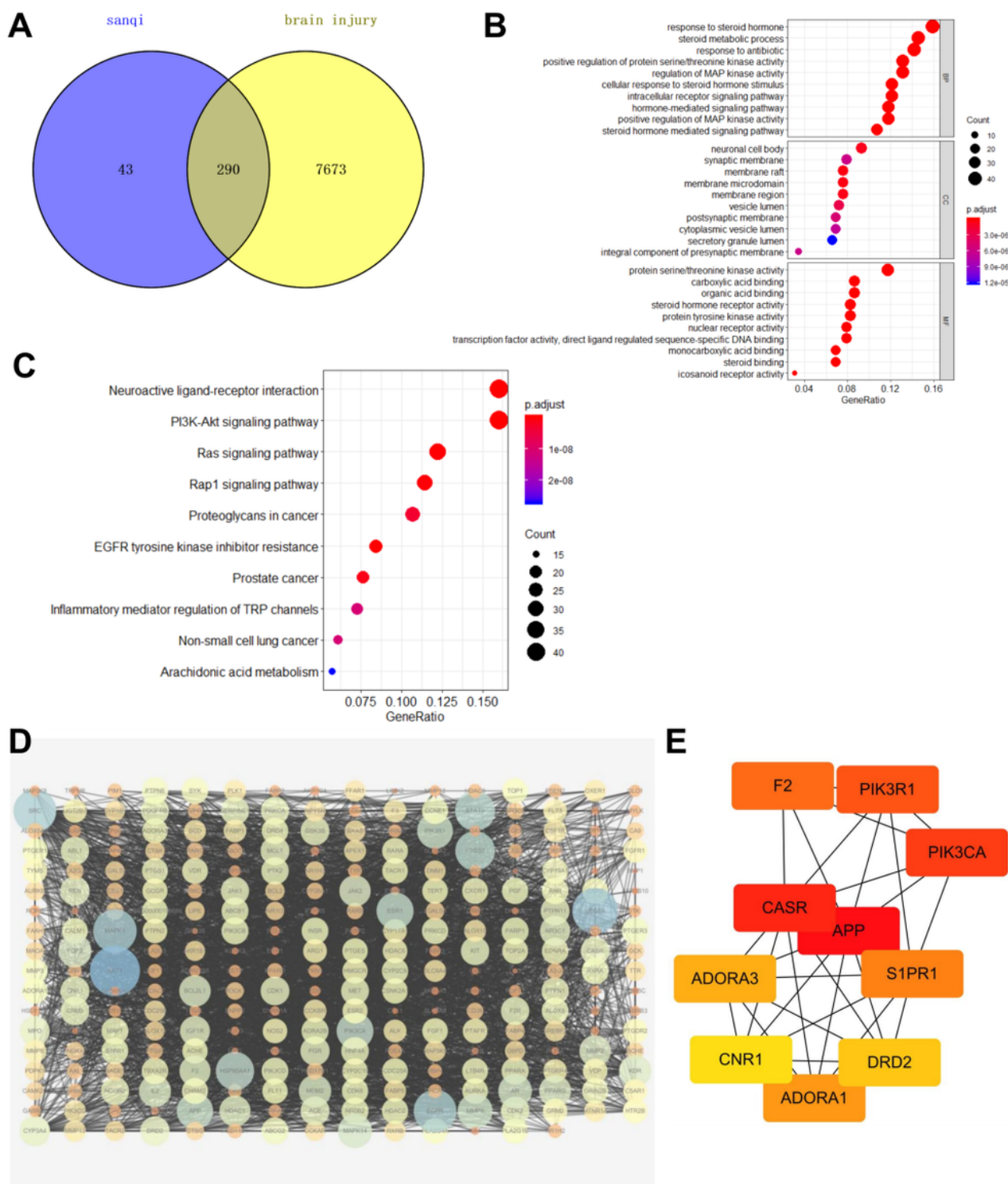


Figure 3

Results of network pharmacological analysis. A) Venn map analysis of active components of Panax notoginseng and craniocerebral injury's targets; B) GO enrichment analysis of potential targets of Panax notoginseng active components in intervention of craniocerebral injury; C) KEGG enrichment analysis of potential targets of Panax notoginseng active components in intervention of craniocerebral injury; D) PPI

network of potential targets of *Panax notoginseng* active components in intervention of craniocerebral injury; E) TOP10 core target networks. The darker the color, the higher the rating.

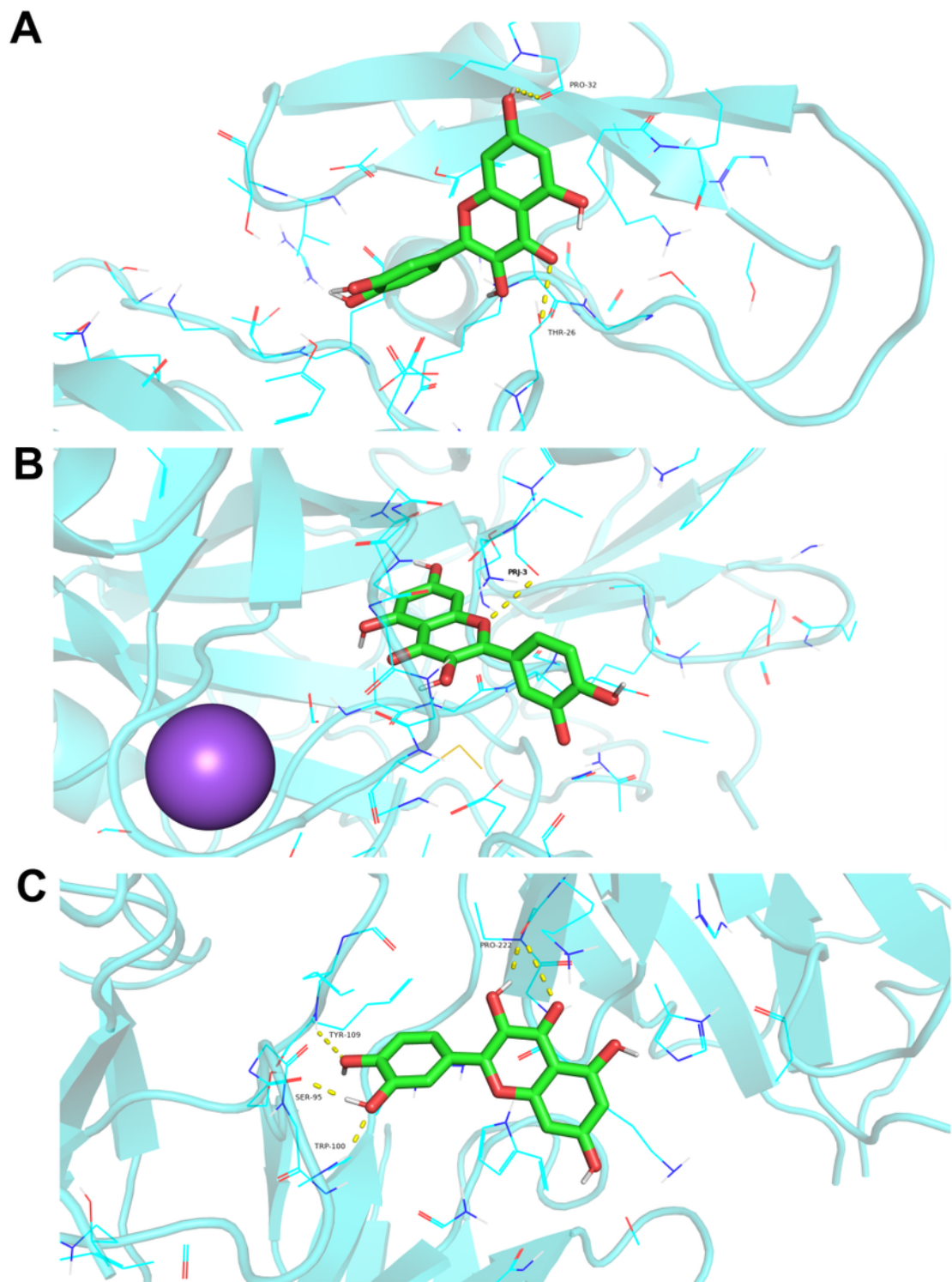


Figure 4

Molecular docking diagram of Quercetin and corresponding targets APP, F2, PIK3R1. A) Molecular docking diagram of quercetin and corresponding target APP; B) Molecular docking diagram of quercetin and

corresponding target F2; C) Molecular docking diagram of quercetin and corresponding target PIK3R1

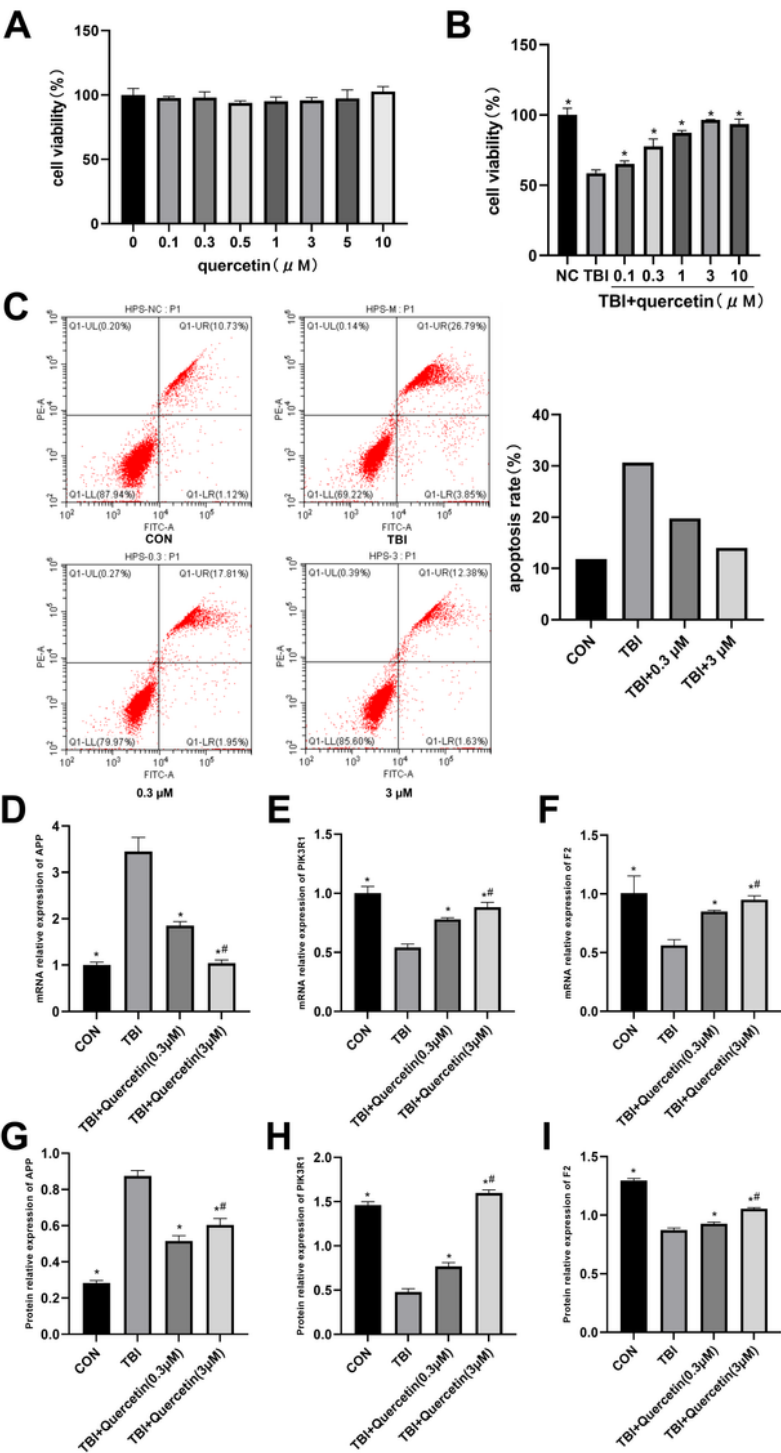


Figure 5

Experimental verification of quercetin intervention in brain injury. A-B)The effect of quercetin on HT22 cell and TBI cell models was detected by CCK-8. A) Effects of quercetin at different concentrations on the activity of HT22 cells; B) Effects of quercetin at different concentrations on the activity of TBI cell models;

C)Effect of quercetin on apoptosis of TBI model cells detected by flow cytometry; D-I Effect of quercetin on mRNA and protein expression levels of related targets in TBI model cells. D-F) mRNA expression levels of APP, PIK3R1 and F2 were detected by QPCR; G-I)Western blot was used to detect the protein expression levels of APP, PIK3R1 and F2.* was compared with TBI group, P<0.05, # was compared with TBI+Quercetin(0.3μM) group, P<0.05

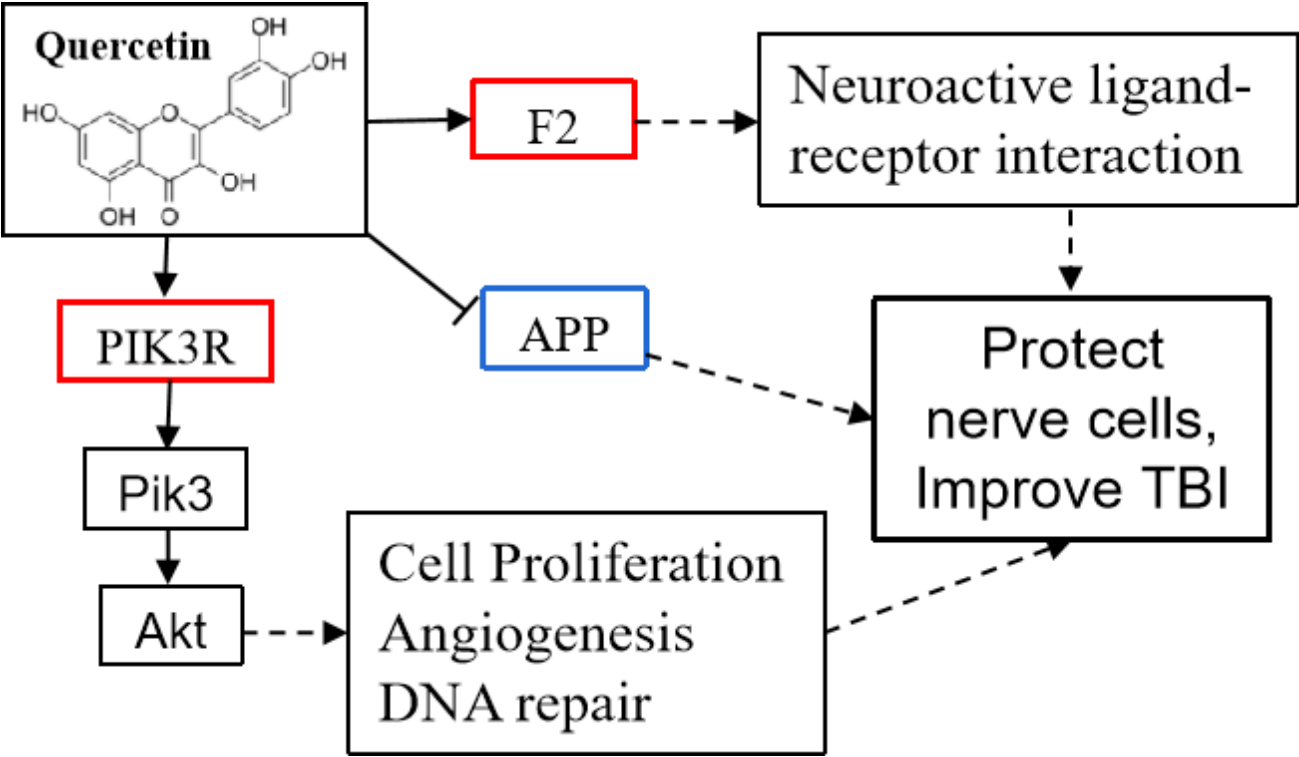


Figure 6

Quercetin improves the signaling pathway of traumatic brain injury; Red box genes represent up-regulated genes and blue box genes represent down-regulated genes