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Original Article

A network pharmacology approach to explore the mechanism of action of Shengqi Fuzheng Injection in treating ischemic stroke

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Abstract: Objective: To investigate the potential active ingredients in Shengqi Fuzheng Injection (SFI) and the drug's potential mechanism of action in the treatment of ischemic stroke (IS) based on network pharmacology. The active ingredients of SFI and the associated genes were obtained by retrieving the database of the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP). Methods: The IS-associated targets were identified as the intersection of differentially expressed genes (DEG) from GSE122709 and GSE140275 profiles. The intersection of SFI- and IS-associated genes were used as the potential targets. The protein-protein interaction (PPI) network among 24 potential targets was constructed using the STRING database. The functional annotation was performed to obtain the pathways of potential targets using the Gene ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis which enriches the function of genes. GO semantic similarity was employed to identify the targeted hub genes of SFI related to the treatment of IS. To unravel the regulation among the potential targets, the transcription factor-targets-pathway network was identified using the Transcription Regulatory Relationships Unraveled Sentence-based Text (TRRUST). Results: There were 28 active compounds identified in SFI. Among them, 24 potential targets of SFI which were related to the treatment of ischemic stroke and involved in various functions and pathways were explored. Analysis of PPI and transcription factor target prediction indicated that JUN, NOS3 and PTGS2 were the key genes responsible for the treatment ischemic stroke. These genes were simultaneously enriched in the oxytocin signaling pathway. Twelve compounds were predicted to target JUN, NOS3 and PTGS2, while formononetin was the only compound associated with all the hub genes. Conclusion: SFI protects nervous system probably via the the regulation of JUN, NOS3 and PTGS2 in the oxytocin signaling pathway by formononetin. Further biological and clinical studies are warranted to validate the above findings.

Keywords: SFI, ischemic stroke, network pharmacology, transcription factor, formononetin

Introduction

Ischemic stroke (IS) has become one of the leading causes of death and adult disability in China, with significant impact on public healthcare expenditure [1]. The annual stroke mortality rate is approximately 157/100,000, of which 43% to 79% are due to ischemic stroke [1]. Despite advances made in the understanding of the causes and risk factors of IS, the exact pathophysiological mechanism of this disease remains unclear, as a result, there is no generally accepted and effective treatments for ischemic stroke up to date [1]. There are currently three classes of drugs used for treatment of IS, namely thrombolytic agents, anticoagulants and antiplatelet agents [2]. However, the use of these therapies was limited by the narrow therapeutic window and side effects. Therefore, it is necessary to explore alternative therapeutic options for the treatment of IS. Traditional Chinese medicine (TCM) may provide promising resources for the development of new anti-IS therapies.

TCM prescriptions consist of numerous compounds from herbs, animals and minerals and boast of a tradition of over thousands of years [3-5]. TCM drugs have long been used for treating IS, and have accumulated abundant theoretical knowledge and treatment experience [6]. For example, Danqi Piantang Jiaonang (DJ)

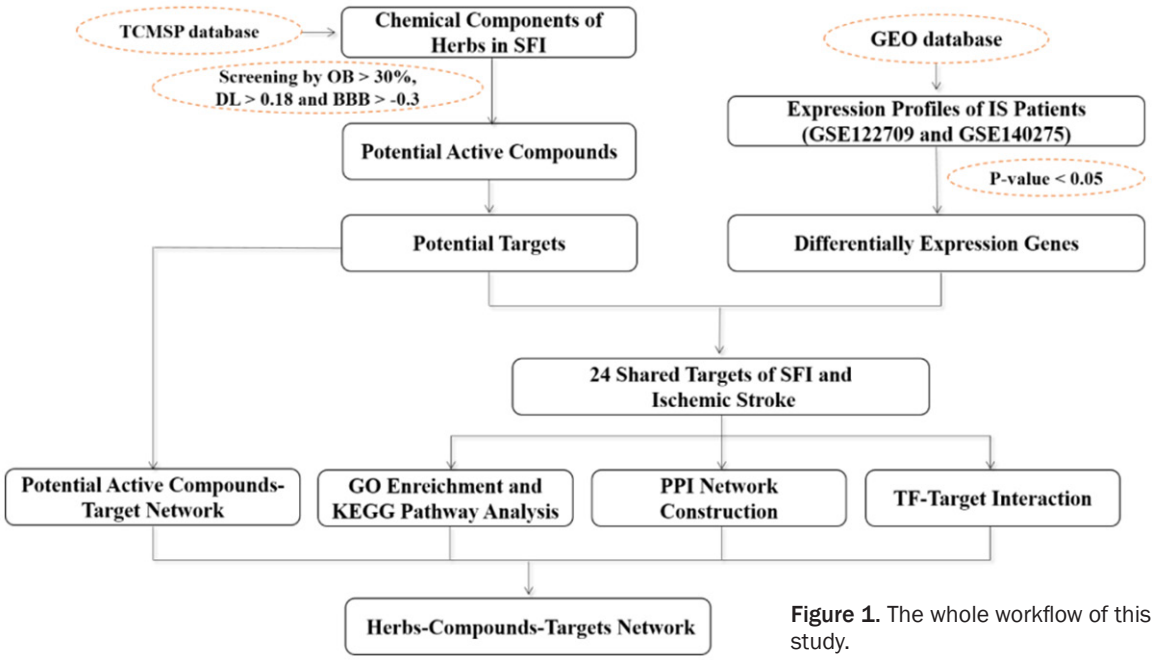

Figure 1. The whole workflow of this study.

capsule has been proved to promote neurological recovery after stroke and has exhibited good tolerability [7]. Ginkgolides injection (GIn), a standard preparation containing ginkgo diterpene lactones extract, has been clinically used for neuroprotective treatment of patients are recovering from cerebral infarction [8]. Shenqi Fuzheng Injection (SFI), contains the extracts of *Radix codonopsis* and *Hedysarum multijugum*, has been widely used as an adjuvant therapy in treating lung cancer, esophagus cancer and colon cancer [9-13]. *Radix codonopsi* and *Hedysarum multijugum* have long been used as immune-modulators in China [14]. Studies have revealed that SFI can obviously improve nerve function, decreases water content and infarction area in brain as well as inhibits Ca^{2+} aggregation in aged rats with cerebral ischemia-reperfusion injury [15]. SFI can effectively attenuate irradiation-induced brain injury by inhibiting the NF-κB signaling pathway and microglial activation [16]. However, these failed to elucidate the potential pharmacological mechanisms of SFI in achieving the therapeutic efficacy. What's more, the multiple and complex active ingredients contained in TCM preparations hinder us to understand the molecular mechanisms related to their efficacy.

With advances in study methods and experimental techniques, the progress in unveiling the mechanism of action of TCM therapies has also been promoted [6]. The approach of network pharmacology provides a novel network mode through encompassing multiple targets, various effects and complex diseases based on polypharmacology and systems biology [17]. As a result, this approach is suitable for fundamental studies focusing on exploring the principle of medicine compatibility for multiple compounds contained in these TCM preparations.

In the current study, we aimed to further understand the neuroprotective effect of SFI in alleviating IS. The flow chart of this study was presented in **Figure 1**. We first searched for the effective ingredients contained in SFI as well as the relevant target genes, and constructed the compounds-target genes network. Then, IS-associated genes were obtained by analyzing the Geodatabases about IS, and potential targets of SFI in treatment of IS were screened. The databases of Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were retrieved and protein-protein interaction (PPI) for the target proteins was analyzed. After that, the transcription factors (TFs) of these genes were predicted to investigate the potential effect of SFI on regulating gene transcription. Finally, we identified the potential hub genes

Table 1. Components of *Radix codonopsis*

Mol ID	Molecule Name	MW	OB (%)	BBB	DL
MOL008407	(8S,9S,10R,13R,14S,17R)-17-[(E,2R,5S)-5-ethyl-6-methylhept-3-en-2-yl]-10,13-dimethyl-1,2,4,7,8,9,11,12,14,15,16,17-dodecahydrocyclopenta[a]phenanthren-3-one	410.75	45.4	1.26	0.76
MOL006554	taraxerol	426.8	38.4	1.18	0.77
MOL001006	poriferasta-7,22E-dien-3beta-ol	412.77	42.98	1.11	0.76
MOL007514	methyl icosa-11,14-dienoate	322.59	39.67	1.1	0.23
MOL004355	spinasterol	412.77	42.98	1.04	0.76
MOL006774	stigmast-7-enol	414.79	37.42	1.04	0.75
MOL000449	stigmasterol	412.77	43.83	1	0.76
MOL003036	ZINC03978781	412.77	43.83	0.96	0.76
MOL003896	7-Methoxy-2-methyl isoflavone	266.31	42.56	0.56	0.2
MOL008391	5alpha-Stigmastan-3,6-dione	428.77	33.12	0.47	0.79
MOL005321	frutinone A	264.24	65.9	0.46	0.34
MOL002879	diop	390.62	43.59	0.26	0.39
MOL002140	perlolyrine	264.3	65.95	0.15	0.27
MOL008397	daturilin	436.64	50.37	0.06	0.77
MOL008411	11-Hydroxyrankinidine	356.46	40	-0.19	0.66
MOL008400	glycitein	284.28	50.48	-0.29	0.24

Table 2. Components of *Hedysarum multijugum*

Mol ID	Molecule Name	MW	OB (%)	BBB	DL
MOL000033	(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5-propan-2-yloctan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	428.82	36.23	1.09	0.78
MOL000296	hederagenin	414.79	36.91	0.96	0.75
MOL000378	7-O-methylisomucronulatol	316.38	74.69	0.84	0.3
MOL000371	3,9-di-O-methylnissolin	314.36	53.74	0.63	0.48
MOL000380	(6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol	300.33	64.26	0.55	0.42
MOL000438	(3R)-3-(2-hydroxy-3,4-dimethoxyphenyl) chroman-7-ol	302.35	67.67	0.34	0.26
MOL000211	mairin	456.78	55.38	0.22	0.78
MOL000398	isoflavanone	316.33	109.99	0.17	0.3
MOL000392	formononetin	268.28	69.67	0.02	0.21
MOL000442	1,7-Dihydroxy-3,9-dimethoxy pterocarpene	314.31	39.05	-0.04	0.48
MOL000387	bifendate	418.38	31.1	-0.06	0.67
MOL000239	jaranol	314.31	50.83	-0.22	0.29

Table 3. The potential targets of each compound of SFI

MOL ID	Molecule.Name	Target Genes
MOL008407	(8S,9S,10R,13R,14S,17R)-17-[(E,2R,5S)-5-ethyl-6-methylhept-3-en-2-yl]-10,13-dimethyl-1,2,4,7,8,9,11,12,14,15,16,17-dodecahydrocyclopenta[a]phenanthren-3-one	NR3C2, PGR
MOL008411	11-Hydroxyrankinidine	CDK-1, ESR1, OPRM1, SCN5A
MOL003896	7-Methoxy-2-methyl isoflavone	Ace, ADRA1B, ADRA1D, ADRB1, ADRB2, AR, CALM, CCNA2, cdk-1, CHRM1, CHRM3, CHRM5, CHRNA7, DPP4, DRD, ERR2, ESR1, GABRA1, GSK3B, HSP90, IGHG1, LTA4H, MAOB, MAPK14, NCOA1, NCOA2, NOS2, NOS3, OPRM1, PDE3A, PIM1, PKIA, PPARG, PRSS1, PTGS1, PTGS2, RXRA, SCN5A, SLC6A3, SLC6A4
MOL008397	daturilin	NR3C1
MOL002879	diop	ADRB2, CHRM3, SCN5A
MOL005321	frutinone A	Ace, ADRB2, AR, CHRNA7, DPP4, GABRA1, HSP90, PDE3A, PIK3CG, PPARG, PTGS1, PTGS2, RXRA
MOL008400	glycitein	APP, AR, CALM, CCNA2, CDK-1, ERR2, ESR1, GSK3B, HSP90, MAPK14, P13, MMP8, NCOA1, NOS2, PDE3A, PIM1, PPARG, PRSS1, PTGS1, PTGS2, RXRA
MOL007514	methyl icosa-11,14-dienoate	NCOA2
MOL002140	perloyrine	PTGS2, RXRA
MOL006554	poriferasta-7,22E-dien-3beta-ol	NCOA2, NR3C2, PGR
MOL004355	spinasterol	NCOA2, NR3C2, PGR
MOL006774	stigmast-7-enol	NCOA2, PGR
MOL000449	stigmasterol	ADH1C, ADRA1A, ADRA1B, ADRA2A, ADRB1, ADRB2, CHRM1, CHRM2, CHRM3, CHRNA7, CTRB, GABRA1, GABRA3, HTR2A, IGHG1, LTA4H, MAOA, MAOB, NCOA1, NCOA2, NR3C2, PGR, PLAU, PTGS1, PTGS2, RXRA, SCN5A, SLC6A2, SLC6A3
MOL003036	ZINC03978781	NCOA2, NR3C2, PGR
MOL000211	mairin	PGR
MOL000033	(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5-propan-2-yloctan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	PGR
MOL000239	jaranol	DPP4, NCOA2, NOS2, ESR2, HSP90, AR, PTGS1, PRSS1, PTGS2, CDK2, SCN5A, CHEK1, CALM
MOL000296	hederagenin	ADRA1B, SLC6A2, GABRA2, LYZ, NCOA2, PTGS1, PDE3A, GABRA5, PTGS2, CHRM2, PGR, CHRM3, GABRA6, GABRA1, ADH1B, ADH1C, GRIA2, RXRA, CHRM1, GABRA3, SCN5A
MOL000371	3,9-di-O-methylnissolin	ADRA2C, NOS2, PDE3A, PTGS1, PTGS2, RXRA, ESR1, ADRB1, ADRA1B, GABRA1, OPRM1, ADRB2, NCOA2, PRSS1, SCN5A, HTR3A, CALM, NOS3, CHRM3, ACHE, CHRM1, F2, ADRA1D
MOL000380	(6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol	CHRM4, ADRA1B, CHRM1, NOS2, NCOA1, RXRA, CALM, F2, HSP90, ADRB2, PTGS1, ADRA1D, ESR1, NCOA2, PRSS1, HTR3A, ACHE, SCN5A, GABRA1, PTGS2, CHRNA7, CHRM3
MOL000387	bifendate	KDR, TOP2, HPS90, KCNMA1, PTGS1, MET, PTGS2
MOL000378	7-O-methylisomucronulatol	MAPK14, KCNMA1, ADRA1D, HTR2A, ADRB1, CCNA2, CHRM4, ESR2, PTGS2, ADRA1A, PDE3A, NCOA2, PTGS1, ESR1, AR, F10, HSP90, DPP4, F2, ADRA2C, CHRM1, CHRM5, SLC6A4, DRD1, ADRA1B, OPRD1, CALM, GSK3B, CHRM2, NOS3, SLC6A3, RXRA, PPARG, CDK2, ADRB2, CHEK1, KCNH2, NOS2, GABRA1, PRSS1, SCN5A, RXRB, CHRM3
MOL000392	formononetin	CDK2, ATP5F1B, ESR2, NOS3, SIRT1, PDE3A, MAOB, HSD3B1, ADRB2, PTGS2, JUN, CCNA2, PPARG, CHRM1, DPP4, HSD3B2, NOS2, PKIA, CHEK1, CALM, GSK3B, PPARG, HSP90, SLC6A4, RXRA, PTGS1, MAPK14, ACHE, ESR1, IL4, AR, PRSS1, ADRA1A, MT-ND6, F2, SLC6A3

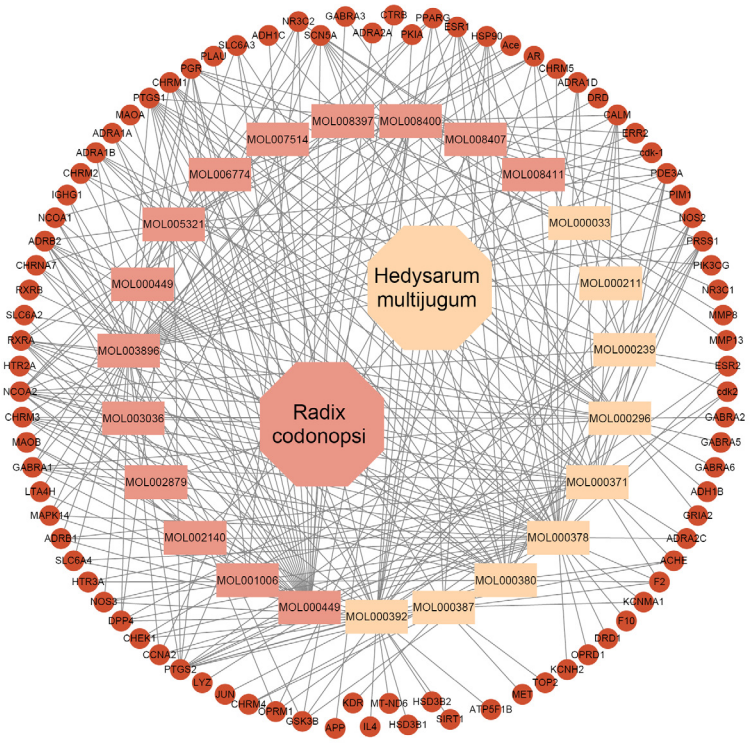

Figure 2. The interaction network of Herbs-compound-targets network. The hexagons represent the main herbs in SFI. The rectangles represent the compounds of the herbs. The ovals represent the potential targets of the compounds.

of SFI involved in the treatment of ischemic stroke.

Materials and methods

Identification of active ingredients of SFI

The compounds' data of *Hedysarum multijugum* (huangqi) and *Radix codonopsis* (dangshen) in the SFI preparation were obtained by searching the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, http://tcmspw.com/tcmsp.php). Compounds with oral bioavailability (OB) >30%, drug likeness (DL) >0.18, and blood-brain barrier (BBB) permeability >-0.3 were selected as the candidate active ingredients of SFI for further analysis.

Prediction of compound-associated targets

The whole compound-target interactions of the compounds (under the criterion of OB >30%, DL >0.18 and BBB permeability >-0.3) in SFI were identified on the "Related Targets" page of the TCMSP database.

Identification of ischemic stroke-associated targets

RNA sequencing-based gene expression profiles in peripheral blood samples collected from ischemic stroke patients and healthy volunteers (GSE122709 and GSE140275) were obtained from Gene Expression Omnibus (GEO). The RNA sequencing data of GSE122709 were obtained from the blood samples collected from 5 ischemic stroke patients within 24 hours after symptom onset, and 5 healthy volunteers [18]. The data of GSE140275 data were obtained from the the blood samples collected from 3 ischemic stroke patients and 3 healthy volunteers as control [19]. The downloaded platforms and a series of matrix files were converted using R programming language (version 3.6.1; https://www.r-project.org/) and annotation software packages. The criteria for screening differentially expression genes (DEGs) were those with $P<0.05$.

GO and KEGG pathway enrichment analysis

Gene Ontology (GO) analysis including biological process, cell component, and molecular function, and KEGG (https://www.kegg.jp/) pathway analysis of the shared genes were performed using the KEGG orthology (KO)-based Annotation System (KOBAS; http://kobas.cbi.pku.edu.cn/program.run.do).

Protein-protein interaction (PPI) network

The compound-target network of SFI, as well as the interaction network of the shared targets of SFI and ischemic stroke, was constructed and visualized using Cytoscape 3.7.2 software with a local STRING database [20]. Those genes with combined score of >500 were selected for PPI network construction. All nodes in the network were ranked by the degree of importance, and a network topological parameter was used for recognizing essential nodes, which repre-

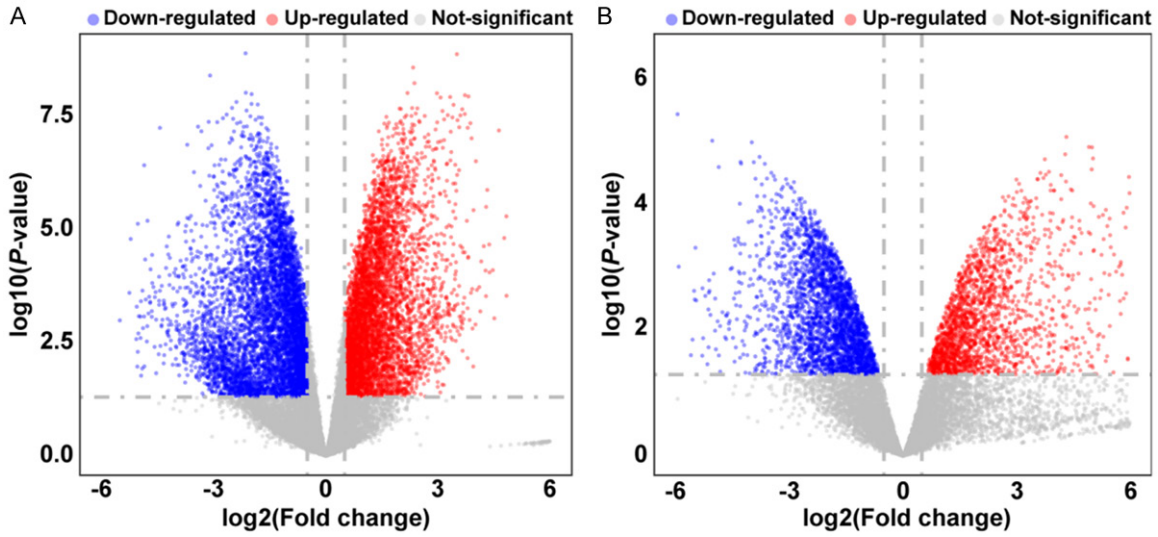

Figure 3. Differentially expressed genes of patients with ischemic stroke. A: The volcano plots of DEGs from GSE122709. B: The volcano plots of DEGs from GSE140275.

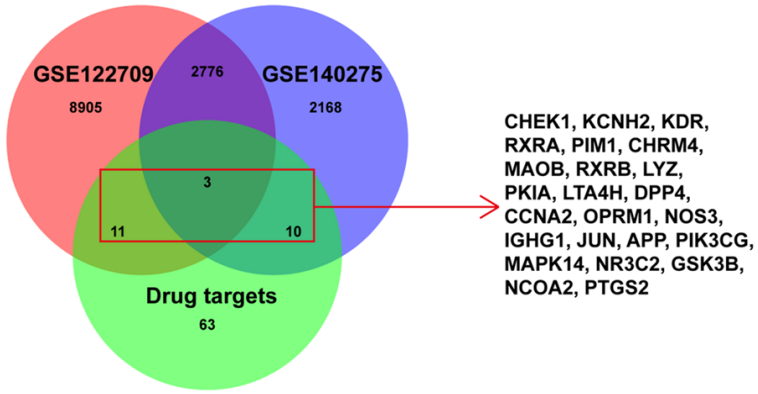

Figure 4. The intersection of the targets of SFI and is chemic stroke.

sented the number of edges connecting to the node. Genes with a degree of importance ≥10 were selected as essential targets.

GO terms or entities annotated with GO terms were compared using GO-based semantic similarity analysis, which is a mature tool for evaluating functional coherence of gene sets and disease or drug analysis [21]. Semantic similarity was calculated by GOSemSim [22].

Construction of TF-targets-pathway network

Pairwise correlation analysis of gene expression was performed using TRRUST (http://www.grnpedia.org/trrust), and the transcription factor-genes relationship among the intersections of SFI targets and IS targets, as well as the related pathways of IS targets, were established in a new network.

Statistical analysis

Comparisons of gene expression level between IS patients and healthy volunteers were performed using the unpaired *t*-test.

Results

Prediction of potential active ingredients and targets in radix codonopsis and hedysarum multijugum

SFI preparation contains the extracted substances of *Radix codonopsis* and *Hedysarum multijugum*. According to the criterion mentioned in Materials and methods, 28 active ingredients in SFI were identified as 'candidate compounds' (**Tables 1** and **2**). The number of candidate compounds contained in *Radix codonopsis* and *Hedysarum multijugum were* 16 and 12, respectively. No common ingredients were predicted for the two herbs. Through searching the TCMSP, 87 SFI-related targets were identified. The potential targets of each compound were also identified using TCMCP database (**Table 3**) and the compound-target network of SFI were established (**Figure 2**).

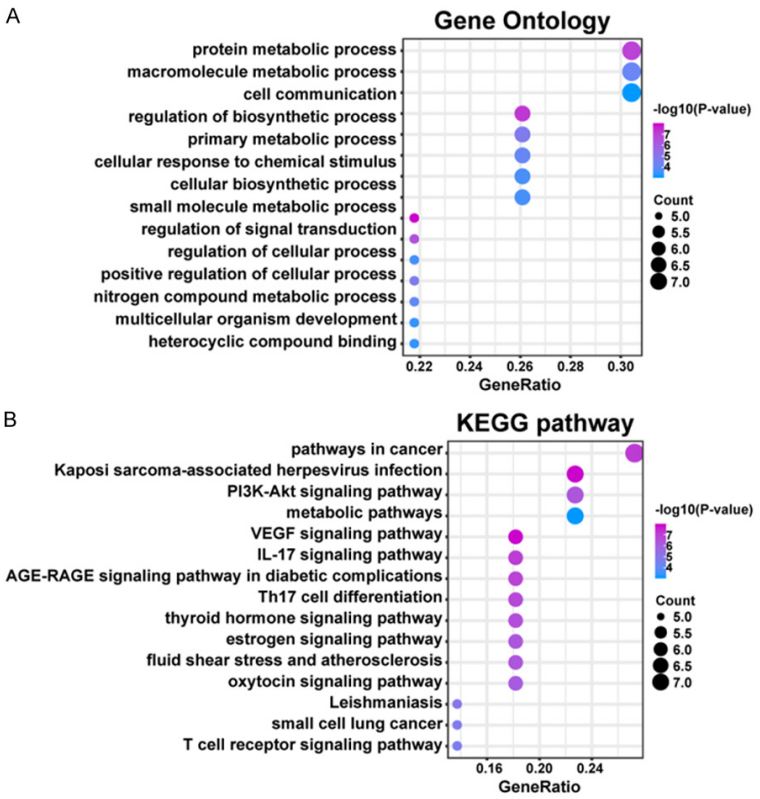

Figure 5. GO and KEGG enrichment analysis of the potential targets of SFI on ischemic stroke. A: GO function enrichment of the shared genes. B: KEGG pathway enrichment of the SFI-ischemic stroke targets. The color of the balls represented -log10 (*P*-value), and the size of the balls represented the counts of genes enriched in the pointed function.

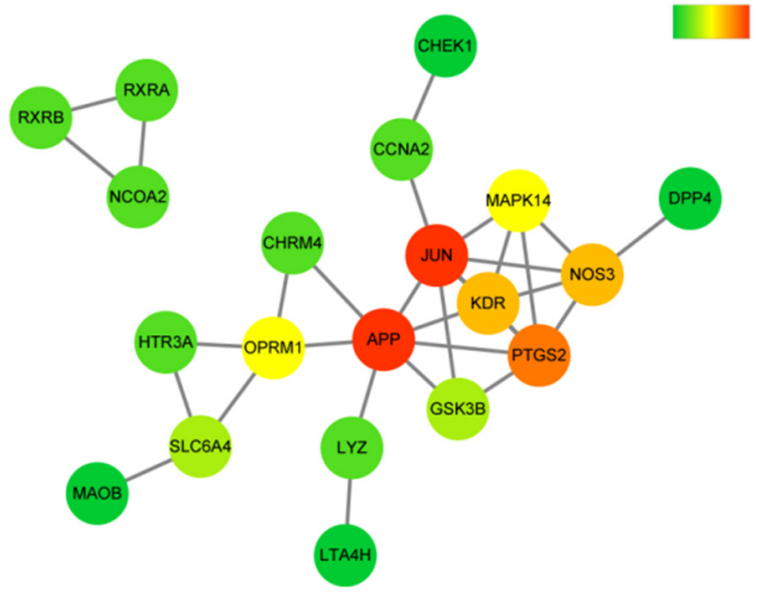

Figure 6. PPI network of the potential targets regulated by SFI. The higher degree value is shown in redder color. Nodes represent the genes. The lines between two nodes represent the interaction.

Some compounds were predicted to regulate a number of genes, including 7-methoxy-2-methyl isoflavone, 7-O-methylisomucronulatol, formononetin, stigmasterol, glycitein, (6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano-[3,2-c]chromen-3-ol, and frutinone A.

Identification of putative targets of ischemic stroke-related genes

A total of 11,695 and 4,957 DEGs were identified from the datasets of gene expression profiles of GSE122709 and GSE140275, respectively, and the DEGs were graphically highlighted using the volcano plots (**Figure 3A**, **3B**). The 24 shared targets of SFI and ischemic stroke were acquired and considered as the potential targets so as to investigate the relationship between the SFI potential targets and ischemic stroke-related genes (**Figure 4**).

GO functional annotation and KEGG pathway enrichment analyses

GO functional annotation was performed on these 24 genes, and the top GO terms were selected based on -$\log_{10}$ (*P*-values) (**Figure 5A**). The results indicated that the 24 genes were primarily involved in the following processes and functions: protein metabolic process, macromolecule metabolic process, cell communication, regulation of biosynthetic process, primary metabolic process, cellular response to chemical stimulus, cellular biosynthetic process, small molecule metabolic process, regulation of signal transduction, regulation of cellular

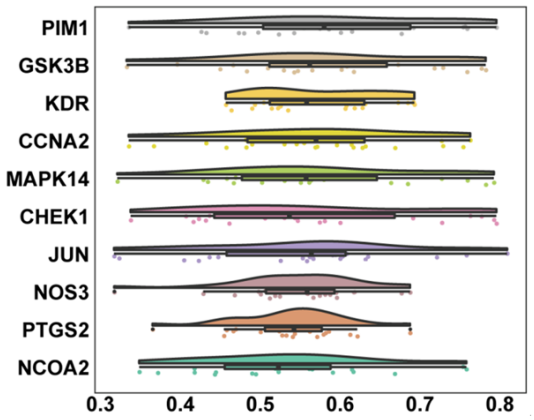

Figure 7. The Top 10 genes in the PPI network ranked by go semantic similarity.

process, positive regulation of cellular process, nitrogen compound metabolic process, multicellular organism development, and heterocyclic compound binding.

KEGG enrichment analysis was conducted to further explore these genes. The results showed that these genes were mapped to 115 pathways, with *P*-value <0.05. As shown in **Figure 5B**, these pathways included those involved in the development and progression of cancer, Kaposi sarcoma-associated herpesvirus infection, PI3K-Akt signaling pathway, metabolic pathways, VEGF signaling pathway, IL-17 signaling pathway, AGE-RAGE signaling pathway in diabetic complications, Th17 cell differentiation, thyroid hormone signaling pathway, estrogen signaling pathway, fluid shear stress and atherosclerosis, and oxytocin signaling pathway. The results suggested that SFI may play a role via the pathways mentioned above in the development and progression of IS.

PPI network of potential targets of SFI in treating ischemic stroke

The interaction network of potential targets of SFI in treatment of IS was constructed by using the Cytoscape 3.7.2 software based on the STRING database. As shown in **Figure 6**, the important targets were painted in red and located centrally in the network. APP, JUN, PTGS2, NOS3, KDR, MAPK14 and OPRM1 were ranked as the top 7 genes according to their degree of importance.

What's more, these 24 genes in the PPI network were ranked according to GO semantic similarity, which measured the strength of the relationship between each protein and its partners by considering the functions and location of proteins. The top 10 ranked genes were shown in **Figure 7**. The intersection between the top genes in the network and genes with the highest GO semantic similarity were JUN, PTGS2, KDR, NOS3, and MAPK14, which were considered as the hub genes of SFI involved in the treatment of ischemic stroke.

The TF-target interaction among potential genes

In order to reveal the regulational relationship among the 24 potential targets, TF-genes interaction was predicted by using the TRRUST database. As shown in **Figure 8**, JUN was predicted to be a transcription factor of APP, NOS3, OPRM1, PTGS2 and itself. Comparison between the hub genes revealed that SFI may exert therapeutic effects via JUN, which regulates the expression of NOS3 and PTGS2. The results of KEGG pathway analysis showed that JUN, NOS3 and PTGS2 were simultaneously enriched in the oxytocin signaling pathway, which might be the key pathway regulated by SFI in the treatment of ischemic stroke.

Finally, a herb-compound-target network with JUN, NOS3 and PTGS2 and 12 compounds of SFI that target these genes was constructed (**Figure 9**).

Discussion

SFI has been proved to protect cells and tissues from cerebral ischemia-reperfusion injury and irradiation-induced brain injury [13, 16]. In this study, based on network pharmacology and bioinformatics analysis, 28 key components of SFI were identified, which corresponded to 87 target proteins. Through functional annotation, pathway enrichment and hub genes screening, the study results indicated that SFI plays a neuroprotective role in ischemic stroke via interfering the transcriptional regulation of JUN/NOS3 and JUN/PTGS2. And this study was the first one to explore the pharmacological mechanisms of SFI in treating ischemic stroke.

In this study, JUN, NOS3 and PTGS2 were identified to be the key genes regulated by SFI in the treatment of ischemic stroke. JUN is a multifunctional transcription factor involved in ischemic stroke [23]. Previous studies have demon-

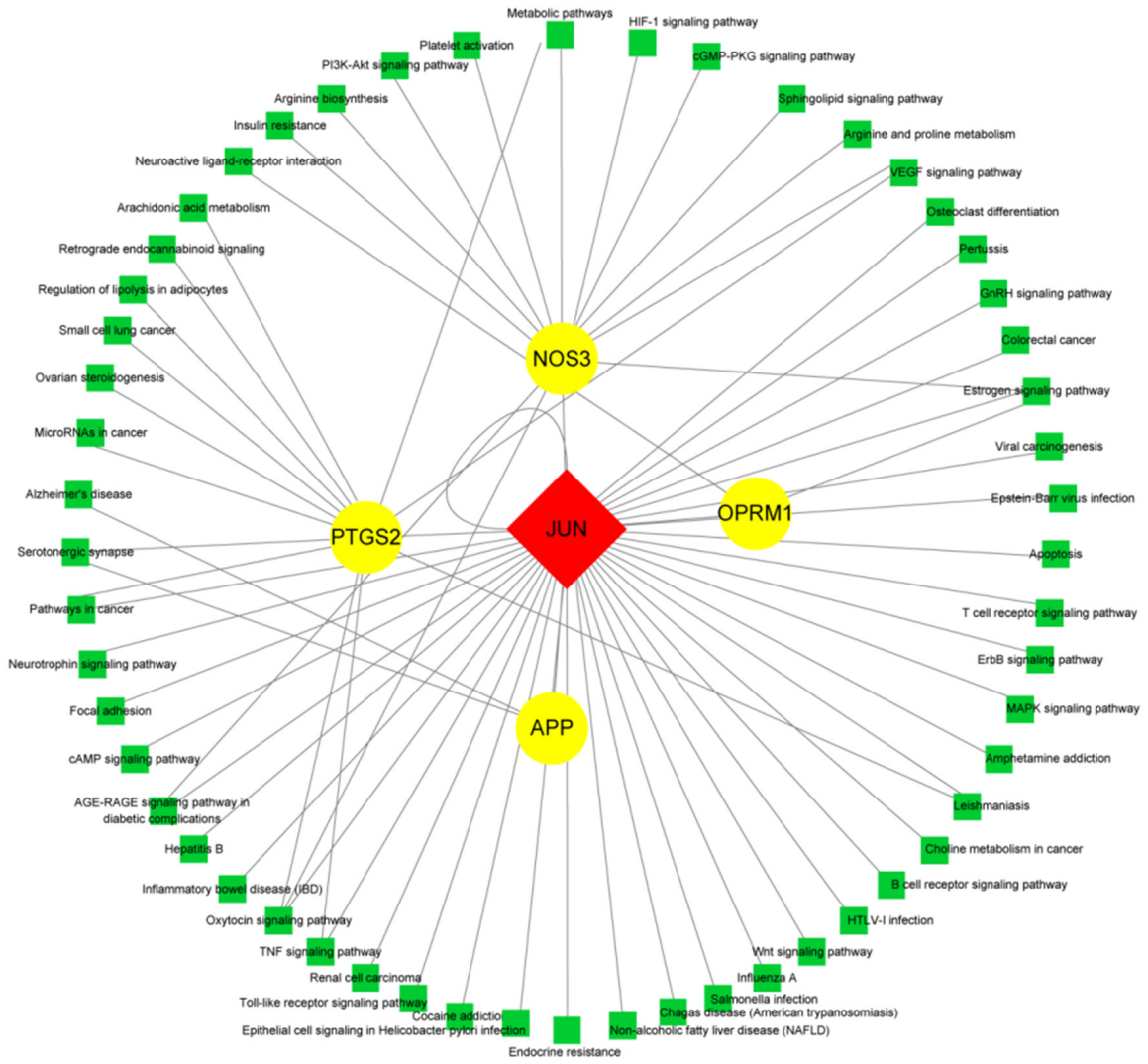

Figure 8. TF-targets-pathway network. Red diamond represented the transcription factors. The potential regulated targets of the TF were represented as yellow circles. The green rectangles were represented pathways associated with these targets.

strated that JUN can be activated by ischemia-induced upregulation of 14-3-3 gamma in astrocytes [24]; NOS3 is associated with severe traumatic brain injury, cardiovascular diseases and ischemic stroke [25-28]. As for PTGS2, previous studies have revealed that knockdown of PTGS2 can induce protective effect in mice with ischemic stroke by suppressing the NF-κB signaling pathway, leading to inhibition of apoptosis and promotion of cell proliferation, migration and angiogenesis of endothelial progenitor cells [29]. Several other studies have indicated that the constitutive expression of hepatocyte-specific gene PTGS2 plays a protective role in hepatic ischemia-reperfusion injury by down-regulating pro-inflammatory cytokines (i.e., IL-1β, IL-6 and TNF-α), promoting anti-apoptosis, and activating AKT and AMPK [30, 31].

The results of KEGG pathway analysis showed that JUN, NOS3 and PTGS2 participated in oxytocin signaling pathway. Oxytocin is a hormone secreted by pituitary and studies have provided evidences for its neuroprotective role. Oxytocin regulates the subunits of $GABA_A$ receptor in providing neuroprotection against reperfusion injury due to oxygen-glucose deprivation- [32]. In the animal model of ischemic stroke, oxytocin could downregulate the overexpression of calpain-1 and reduce the infarct volume of the cerebral cortex and striatum [33]. Thus, it was primarily inferred that the most important mechanism of SFI in the treat-

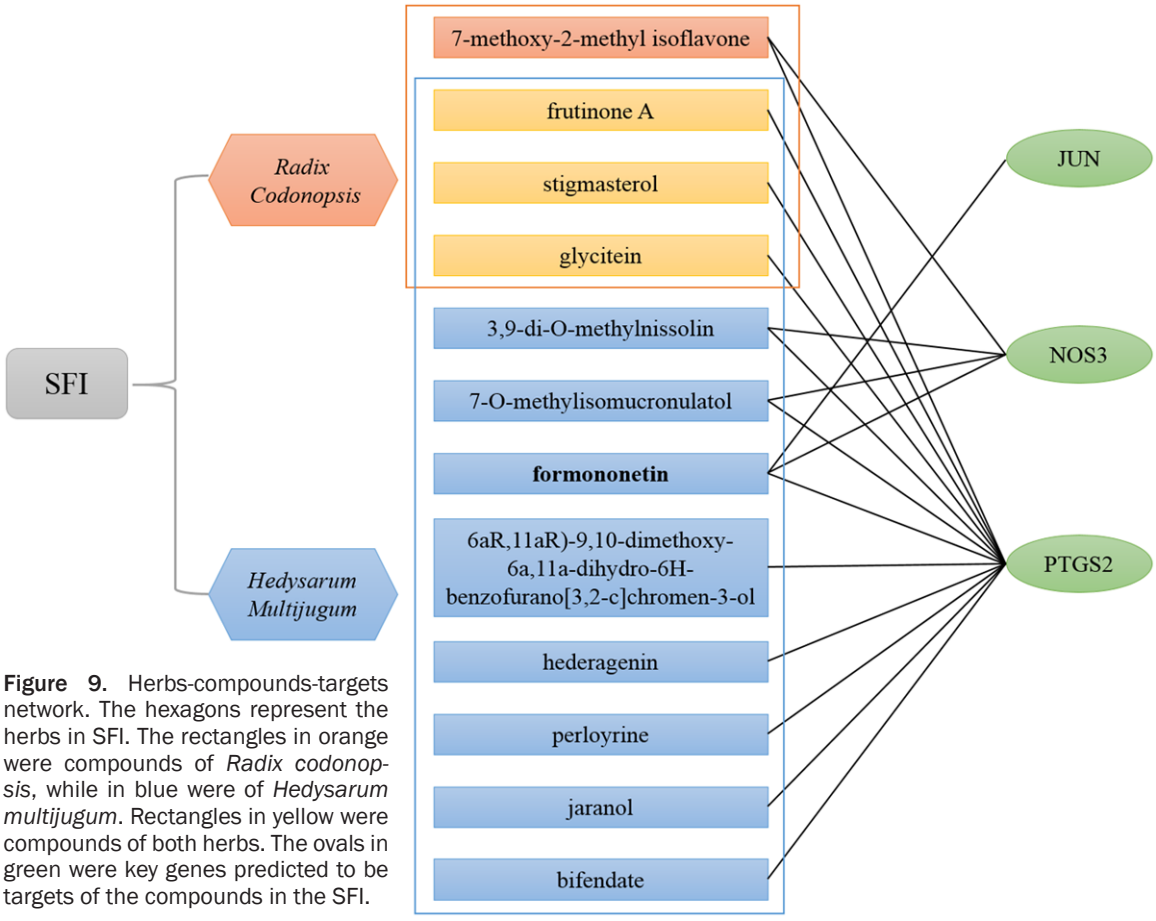

Figure 9. Herbs-compounds-targets network. The hexagons represent the herbs in SFI. The rectangles in orange were compounds of *Radix codonopsis*, while in blue were of *Hedysarum multijugum*. Rectangles in yellow were compounds of both herbs. The ovals in green were key genes predicted to be targets of the compounds in the SFI.

ment of ischemic stroke may be the regulation of oxytocin signaling pathway.

Besides the oxytocin signaling pathway, JUN and PTGS2 were enriched in the pathways involved in the pathogenesis of tumors, Kaposi sarcoma-associated herpesvirus infection, TNF signaling pathway, C-type lectin receptor signaling pathway, IL-17 signaling pathway and Leishmaniasis. The IL-17 signaling pathway was reported to mediate excessive autophagy and aggravate neuronal ischemic injuries [34]. JUN and NOS3 were enriched in the AGE-RAGE signaling pathway in diabetic complications and the estrogen signaling pathway as well. The expression of sRAGE in the plasma of early post-stroke patients, the key gene involved in the AGE-RAGE signaling pathway, may play a protective role in ischemic stroke [35]. It is likely that SFI has multiple functions in the treatment of ischemic stroke.

In this study, we identified 28 active ingredients in SFI. Formononetin, a compound contained in *hedysarum multijugum*, was predicted to simultaneously target JUN, NOS3 and PTGS2 (**Figure 8**). As a typical phytoestrogen, formononetin has been reported as a novel agent because of its diverse biological activities. For example, formononetin modulates osteogenic and myogenic differentiation through regulating p38MAPK-JAK-STAT and Smad-1/5/8 signaling pathways [36]. This ingredient can significantly upregulate the expression of microRNA miR-149 and suppress the proliferation and invasion of colon cancer cells, leading to the downregulation of ephrin type-B receptor 3 (EphB3) and inhibition of PI3K/AKT and STAT3 signaling pathways [37]. Studies have shown that formononetin could downregulate RANKL-induced expression of phosphorylated c-JUN [38]. Further studies are warranted to investigate the function of formonenetin using ischemic stroke models, which might be helpful to understand the exact mechanism of action of SFI in the treatment of ischemic stroke.

Taken together, SFI probably protects the nervous system against ischemic stroke through the regulation of JUN, NOS3 and PTGS2 in the

oxytocin signaling pathway by formonetin. Further biological and clinical studies are needed to verify the above findings.

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Disclosure of conflict of interest

None.

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References

[1] Liu L, Wang D, Wong KS and Wang Y. Stroke and stroke care in China: huge burden, significant workload, and a national priority. Stroke 2011; 42: 3651-3654.

[2] Bansal S, Sangha KS and Khatri P. Drug treatment of acute ischemic stroke. Am J Cardiovasc Drugs 2013; 13: 57-69.

[3] Wang W, Jiang B, Sun H, Ru X, Sun D, Wang L, Wang L, Jiang Y, Li Y, Wang Y, Chen Z, Wu S, Zhang Y, Wang D, Wang Y and Feigin VL. Prevalence, incidence, and mortality of stroke in China: results from a nationwide population-based survey of 480 687 adults. Circulation 2017; 135: 759-771.

[4] Wang P, Dai L, Zhou W, Meng J, Zhang M, Wu Y, Huo H, Xiong X and Sui F. Intermodule coupling analysis of Huang-Lian-Jie-Du decoction on stroke. Front Pharmacol 2019; 10: 1288.

[5] Li S and Zhang B. Traditional Chinese medicine network pharmacology: theory, methodology and application. Chin J Nat Med 2013; 11: 110-120.

[6] Guoqiong C, Zhengping H and Fengang M. Research progress of traditional Chinese medicine treating ischemic stroke. Liaoning J Tradit Chin Med 2019; 46: 2666-2671.

[7] Chen C, Venketasubramanian N, Gan RN, Lambert C, Picard D, Chan BP, Chan E, Bousser MG and Xuemin S. Danqi Piantang Jiaonang (DJ), a traditional Chinese medicine, in poststroke recovery. Stroke 2009; 40: 859-863.

[8] Geng JL, Aa JY, Feng SQ, Wang SY, Wang P, Zhang Y, Ouyang BC, Wang JK, Zhu YJ, Huang WZ, Wang ZZ, Xiao W and Wang GJ. Exploring the neuroprotective effects of ginkgolides injection in a rodent model of cerebral ischemia-reperfusion injury by GC-MS based metabolomic profiling. J Pharm Biomed Anal 2017; 142: 190-200.

[9] Dong XR, Wang JN, Liu L, Chen X, Chen MS, Chen J, Ren JH, Li Q and Han J. Modulation of radiation-induced tumour necrosis factor-alpha and transforming growth factor beta1 expression in the lung tissue by Shengqi Fuzheng injection. Mol Med Rep 2010; 3: 621-627.

[10] Wang J, Tong X, Li P, Cao H and Su W. Immunoenhancement effects of Shenqi Fuzheng Injection on cyclophosphamide-induced immunosuppression in Balb/c mice. J Ethnopharmacol 2012; 139: 788-795.

[11] Dong J, Su SY, Wang MY and Zhan Z. Shenqi fuzheng, an injection concocted from Chinese medicinal herbs, combined with platinum-based chemotherapy for advanced non-small cell lung cancer: a systematic review. J Exp Clin Cancer Res 2010; 29: 137.

[12] Ge L, Mao L, Tian JH, Shi FY, Lou L, Qiu X, Li JL and Yang KH. Network meta-analysis on selecting Chinese medical injections in radiotherapy for esophageal cancer. Zhongguo Zhong Yao Za Zhi 2015; 40: 3674-3681.

[13] Xu R, Lin L, Li Y and Li Y. ShenQi FuZheng Injection combined with chemotherapy in the treatment of colorectal cancer: a meta-analysis. PLoS One 2017; 12: e0185254.

[14] Qi GY, Liu P and Gao BL. Shenqi fuzheng injection alleviates the transient worsening caused by steroids pulse therapy in treating myasthenia gravis. Evid Based Complement Alternat Med 2013; 2013: 816829.

[15] Cai YM, Hu HT and Ma XY. Protective effect of shenqi fuzheng injection on cerebral ischemia/reperfusion injured aged rats. Zhongguo Zhong Xi Yi Jie He Za Zhi 2006; 26 Suppl: 10-14.

[16] Zhang J, Tong F, Cai Q, Chen LJ, Dong JH, Wu G and Dong XR. Shenqi fuzheng injection attenuates irradiation-induced brain injury in mice via inhibition of the NF-κB signaling pathway and microglial activation. Acta Pharmacol Sin 2015; 36: 1288-1299.

[17] Zhang HR, Peng JH, Cheng XB, Shi BZ, Zhang MY and Xu RX. Paeoniflorin atttenuates amyloidogenesis and the inflammatory responses in a transgenic mouse model of Alzheimer's disease. Neurochem Res 2015; 40: 1583-1592.

[18] Zhu WL, Tian LL, Yue XY, Lu JY, Fu Y and Yan YP. LncRNA expression profiling of ischemic stroke during the transition from the acute to subacute stage. Front Neurol 2019; 10: 36.

[19] Li S, Zheng H, Chen L, Xu C, Qu X, Qin Z, Gao J, Li J and Liu J. Expression profile and potential functions of circulating long noncoding RNAs in acute ischemic stroke in the Southern Chinese Han population. Front Mol Neurosci 2019; 12: 290.
[20] von Mering C, Huynen M, Jaeggi D, Schmidt S, Bork P and Snel B. STRING: a database of predicted functional associations between proteins. Nucleic Acids Res 2003: 31: 258-61.
[21] Mazandu GK, Chimusa ER and Mulder NJ. Gene Ontology semantic similarity tools: survey on features and challenges for biological knowledge discovery. Brief Bioinform 2017; 18: 886-901.
[22] Yu G, Li F, Qin Y, Bo X, Wu Y and Wang S. GOSemSim: an R package for measuring semantic similarity among GO terms and gene products. Bioinformatics 2010; 26: 976-978.
[23] Zhang C, Wu H, Zhu X, Wang Y and Guo J. Role of transcription factors in neurogenesis after cerebral ischemia. Rev Neurosci 2011; 22: 457-465.
[24] Dong Y, Liu HD, Zhao R, Yang CZ, Chen XQ, Wang XH, Lau LT, Chen J and Yu AC. Ischemia activates JNK/c-Jun/AP-1 pathway to up-regulate 14-3-3gamma in astrocyte. J Neurochem 2009; 109 Suppl 1: 182-188.
[25] Grewal RP, Dutra AV, Liao YC, Juo SH and Papamitsakis NI. The intron 4c allele of the NOS3 gene is associated with ischemic stroke in African Americans. BMC Med Genet 2007; 8: 76.
[26] Türkanoğlu Özçelik A, Can Demirdöğen B, Demirkaya Ş and Adalı O. Importance of NOS3 genetic polymorphisms in the risk of development of ischemic stroke in the Turkish. Genet Test Mol Biomarkers 2014; 18: 797-803.
[27] Robertson CS, Gopinath SP, Valadka AB, Van M, Swank PR and Goodman JC. Variants of the endothelial nitric oxide gene and cerebral blood flow after severe traumatic brain injury. J Neurotrauma 2011; 28: 727-737.
[28] Kobashi G, Yamada H, Ohta K, Kato E, Ebina Y and Fujimoto S. Endothelial nitric oxide synthase gene (NOS3) variant and hypertension in pregnancy. Am J Med Genet 2001; 103: 241-244.
[29] Zhou Z, Lu C, Meng S, Dun L, Yin N, An H, Xu H, Liu G and Cai Y. Silencing of PTGS2 exerts promoting effects on angiogenesis endothelial progenitor cells in mice with ischemic stroke via repression of the NF-kappaB signaling pathway. J Cell Physiol 2019; 234: 23448-23460.
[30] Motiño O, Francés DE, Casanova N, Fuertes-Agudo M, Cucarella C, Flores JM, Vallejo-Cremades MT, Olmedilla L, Pérez Peña J, Bañares R, Boscá L, Casado M and Martín-Sanz P. Protective role of hepatocyte cyclooxygenase-2 expression against liver ischemia-reperfusion injury in mice. Hepatology 2019; 70: 650-665.
[31] Tolba RH, Fet N, Yonezawa K, Taura K, Nakajima A, Hata K, Okamura Y, Uchinami H, Klinge U, Minor T, Yamaoka Y and Yamamoto Y. Role of preferential cyclooxygenase-2 inhibition by meloxicam in ischemia/reperfusion injury of the rat liver. Eur Surg Res 2014; 53: 11-24.
[32] Kaneko Y, Pappas C, Tajiri N and Borlongan CV. Oxytocin modulates GABAAR subunits to confer neuroprotection in stroke in vitro. Sci Rep 2016; 6: 35659.
[33] Etehadi Moghadam S, Azami Tameh A, Vahidinia Z, Atlasi MA, Hassani Bafrani H and Naderian H. Neuroprotective effects of oxytocin hormone after an experimental stroke model and the possible role of calpain-1. J Stroke Cerebrovasc Dis 2018; 27: 724-732.
[34] Liu T, Han S, Dai Q, Zheng J, Liu C, Li S and Li J. IL-17A-mediated excessive autophagy aggravated neuronal ischemic injuries via Src-PP2B-mTOR pathway. Front Immunol 2019; 10: 2952.
[35] Tang SC, Wang YC, Li YI, Lin HC, Manzanero S, Hsieh YH, Phipps S, Hu CJ, Chiou HY, Huang YS, Yang WS, Mattson MP, Arumugam TV and Jeng JS. Functional role of soluble receptor for advanced glycation end products in stroke. Arterioscler Thromb Vasc Biol 2013; 33: 585-594.
[36] Soundharrajan I, Kim DH, Kuppusamy P and Choi KC. Modulation of osteogenic and myogenic differentiation by a phytoestrogen formononetin via p38MAPK-dependent JAK-STAT and Smad-1/5/8 signaling pathways in mouse myogenic progenitor cells. Sci Rep 2019; 9: 9307.
[37] Wang AL, Li Y, Zhao Q and Fan LQ. Formononetin inhibits colon carcinoma cell growth and invasion by microRNA-149-mediated EphB3 downregulation and inhibition of PI3K/AKT and STAT3 signaling pathways. Mol Med Rep 2018; 17: 7721-7729.
[38] Huh JE, Lee WI, Kang JW, Nam D, Choi DY, Park DS, Lee SH and Lee JD. Formononetin attenuates osteoclastogenesis via suppressing the RANKL-induced activation of NF-kappaB, c-Fos, and nuclear factor of activated T-cells cytoplasmic 1 signaling pathway. J Nat Prod 2014; 77: 2423-2431.