



Shennao fuyuan decoction and hUCMSC-exo combination therapy promotes lipid and atherosclerosis pathway activation and improves brain injury in middle cerebral artery occlusion rats

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Human umbilical cord mesenchymal stem cell-derived exosomes (hUCMSC-exo) decrease the mortality rates associated with brain injuries. Its combination with Shennao fuyuan decoction (SFD), a traditional Chinese medicine formulation for ischemic stroke treatment, additionally augments neural deficit repair in rats post-cerebral ischemia. Nonetheless, there is a notable need for more comprehensive research into the exact mechanisms by which this combined treatment ameliorates brain injuries. Consequently, we conducted thorough research on the mechanisms of action of these entities, utilizing network pharmacology approaches and a rat model of middle cerebral artery occlusion (MCAO). Our findings identified the hsa05417: Lipid and atherosclerosis pathway as the primary channel through which SFD impacts the cardiovascular system. The integrated treatment of SFD and hUCMSC-exo attenuated TNF and HSP90AA1 expression and elevated IL6 and GSK3B levels in the brains of rats subjected to MCAO. Moreover, the discrete utilization of hUCMSC-exo in conjunction with SFD reduced cerebral infarction and diminished tissue damage in rats with MCAO. Our investigation has revealed that the coordinated use of hUCMSC-exo and SFD may influence the TNF, HSP90AA1, IL6 and GSK3B targets, thereby managing the lipid and atherosclerosis pathway and lessening cerebral harm in rats afflicted by MCAO.

Keywords: Atherosclerosis, Cerebral infarction, Exosomes, Traditional Chinese medicine

Middle cerebral artery occlusion (MCAO) leads to focal cerebral hypoperfusion, resulting in ischemic stroke¹. Ischemic stroke is a leading cause of death and disability worldwide². Malignant middle cerebral artery infarction refers to a large, space-occupying infarction that is accompanied by massive edema and cerebral herniation and often leads to death³. Current management options include medical treatment, with or without decompression hemicraniectomy, and subsequent duraplasty⁴. Surgical decompression has been shown to improve the treatment of malignant edema after acute ischemic stroke, resulting in a significant reduction in death or severe disability and a decrease in death or moderate disability⁵. However, the mortality rate hovers as high as 30%, even if all kinds of decompressing therapies, such as hyperventilation, mannitol, hypertonic saline, and decompressive craniectomy, were performed⁶. In recent years, there has been an increasing interest in investigating the mechanisms of traditional Chinese medicine (TCM) for treating MCAO⁷.

Cerebral infarction is classified as an "ischemic stroke" in TCM, which has been treating such conditions for centuries⁸. Transitioning from achieving clinical treatment goals to deeply understanding the causes of the disease, traditional Chinese medicine has demonstrated considerable clinical benefits in managing ischemic stroke, featuring multiple pharmacological targets corresponding with the progressive course of the disease^{9,10}. Buyang huanwu decoction is a classic herbal formula of TCM. The Shennao fuyuan decoction (SFD) was mainly composed of Buyang huanwu decoction, and Chinese herbal was added and subtracted. The SFD has been recognized as an efficacious TCM remedy for ischemic stroke, with over two decades of availability in China¹¹. SFD derives its composition from Buyang huanwu decoction, consisting of a blend of traditional herbs, including *Rehmanniae Radix praeparata* (processed root of *Rehmannia glutinosa* Libosch.), *Corni Fructus* (pulp of ripe fruit of *Cornus officinalis* Sieb. Et Zucc.), *Astragali radix* (root of *Astragalus membranaceus* (Fisch.) Bge.var. *mongholicus* (Bge.) Hsiao), *Dioscoreae rhizoma* (root of *Dioscorea opposita* Thunb.), *Rhodiolae crenulatae radix et rhizoma* (root

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and rhizome of *Rhodiola crenulata* (Hook. f. et Thoms.) H. Ohba), *Moutan cortex* (root bark of *Paeonia suffruticosa* Andr.), *Angelicae sinensis radix* (root of *Angelica sinensis* (Oliv.) Diels), *Paeoniaeradix rubra* (root of *Paeonia lactiflora* Pall) and *Pheretima* (Chinese name Dilong)^{11,12}.

Network pharmacology has been extensively adopted for screening TCM compounds in ischemic stroke¹³. For example, the Taohong siwu decoction demonstrates pronounced neuroprotective effects on focal cerebral ischemia precipitated by MCAO¹⁴. The chief active elements of *Cistanche deserticola* are the total glycosides, which counteract brain injury from MCAO/R predominantly through the Nrf-2/Keap-1 pathway¹⁵. Presently, conventional treatments that rely on antithrombotic and neuroprotective modalities are severely constrained by their limited safety and effectiveness¹⁶. Consequently, investigating the application of SFD could offer a novel viable strategy for MCAO intervention.

Mesenchymal stem cells (MSC) secrete exosomes, which have been implicated in brain diseases¹⁷. MSC-derived exosome miR-542-3p has shown potential in preventing ischemia-induced glial inflammation by downregulating TLR-4, suggesting a possible therapeutic strategy for delivering exosomes to treat cerebral ischemia injury¹⁸. Furthermore, the combined implantation of SFD and human umbilical cord mesenchymal stem cell-derived exosomes (hUCMSC-exo) has significantly improved the repair of nerve defects after cerebral ischemia in rats¹⁹. This neuroprotective effect may be attributed to promoting BDNF and bFGF expression. Based on this, we hypothesized that combining exosome and TCM compound therapy could enhance the pharmacokinetic behavior of drugs *in vivo*, leading to effective drug accumulation at the target site, improved therapeutic effects, and reduced side effects. The temporary or permanent intracavitary line occlusion-MCAO model is commonly used to simulate ischemic stroke in humans²⁰. Therefore, in this study, we investigated the potential effects of Shennao fuyuan decoction (SFD) and hUCMSC-exo in combination for the treatment of middle cerebral artery occlusion (MCAO) in rats.

Materials and Methods

Animal model preparation

The animal experiments were approved by the Ethics Committee of the First Affiliated Hospital of

Hunan University of Chinese Medicine (LLBH-202101060004). Male *Sprague-Dawley* (SD) rats were obtained from Hunan Slyke Jingda Animal Research Center to create the MCAO rat model. The animals were randomly divided into four groups: the control group (Sham), the model group (MCAO), the SFD group (SFD), and the SFD+hUCMSC-exo (SFD+hUCMSC-exo) group. The MCAO rat model was constructed as follows:²¹ the rat was placed supine, and a midline incision was made in the neck to expose the external carotid artery (ECA) and the common carotid artery (CCA). The ECA was ligated to separate the internal carotid artery. One clamp was placed on the CCA, and another on the internal carotid artery. The ECA was then cut, and a 4-0 silicon-coated monofilament suture was inserted into the ECA. The clamp on the internal carotid artery was removed, and the sutures were advanced (18-22 mm) until resistance was felt. After 2 h of occlusion, the sutures and CCA clips were removed, and the ECA was closed. The neck wound was stitched closed, and the animal was allowed to recover.

Preparation of SFD

The SFD formulation consists of the following ingredients: 10 g of *Rehmanniae radix praeparata*, 30 g of *Astragali Radix*, 10 g of *Corni fructus*, 15 g of *Dioscoreae rhizoma*, 20 g of *Rhodiola crenulatae radix et rhizomae*, 10 g of *Moutan cortex*, 10 g of *Paeoniaeradix rubra*, 10 g of *Angelicae sinensis radix*, and 10 g of *Pheretima*. The Pharmacy Department of the First Hospital of the Hunan University of Chinese Medicine verified these medicinal materials as genuine, following the standards set in the 2020 edition of the Pharmacopoeia of the People's Republic of China. For the water-soluble extraction process, 0.5 kg of SFD powder were steeped in 3 kg of water for one hour and heated at 110°C for another hour. After the first filtration, the remaining solids were reconstituted in 3 kg of fresh water and heated at 110°C for one hour. The filtrates from both processes were then combined through a second round of filtration. After the filtration, the SFD liquid extract was evaporated, under a vacuum at 60°C, into a dry powder form. This dried extract powder was reconstituted in distilled water for the following experimental procedures.

Extraction and identification of exosomes

Human umbilical cord mesenchymal stem cells (HUM-iCell-e011) were sourced from iCell Bioscience Inc. The hUCMSCs were cultured in a

specialized medium (ZQ-1320, provided by Shanghai Zhongqiao Xinzhou Biological Technology Co., Ltd). Upon reaching a 70-80% confluency, the cells were digested with 0.25% trypsin (which contained 0.02% EDTA, product C0201 from Beyotime Biotechnology) to prepare a cell suspension. Subsequently, cells underwent centrifugation as part of the subculturing process. Following a 48 h culture period, the cell supernatant was harvested and centrifuged to remove cellular debris. Exosomes were extracted using the ExoQuick solution (EXOQ5A-1; SBI System Biosciences, USA) according to the manufacturer's instructions. A sample volume of 10 μ L from the exosome suspension was deposited onto a film-covered copper grid and subsequently heated in the oven for 1 to 5 min. Then, a 10 μ L aliquot of phosphotungstic acid staining solution was dispensed onto the copper grid prepared with the sample. The grid was left to air-dry prior to observation under an electron microscope. Furthermore, markers, including CD9, CD63, calnexin and TSG101, were detected in the exosomes isolated from hUCMSC-exo.

Animal experiments and grouping

The SFD administration in rats was derived from the equivalency of a daily intake of 145 g SFD powder for an adult weighing 60 kg. The dosing formula employed was: rat dose (g) = human daily dose (g) multiplied by the ratio of rat body mass to body surface area to that of the human mass to body surface area. Each group received a dosage volume of 20 mL/kg, equivalent to 40 g of SFD powder per kg. Daily, the rats were given an intragastric dose of SFD water extract, which had been diluted with distilled water to the specified volume. In the group receiving SFD and exosomes, rats were administered an intraperitoneal injection of 200 μ g of exosome²² and SFD for seven consecutive days.

Network pharmacology

Active compounds along with their targets from *Rehmanniae radix praeparata*, *Astragali radix*, *Corni fructus*, *Dioscoreae rhizoma*, *Moutan cortex*, *Paeoniaeradix rubra* and *Angelicae sinensis radix* were retrieved from the Traditional Chinese Medicine Systems Pharmacology Database (TCMSP database) applying thresholds of Oral Bioavailability (OB) \geq 30% and Drug Likeness (DL) \geq 0.18²³. Supplementation was derived from the Batman-TCM database for Pheretima's active compounds²⁴. The active compounds of *Rhodiola crenulatae radix et rhizoma* were sourced from The Encyclopedia of

Traditional Chinese Medicine 2.0 (EMCT database). The term "middle cerebral artery" was used as a keyword to assess related genes within the GeneCards, NCBI, OMIM and CTD databases. After entering the chosen drug and disease targets into Venny 2.1, we identified 233 mutual targets that were earmarked for ensuing analysis. The String database was utilized to construct a protein-protein interaction (PPI) network from the common drug-disease targets, specifying "Homo sapiens" as the biological species and a high-confidence score (>0.9). The PPI network data were integrated into Cytoscape 3.8.0, where NetworkAnalyzer facilitated topology analysis and organized nodes according to degree²⁵. Enrichment analysis of common targets within the KEGG pathways was undertaken to elucidate the biological pathways involved. Outcome significance was ascertained through screening, with a P-value threshold set below 0.05.

2,3,5-Chlorotriphenyltetrazolium (TTC) staining

After administering anesthesia, the brains of rats were quickly removed through decapitation. Any blood stains were cleaned using 0.9% saline solution. The brain tissue was then frozen for 30 min in a refrigerator set at -20°C . Coronal sections with a standard thickness of 2.0 mm were prepared. These sections were immersed in a pre-prepared solution of 1% TTC (AWI0490a, Abiowell) and incubated in a light-free environment. The sections were turned every 5 min during incubation to ensure consistent staining. The staining process could be stopped once the normal area was uniformly bright red while the infarcted part remained unstained and white. The stained brain sections were then fixed in 4% paraformaldehyde (AWI0056a, Abiowell) and subsequently observed. The Zea Longa method, precisely the 5-level 4-point method as given in Ding *et al.*²⁶, was employed to quantify the score of neural dysfunction in the rats.

Hematoxylin-eosin (HE) staining

Tissue slices were roasted at 60°C for 12 h. Then, the sections were dewaxed with water. Hematoxylin (Abiowell) and eosin (Abiowell) were used to stain the sections consecutively. Finally, the histopathology was observed using a microscope (BA210T, Motic).

Immunofluorescence (IF)

The tissue sections were first treated with sodium borohydride solution and Sudan black dye solution after being prepared for thermos-repair antigen. Following this, the sections were sealed with a 5%

BSA solution. BrdU (ab8152, 1:50, Abcam) and DCX (ab18723, 1:50, Abcam) were applied to the sections. A fluorescent antibody labeled with anti-IgG was then added to the sections. The sections were observed under a microscope, and simultaneously, the number of BrdU+DCX+ neurons was statistically analysed.

Western blot

Brain tissues of rats were collected, and the proteins were separated using radioimmunoprecipitation assay, lysis buffer, and SDS-PAGE methods. The isolated proteins were then transferred to polyvinylidene fluoride membranes. Subsequently, they were incubated at 4°C overnight with the following antibodies: anti-CD9 (ab236630, 1:1000, Abcam, USA), anti-CD63 (25682-1-AP, 1:500, Proteintech, USA), anti-Calnexin (ab22595, 1 µg/mL, Abcam, USA), anti-TSG101 (ab83, 1:2000, Abcam, USA), TNF- α (ab6671, 1:1000, Abcam, USA), IL-6 (ab6672, 1:1000, Abcam, USA), (ab79849, 1:1000, Abcam, USA), anti-GSK-3 β (22104-1-AP, 1:2000, Proteintech, USA). Afterward, they were incubated with anti-IgG (1:500/6000, Proteintech, USA) at 37°C for 90 min. Chemiluminescence (Millipore, USA) was used for visualization, and imaging analysis was performed by GE Healthcare (Life Sciences, USA).

Data analysis and statistics

GraphPad Prism 8.0 statistical software was utilized to analyze the data in this study. The measurement data were expressed as mean \pm standard deviation. Initially, a normality and variance homogeneity test was performed, which confirmed that the data followed a normal distribution and exhibited homogeneity of variance. For inter-group comparison, an unpaired t-test was employed. A one-way ANOVA of repeated measurement data was also conducted for inter-group comparison. Subsequently, Tukey's test was performed. A significance level of $P < 0.05$ was considered statistically significant, and $P < 0.01$ was considered highly statistically significant (* $P < 0.05$; ** $P < 0.01$).

Results

Isolation and identification of hUCMSC-exo

Examination through transmission electron microscopy displayed exosomes as saucer-like vesicles spanning 40 to 150 nm in diameter (Fig. 1A). Exosomal markers CD9, CD63, and TSG101 were detected in the exosomes, in contrast to Calnexin, which showed no expression (Fig. 1B). The outcomes

validate the successful isolation of hUCMSC-exo, rendering them appropriate for ensuing experimental assays.

Amelioration of cerebral infarction in MCAO rats by combined SFD and hUCMSC-exo treatment

Compared to the Sham group, a greater incidence of white infarcts was evident in the cerebral tissues of MCAO rats, particularly extensive infarctions in the right cerebral region. Deployment of either SFD alone or in conjunction with exosomes diminished the extent of infarcted cerebral tissue in MCAO rats, with the combined treatment exhibiting enhanced effectiveness. Concurrently, the MCAO group demonstrated heightened scores for neurologic dysfunction, which were reduced following treatment with SFD alone or SFD paired with exosomes (Fig. 2A). Brain cells of MCAO rats exhibited pronounced edema, as evidenced by cytoplasm stained lightly and assumed a faint red hue. Nissl substance alongside neurons exhibited vanishing, interspersed with an increase in interneuronal space, with some areas showing notable background transparency (Fig. 2B). MCAO rats treated with SFD alone or in conjunction with exosomes generally retained normal morphology of brain tissue cells and exhibited loosely arrayed nerve fibers without marked tissue damage (Fig. 2B). These findings suggest that the concomitant application of SFD and hUCMSC-exo contributes to the amelioration of cerebral infarction and associated tissue impairment in MCAO rats.

Network pharmacology analysis

Through network pharmacology analysis, a total of 79 common targets between SFD and MACO were identified (Fig. 3A). A protein-protein interaction (PPI) network was constructed using The String

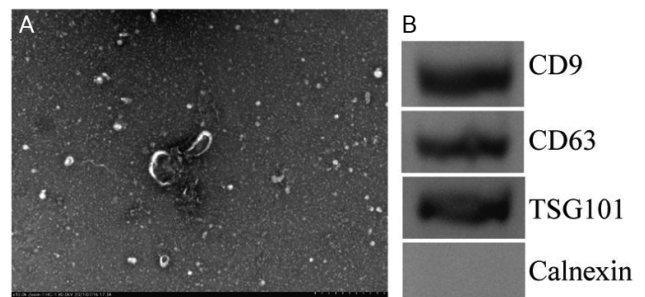


Fig. 1 — Extraction and identification of hUCMSC-exo. (A) Observation of vesicle structure of hUCMSC-exo by transmission electron microscopy [$\times 10.0k$, Scale bar = 1 μ m]; and (B) Western blotting-based detection of CD9, CD63 TSG101, and Calnexin expression levels in hUCMSCs-exo.

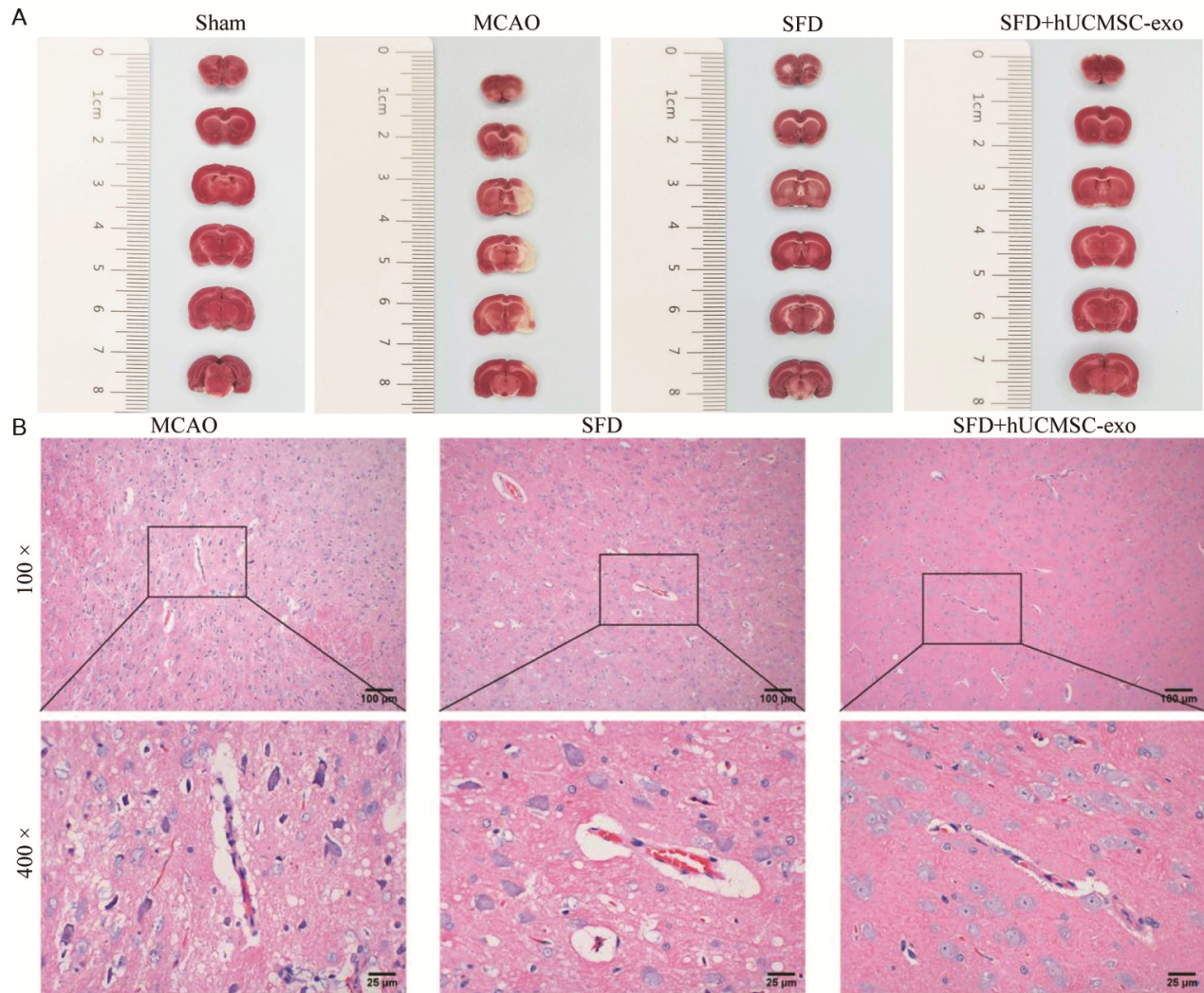


Fig. 2 — SFD combined with hUCMSC-exo improved MCAO. (A) TTC staining; and (B) HE staining [100X and 400X, Scale bar = 100 μm and 25 μm].

database. Topological analysis revealed that the top 10 highly connected targets in the PPI network were TNF, IL6, IL1B, MMP9, AKT1, PTGS2, PPARG, TGFB1, CCL2, and CASP3 (Fig. 3B). GO analysis of the DAVID database was conducted on these 79 common targets, and the top 10 results for biological processes (BP), cellular components (CC), and molecular functions (MF) are depicted in Fig. 3C. Furthermore, KEGG pathway analysis (Fig. 3D) demonstrated that the most significant pathway influencing the cardiovascular system is hsa05417: Lipid and atherosclerosis, involving 27 common targets. Additionally, we constructed a regulatory network illustrating the interactions between active ingredients of traditional Chinese medicine and targets (Fig. 3E). The results indicated that the top 10 highly connected targets in this network were PTGS2,

PTGS1, HSP90AA1, DPP4, ESR1, NOS2, ADRB2, WNT3A, F7 and GSK3B. Among the 25 targets in the lipid and atherosclerosis pathway, 8 targets (TNF, CASP3, CCL2, AKT1, MMP9, IL6, IL1B, PPARG) are among the top 10 targets in the PPI network. GSK3B and HSP90AA1 are among the top 10 targets in the TMC-active ingredients-targets network (Fig. 3F).

Synergistic effect of SFD and hUCMSC-exo on neurogenesis in cerebral tissue of MCAO rats

The synergistic treatment with SFD and hUCMSC-exo resulted in an increased count of DCX(+)-positive cells in the cerebral tissues of MCAO rats relative to the control group (Fig. 4). The study's outcomes suggest that the concomitant use of SFD and hUCMSC-exo advances brain injury recovery in MCAO rats by bolstering neurogenic processes in the brain.

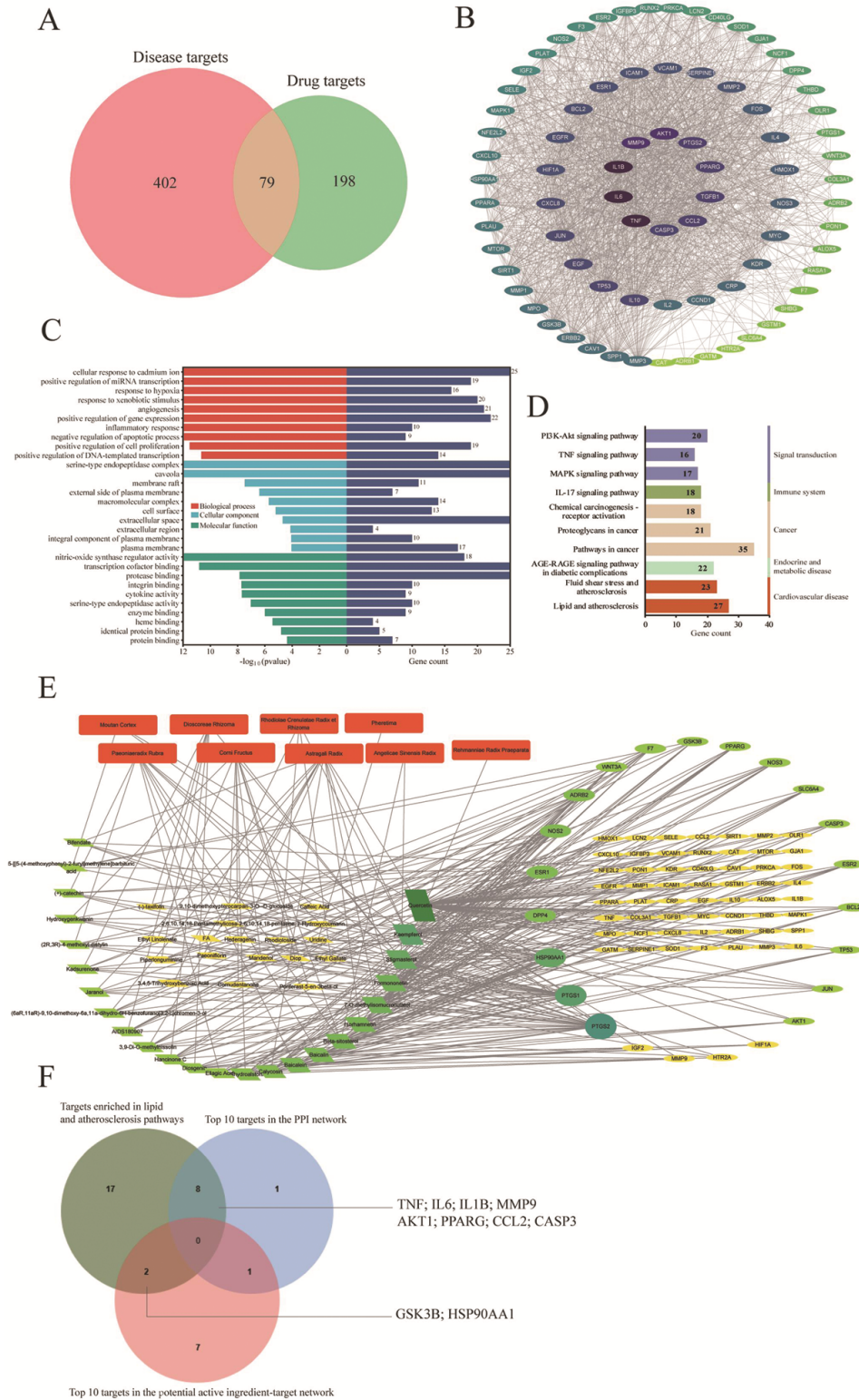


Fig. 3 — Network pharmacological analysis. (A) Common targets of SFP and MCAO; (B) PPI network of the common targets; (C & D) GO analysis and KEGG pathway analysis of the common targets using a bar graph to rank the importance from top to bottom; (E) TCM-active compound-target network. 9 TCMs are shown as red rectangles, 42 active compounds, 42 active compounds and 79 targets are shown as circles. The higher the degree of the target gene, the darker the colour; and (F) Venn diagram of targets enriched in lipid and atherosclerosis pathway, top 10 degree targets in PPI network and top 10 degree targets in TCM-active compound-target network.

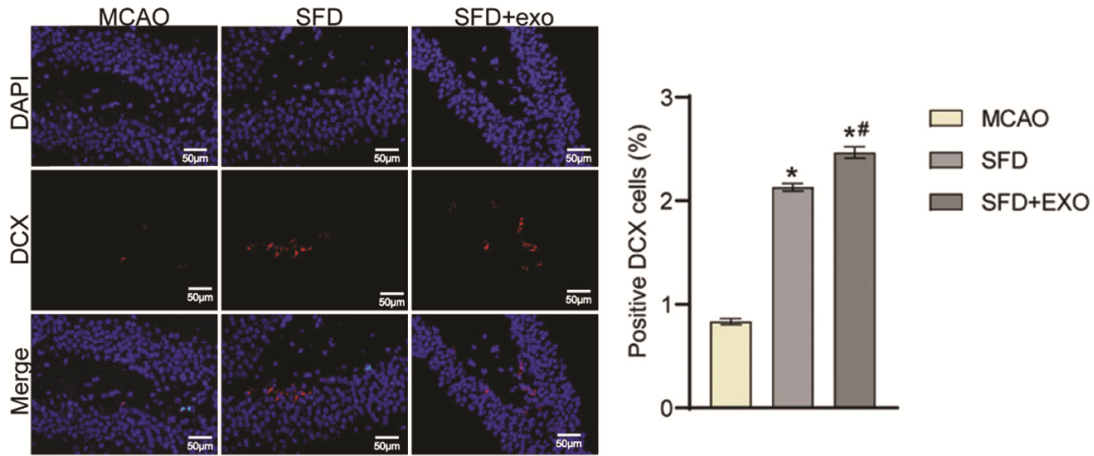


Fig. 4 — Number distribution of DCX+ neurons was observed by IF [400X, Scale bar = 25 μm]

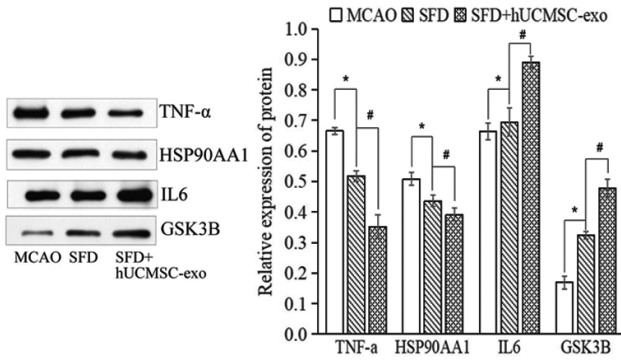


Fig. 5 — Lipid and atherosclerosis pathway protein expression was detected by western blot. (A) Western blot for TNF-α, HSP90AA1, IL6 and GSK3B; and (B) Bar graphs summarize the quantifications by densitometry. [*P <0.05 compared to the MCAO group; #P <0.05 compared to the SFD group]

Activation of lipid and atherosclerosis pathway in MCAO rats through SFD and hUCMSC-exo combination therapy

Lipid and atherosclerosis pathways involve 27 common targets. Among these targets, 8 key targets (TNF, CASP3, CCL2, AKT1, MMP9, IL6, IL1B, PPARG) were found to be among the top 10 degree nodes in the protein-protein interaction (PPI) network. This study chose TNF and IL6, which displayed the highest degree values, for Western blot validation experiments. GSK3B and HSP90AA1 were included in the top 10 degree targets in the Chinese herbal medicine-active ingredient-targets network and were subjected to Western blot validation experiments. The experimental results demonstrated that compared to the MCAO group, treatment with SFD and hUCMSC-exo down-regulating the expression of TNF and HSP90AA1 in the brain tissues of MCAO rats while up-regulating the expression of IL6 and GSK3B (Fig. 5). Therefore, the synergistic application of hUCMSC-exo and SFD enhances lipid and

atherosclerosis pathways, positively improving MCAO patients with cerebral infarction.

Discussion

Previous studies have demonstrated that hUCMSC-exo has the potential to effectively reduce mortality and growth retardation caused by severe brain injury²⁷. The anti-inflammatory effects of engrafted MSCs are the primary mechanism responsible for their beneficial effects on the injured brain²⁸. Additionally, hUCMSC-exo has been found to protect against atherosclerosis and its complications by regulating inflammation^{29,30}. The lipid and atherosclerosis pathway involves several vital lipid metabolism and inflammation targets, including TNF, IL-6, NF-κB, and IL-8²⁹. These targets regulate the activity of lectin-like oxidized low-density lipoprotein (LDL) receptor-1 (LOX-1), which promotes atherosclerosis and plaque formation³¹. Previous experiments have demonstrated that traditional Chinese medicine and core ingredients in SFD formulas, such as *Rehmanniae radix praeparata*³², quercetin³³, and Kaempferol³⁴, can regulate the activity of LOX-1. As widely known, TCM is an ancient system of medicine that continues to be widely practiced in China and surrounding areas as a complementary and alternative medicine³⁵.

The theory of Loewe additivity suggests that drugs with similar modes of action on the same target or pathway can produce synergistic effects³⁶. In the light of this theory, we conducted a study to examine the impact of SFD and hUCMSC-exo combination therapy on the lipid and atherosclerosis pathway. Our study confirmed that compared to the MCAO group, the treatment involving both SFD and hUCMSC-exo

resulted in the downregulating of TNF and HSP90AA1 expression, as well as the upregulating of IL6, GSK3B expression in the brain tissues of MCAO rats.

Tumor necrosis factor-alpha (TNF- α) is a pro-inflammatory cytokine. Brain injury, such as stroke or head trauma, leads to a substantial increase in TNF levels³⁷. TNF is crucial in regulating infection control, autoimmunity, allergic diseases, and immune processes related to antitumor activity³⁸. The impact of TNF on vascular endothelium involves various mechanisms, such as promoting the expression of tissue factors and leukocyte adhesion molecules, activating matrix metalloproteinases, and inducing oxidative stress through xanthine oxidase³⁹. These activities activate specific sections of blood vessels, leading to localized inflammation, thrombosis, and bleeding⁴⁰.

When examining the potential therapeutic targeting of TNF- α for stroke, it has been observed that its inhibition of TNF- α can effectively decrease focal ischemic damage and enhance clinical outcomes⁴¹. Etanercept, a specific TNF inhibitor, has demonstrated potential in preventing the exacerbation of cerebral ischemic injury in individuals with diabetes. This favorable outcome is primarily attributed to its anti-inflammatory properties⁴².

Heat Shock Protein 90 Alpha Family Class A Member 1 (HSP90AA1) has been found to have the ability to bind bacterial lipopolysaccharide (LPS) and facilitate the inflammatory response triggered by LPS, which includes the secretion of TNF by monocytes⁴³. Additionally, Interleukin 6 (IL6) has been observed to promote angiogenesis following a stroke and aid in reducing the damage caused by the stroke⁴⁴. The binding of IL6 to the soluble IL6 receptor (sIL6R), also known as trans-signalling, has stimulated a pro-inflammatory response. This interaction exhibits potential as a cardiovascular (CV) risk marker and could be utilized in selecting patients for anti-inflammatory therapy⁴⁵.

GSK3B or GSK-3 β , has emerged as a potential therapeutic target for treating cerebral ischemia. Studies have demonstrated that GSK3B can independently regulate Nrf2 activity, reducing infarct volume, brain edema, and neurological deficit^{46,47}. In conclusion, the available evidence strongly suggests a close association between TNF, IL6, GSK3B, HSP90AA1 and MCAO disease.

MSC-derived small extracellular vesicles have been found to promote stroke recovery⁴⁸. In a study, gold nanoparticles coated with stem cells and modafinil were observed to impact MCAO brain injury positively. This was attributed to their ability to increase neurotrophic factors and reduce apoptosis of brain cells⁴⁹. Additionally, MSC-exo with miR-223-3p overexpression decreased the volume of cerebral infarction induced by MCAO/R and improved neurological deficits⁵⁰. The combined treatment of intravenous recombinant tissue plasminogen activator and MSC infusion showed inhibitory effects on endothelial dysfunction and bleeding events in rats with transient MCAO⁵¹. Our study further confirmed that combining hUCMSC-exo and SFD could effectively improve cerebral infarction and tissue injury in MCAO rats, suggesting a potential novel approach for treating MCAO.

MSC-exo treatment increased peri-infarction angiogenesis and subventricular neurogenesis (DCX/BrdU) in young and old rats⁵². Transplantation of bone marrow (BM)-MSCs significantly elevated the number of IGF-1+CD68+ and BDNF+Iba-1+ double-positive cells in the ischemic core cortex on day two⁵³. Additionally, MSC-exo reduced the infiltration of leukocytes, mainly neutrophils, monocytes, and macrophages, in the brains of aged MCAO mice⁵⁴. Our study observed that SFD combined with hUCMSC-exo promoted the number of DCX(+) neurons in the brain tissue of MCAO rats, which is consistent with previous research. However, our study primarily focused on examining the therapeutic effects of SFD on MCAO rats while also investigating the additional impact of hUCMSC-exo on SFD. It is important to note that there was no separate treatment group specifically for hUCMSC-exo, which is a limitation of our study. Previous research has indicated that the therapeutic benefits of conditioned culture medium derived from hMSCs are attributed, at least in part, to improved neurogenesis and angiogenesis, leading to faster recovery from ischemic brain injury⁵⁵. Our study further confirms the role of hUCMSC-Exo in this regard, as evidenced by the higher efficacy of SFD+hUCMSC-exo compared to SFD alone. hUCMSC-exo is known to have high proliferative capacity and low immunogenicity, and it contains various components such as proteins, mRNA, miRNA, and DNA⁵⁶. Given the complexity of the mechanism of action of hUCMSC-exo, further exploration is necessary in future studies.

Conclusion

The above results have demonstrated that combining hUCMSC-exo with the Shennao fuyuan decoction (SFD) has the potential to modulate the TNF, HSP90AA1, IL6 and GSK3B targets, consequently regulating the lipid and atherosclerosis pathway and mitigating cerebral damage in rats induced with the middle cerebral artery occlusion (MCAO).

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Conflict of Interest

Authors declare no competing interests.

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