Zhongfenggao Protects Brain Microvascular Endothelial Cells from Oxygen–Glucose Deprivation/Reoxygenation-Induced Injury by Angiogenesis

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INTRODUCTION

Zhongfenggao (ZFG) is prescribed for the treatment of cerebrovascular diseases in critical projects of the State Administration of Traditional Chinese Medicine. ZFG has been found to nourish qi, activate blood circulation, remove blood stasis, dredge collaterals, and strengthen the brain and mind. The present study investigated the effects of ZFG on oxygen–glucose deprivation–reoxygenation (OGD/R) induced injury to brain microvascular endothelial cells (BMECs), and the mechanisms underlying such effects. BMECs are essential target cells of ischemic stroke. In order to simulate ischemic-like conditions in vitro, BMECs were exposed to glucose deprivation and hypoxia for 2h. Results indicate that ZFG may protect OGD/R-induced injury to BMECs by promoting angiogenesis. Further, we observed that ZFG significantly inhibited apoptosis induced by OGD/R injury. ZFG significantly promoted migration and microtubule formation in BMECs under OGD/R conditions. Additionally, ZFG increased levels of the vascular endothelial growth factor (VEGF) significantly and activated the Notch and Wnt signaling pathways. These results provide novel insights into the mechanisms underlying the therapeutic action of ZFG which shows promise as a potential drug candidate for treating cerebral ischemia–reperfusion.

Key words Zhongfenggao; stroke; oxygen–glucose deprivation–reoxygenation; Notch; Wnt

INTRODUCTION

Stroke, also known as cerebral apoplexy, is associated with a high level of morbidity and disability, as well as with many other complications. Together with coronary heart disease and cancer, stroke is ranked by the medical profession as one of the three leading diseases threatening human health. Ischemic stroke, which is the main pathological form of stroke, accounts for 87% of all strokes. Although restoring blood flow and reoxygenation during reperfusion is crucial for rescuing stroke victims, paradoxically, it initiates a cascade of events that may lead to additional cell injury known as reperfusion injury, which frequently exceeds the original ischemic insult.

The mechanism underlying cerebral ischemia–reperfusion (I/R) injury is complex. Studies indicate that while the early stages may be related to excitability caused by amino acid toxicity, intracellular Ca$^{2+}$ overload and free radical damage, the middle stages may be mainly associated with inflammatory response and neuronal apoptosis. Angiogenesis may provide critical neurovascular substrates for neuronal remodeling. In terms of treatment, thrombolytic therapy is the only effective treatment, but clinical use is limited due to time window constraints and the risk of intracranial hemorrhage. Neuroprotection is a crucial method for treating ischemic stroke, but it has little effect as a treatment under clinical conditions. Therefore, it may be necessary to study the pathogenesis of ischemic stroke as also to explore potential treatment methods for this disease. It has been reported that nerve function may be severely compromised due to excessive neuronal loss and vascular damage following ischemic stroke. Therefore, the difficulties in attaining substantial tissue repair and functional reconstruction remain critical factors hampering the clinical treatment of ischemic stroke.

Angiogenesis, which plays a vital role in promoting local blood circulation, protecting neurons and improving neurological function, is an important process associated with self-repair and remodeling following cerebral ischemia injury. Many factors including angiogenin, growth factors, matrix release enzymes, anti-angiogenic proteins as well as many other molecules and signal transduction pathways, are involved in the regulation of this process. Presently, increasing attention is being directed towards saving vascular endothelial cell nerve cells, and glial cells in the ischemic penumbra, which are on the verge of death. Cerebral vascular endothelial cells are susceptible to ischemia. Therefore, acute cerebral ischemia may lead to cerebral vascular endothelial dysfunction, resulting in an increase in the permeability of the blood–brain barrier (BBB) causing vascular edema followed by vasospasm and ischemic stroke. Endothelial cell-mediated angiogenesis plays a vital role in the recovery of cerebral ischemia victims. Brain microvascular endothelial cells (BMECs) are important target cells of ischemic stroke, and therefore, protecting...
BMECs against cerebral I/R induced damage may be useful for ameliorating ischemic stroke.

The primary form of pathogenesis-related to ischemic stroke is caused by blood stasis due to qi deficiency. The method of nourishing qi and activating blood circulation is the basic method of treatment for ischemic stroke in Traditional Chinese Medicine (TCM), which has a definite and irreplaceable function. As a hospital preparation, Zhongfenggao (ZFG) has a basis in the ancient "Fushousan" consisting of Angelica and Chuanxiong. Other compatible drugs such as Astragalus, Salvia miltiorrhiza, Radix Paeoniae Rubra, and rhizoma, licorice among others, have displayed strong effects on replenishing qi, promoting blood circulation and dispersing blood stasis as well as dredging collateral.

Our previous clinical and experimental studies indicated that ZFG may effectively reduce blood viscosity, inhibit carotid atherosclerosis and platelet aggregation, reduce calcium overload, and inhibit neuronal apoptosis. In addition, ZFG has demonstrated beneficial curative capabilities such as neuroprotective functions and improvement of microcirculation under clinical conditions. However, the mechanisms underlying ZFG function in the treatment of cerebral I/R injury have not yet been fully resolved. Therefore, we investigated whether ZFG-medicated serum ameliorates ischemic stroke by promoting angiogenesis and whether Notch and Wnt signaling pathways were associated with the molecular mechanisms underlying ZFG function.

MATERIALS AND METHODS

Chemicals and Materials ZFG (lot no.: 20161018) was obtained from Gansu Provincial Hospital of Traditional Chinese Medicine. Ingredients of ZFG as prescribed by the Department of Pharmacognosy of Gansu province included Angelica (Zhongtian Pharmaceutical Co., Ltd., Gansu, China), chuanxiong (Tianxin Pharmaceutical Co., Ltd., Gansu, China), Glycyrrhiza (Zhongtian Pharmaceutical Co., Ltd., Gansu, China), Astragalus membranaceus, Salvia miltiorrhiza, Red Peony and notopter -

BMEC Preparation and Cell Culture One week old Male Sprague-Dawley (SD) rats were purchased from the Laboratory Animal Center (Gansu, China) and fed ad libitum prior to experimentation. The experimental protocol was authorized by Gansu University of Chinese Medicine Animal Care and Ethics Committee, and animal care and treatment were conducted in conformity with National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). BMECs were isolated as previously described. Briefly, mouse cerebral cortex was isolated and digested in 0.25% trypsin at 37°C for 30 min and centrifuged. The second band containing micro-vessels was collected and plated onto collagen-coated dishes. The cells were cultured in DMEM supplemented with 10% FBS, 1% non-essential amino acids, 1% glutamine, 100 U/mL penicillin and 100 U/mL streptomycin, and maintained at 37°C with 5% CO₂. BMECs (purity quotient: ≥ 95%) were grown to approximately 90% confluency before use.

Oxygen–Glucose Deprivation and Reoxygenation (OGD/R) To simulate ischemic-like conditions in vitro, cell cultures were exposed to glucose deprivation and hypoxia for 2 h. BMEC cells were incubated in a microaerophilic system (ThermoFisher, Waltham, MA, U.S.A.) with 5% CO₂ and 1% O₂ balanced with 94% N₂ for 2 h. OGD-treated cells were induced in an airtight chamber in serum-free and glucose-free medium.

Preparation of ZFG-Medicated Serum Rat medicated serum was prepared according to previous protocols. Briefly, 20 SD rats (220–250 g) were purchased from the Laboratory Animal Center (Gansu, China) and randomly divided into a ZFG group (n = 10) and a control group (n = 10). The rats were housed in an air-conditioned room (20–25°C) and subjected to a 12 h light–dark cycle while being provided with standard chow and free access to tap water. Rats in the ZFG group were administrated ZFG (1 mL/100 g) via gastric perfusion once a day for 3 d, whereas the control group was treated with the same volume of saline. One hour following the final administration, blood samples were collected from the abdominal aorta and maintained at room temperature for 24 h, and centrifuged at 1200 × g for 20 min at 4°C in order to obtain medicated serum. The serum was inactivated by heating for 30 min at 56°C and filtered through 0.22 µm filter and stored at −20°C until use.

Grouping and Drug Treatment Experimental cells were randomly divided into seven groups: (1) Normal control group (NC)-cells cultured in 10% FBS-containing DMEM medium under normoxic conditions; (2) OGD/R-group-cells subjected to OGD/R injury only; (3) OGD/R + serum control group (SC)-cells subjected to OGD/R injury and DMEM containing 10% rat blank serum added to the reoxygenation medium; (4) OGD/R + ZFG-group-cells subjected to OGD/R injury and DMEM containing 10% ZFG-medicated serum added to the reoxygenation medium; (5) OGD/R + ZFG + DAPT group-cells subjected to OGD/R injury and DMEM containing 10% ZFG-medicated serum and Notch1 signaling inhibitor DAPT (2 µM) added to the reoxygenation medium; (6) OGD/R + ZFG + DAPT + DK1 group-cells subjected to OGD/R injury and DMEM containing 10% ZFG-medicated serum and Wnt signaling pathway antagonist Dickkopf-1 (DKK1, 100 ng/mL) added to the reoxygenation medium; (7) OGD/R + ZFG + DAPT + DK1 group-cells subjected to OGD/R injury and DMEM containing 10% ZFG-medicated serum and DAPT and DKK1 added to the reoxygenation medium.

Cell Viability Assay Cells were plated in 96-well plates (100 µL/well, 5 × 10⁵ cells) and grown overnight. After 24 h, the adherent cells were incubated under OGD condition for 2 h, and then the medium was replaced with standard re-oxygenation medium (2 h), containing different drugs, and allowed to grow for 1, 3, 7, and 14 d. The drug groups were

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as follows (1, 2.5, 5, 10, 20%). Finally, the CCK-8 reagent was added to each well, incubated for 3 h at 37°C, and measured at 450 nm using a microplate reader (Molecular Devices, San Jose, CA, U.S.A.).

Determination of Cellular Apoptosis BMECs (5 × 10⁵ cells/mL) were seeded at 6-well plates and incubated for 24 h at 37°C. After incubation, the cells were collected and treated according to the manufacturer’s instructions using Annexin V-FITC Apoptosis Detection Kit (Nanjing KeyGen Biotech. Co., Ltd., Nanjing, China). The percentages of apoptotic cells were analyzed using a flow cytometer (Becton Dickinson, Franklin Lakes, NJ, U.S.A.).

Measurement of VEGF Level Cells (2 × 10⁵ cells/mL) were plated in 6-well plates, incubated at 37°C for 24 h and treated using the method described above. The level of VEGF was determined using a double-antibody sandwich. Enzyme-linked immunosorbent assay (ELISA) were performed according to the manufacturer’s instructions.

Transmission Electron Microscopy (TEM) OGD/R-induced apoptotic morphologic alterations were observed using transmission electron microscopy. Briefly, following treatment by OGD for 2 h, BMECs were fixed for 30 min in 2.5% (vol/vol) glutaraldehyde and 0.1 mol/L phosphate-buffered saline (PBS) at a pH of 7.4. After post-fixation in 1% (wt/vol) osmium tetroxide, cells were dehydrated via a graded series of ethanol and propylene oxide and embedded in Epon. Finally, they were processed for imaging on a Tecnai 10 transmission electron microscope (Philips, Amsterdam, the Netherlands). Typically, apoptotic morphology manifests as cellular shrinkage and nuclear condensation, whereas necroptotic cells show swelling and rapid plasma membrane expansion.

Transwell Migration Assay Cell migration was measured using Millicell hanging cell culture inserts with a 8-µm-pore polyethylene terephthalate membrane filter (Merck Millipore, Darmstadt, Germany). Typically, for the purpose of migration assay, cells were seeded at 1 × 10⁵/well in the upper chambers of a graded series of wells containing 10% FBS with 200 µL serum-free basic medium. Next, 600 µL medium containing 10% FBS was placed in the lower wells serving as a source of chemoattractants. The cells were incubated for 12 h at 37°C. Cells which migrated to the lower surface of the filter were fixed with 70% methanol and stained with 0.5% crystal violet solution. The number of migrated cells was determined by counting stained cells, following which the average cell number per field was calculated for each well. The counting was triple blinded, including one who was blinded to the results.

Microtubule Formation Assay Microtubule formation assay was performed as previously described.²⁰ Briefly, 48-well plates were coated at 4°C with 100 µL Matrigel solution (BD Bioscience, New Bedford, MA, U.S.A.) and diluted 1:1 in cell culture medium. The plate was allowed to solidify for 1 h at 37°C before cell seeding. Endothelial cells in conditioned medium were seeded at approximately 2 × 10⁴ per well. Following incubation at 37°C for 16 h, photographs of three randomly selected fields of each well were taken. The angiogenesis index was calculated according to a previously described formula.²⁰

Western Blot Analysis BMECs were plated in 6-well plates, treated, and the proteins extracted from cells were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes. Western blot was performed using antibodies against Notch1, DLL4, Hes1, Wnt3a, β-catenin, CyclinD1, and Nrarp. Protein quantification was measured in optical density units using Image Lab software (Bio-Rad, Hercules, CA, U.S.A.) and normalized to the expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Information for each antibody is listed (Table 1). The methods used for the determination were as previously described.²¹

RNA Extraction and Quantitative (q)RT-PCR Analysis Total RNA was extracted from the BMECs using TRIzol (Invitrogen, Carlsbad, CA, U.S.A.) following the manufacturer’s protocol. The purity of the RNA was determined and the RNA was reverse-transcribed (TaKaRa Biotechnology Co., Ltd., China). Real-time quantitative PCR was performed using a SYBR PrimeScript RT Kit as described by the manufacturer (TaKaRa). Amplifications were performed under the following conditions: initial denaturation at 50°C for 3 min and 95°C for 15 min; 40 thermal cycles (95°C for 10s, 60°C for 20s and 72°C for 20s), and melt cure 70 to 95°C on a CFX Connect Real-Time System (Bio-Rad). Sequences of the primers are shown (Table 2). GAPDH was used as an internal control and the comparative Ct method (2^ΔΔCt) was used to analyze differences between groups.²²

Table 1. Antibody Source, Dilution and Manufacturer Information

<table>
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<th>Antibody</th>
<th>Source</th>
<th>Dilution</th>
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<tr>
<td>Notch1</td>
<td>Rabbit</td>
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<td>DLL4</td>
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<td>1:1000</td>
<td>Abcam, U.K.</td>
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<td>Hes1</td>
<td>Rabbit</td>
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<tr>
<td>Wnt3a</td>
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<td>Abcam, U.K.</td>
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<tr>
<td>Nrarp</td>
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<td>GAPDH</td>
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Table 2. Primer Sequences Used for Real-Time PCR

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<th>Reverse primer (5’→3’)</th>
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<td>Rat-Notch1</td>
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<td>TGCCACCTCCTCCATATT</td>
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<td>Rat-DLL4</td>
<td>ACCGTCCTTCAAGGCTG</td>
<td>TACACCTCAGTGCGATTC</td>
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<td>Rat-Hes1</td>
<td>ATGACCGTCATGATATAGG</td>
<td>TTTGAATGCGATCCAAATCT</td>
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<td>Rat-Wnt3a</td>
<td>TGGTGGATCTTGCTG</td>
<td>CTGGATGCAGCGGGCCTTG</td>
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<tr>
<td>Rat-β-catenin</td>
<td>ATGCGCTCTCAAGGCTG</td>
<td>CCGAATGTGGCTGCTG</td>
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<tr>
<td>Rat-CyclinD1</td>
<td>AACACTCCTGAGCGCTGG</td>
<td>ATCCGCGCTTGGGATTTT</td>
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<td>Rat-Nrarp</td>
<td>CAGGCGAGCTGCTCACCT</td>
<td>GAATTTGACATGCAGCTCCAACAG</td>
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<tr>
<td>Rat-GAPDH</td>
<td>CAGTGCCACGCTGCTC</td>
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Statistical Analysis All values are expressed as mean ± standard deviation (S.D.). A two-tailed Student’s t-test was used to compare the means between two groups. All statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Prism Software, La Jolla, CA, U.S.A.). Statistical significance was set at \( p < 0.05 \).

RESULTS

ZFG-Medicated Serum Treatment Restored Cell Damage after OGD/R Injury The effects of ZFG on the viability of BMECs were investigated using the CCK-8 assay. ZFG-medicated serum, at concentrations of 1, 2.5, 5, 10, and 20%, was treated within 24 h to determine the appropriate concentration of ZFG-medicated serum. Next, we detected cell viability after 1, 3, 7, and 14 d of incubation with ZFG-medicated serum, Notch signaling inhibitor DAPT and Wnt signaling antagonist DKK1 under OGD/R conditions. The CCK-8 assay indicated that OGD/R significantly decreased the viability of BMECs whereas ZFG-medicated serum (10%) significantly recovered viability (Fig. 1A). Incubation with ZFG-medicated serum (10%) for 3, 7, and 14 d exerted a reversal effect on the cell viability of BMECs compared to that observed in the OGD/R group, whereas inhibitor groups reversed the effects of ZFG-medicated serum (10%) (Fig. 1B).

ZFG-Medicated Serum Treatment Inhibited the BMECs Apoptosis Induced by OGD/R We tested apoptosis in BMECs following 14 d of ZFG-medicated serum (10%) and inhibitors incubation under OGD/R condition using flow cytometry, and apoptotic morphologic alterations and ultrastructure of BMECs after 14 d of ZFG-medicated serum (10%) incubation under OGD/R conditions using TEM. Enhancement of early apoptosis in the OGD/R group was significantly suppressed by ZFG-medicated serum. Similarly, the inhibitor groups markedly reversed the effects of ZFG-medicated serum (Figs. 2A, B). Normal BMECs displayed large oval nuclei with clear nuclear membranes, while cells in the OGD/R group, displayed irregular nuclear membranes as well as chromatin condensation. By contrast, the above changes were alleviated in the ZFG-medicated serum treatment group, whereas inhibitor groups reversed the effects of ZFG-medicated serum (Fig. 2C). These results indicated that ZFG-medicated serum treatment may alleviate endothelial cell damage.

ZFG-Medicated Serum Treatment Promotes Migration and Microtubule Formation of BMECs Subsequently, we investigated the effect of ZFG-medicated serum treatment on angiogenesis following OGD/R injury after 14 d of ZFG-medicated serum (10%) incubation. The transwell assay revealed that the number of cells penetrating the membrane was significantly decreased in the OGD/R group as compared to that in the NC group. Conversely, ZFG-medicated serum treatment improved the penetrating ability of BMECs under OGD/R condition (Figs. 3A, B). Microtubule formation assay indicated that less tube-like structures, as well as poorly formed cellular networks, were present in the groups under OGD/R condition than those under normal conditions. However, ZFG-medicated serum treatment significantly increased tube formation. Further, the inhibitor groups reversed the effects of ZFG-medicated serum on migration and microtubule formation of BMECs (Fig. 3C). These results suggest that ZFG-medicated serum may have a boosting effect on angiogenesis.

ZFG-Medicated Serum Treatment Increased the VEGF Level of BMECs VEGF plays an essential role in neovascular remodeling in ischemic stroke.\(^{23}\) We estimated VEGF
levels using ELISA. BMECs under OGD/R showed a markedly higher expression level of VEGF compared to those under normoxic condition. And the expression level of VEGF significantly increased after 7 and 14 d incubated with 10% ZFG-medicated serum. Treatment with ZFG-medicated serum increased the VEGF levels of BMECs under OGD/R condition, whereas the inhibitor groups reversed the effects of ZFG in 7 and 14 d groups (Fig. 4). The results revealed that pre-treatment with 10% ZFG-medicated serum for 1, 3, 7 and 14 d showed time-dependent effects.

**Notch and Wnt Signal Pathways Involved in the Protective Effects of ZFG-Medicated Serum on OGD/R-Induced BMEC Injury**

Notch and Wnt signal pathways were detected using protein and gene measurements. The expression...
levels of Notch signaling pathway associated proteins, Notch1, DLL4, and Hes1 were significantly increased in BMECs under OGD/R condition; ZFG-mediated serum treatment exerted inhibitory effects on these proteins. Expression levels of the Wnt signaling pathway associated proteins, Wnt3a, and β-catenin, were significantly increased while Cyclin D1 expression was significantly decreased in BMECs under OGD/R condition; ZFG-mediated serum treatment increased Wnt3a and β-catenin proteins expression and decreased Cyclin D1 expression. Notch-regulated ankyrin repeat protein (Nrarp) acts as a molecular link between Notch and Wnt signaling in endothelial cells to control the stability of new vessel connections in angiogenesis (Figs. 5A, B). Our results indicated that Nrarp expression was significantly increased in BMECs under OGD/R condition, whereas ZFG-mediated serum treatment exerted an inhibitory effect. And the results revealed that treatment with 10% ZFG-medicated serum for 1, 3, 7, and 14 d showed time-dependent effects.

DISCUSSION

Of all organs, the brain is most sensitive to hypoxia, and cerebral ischemia may damage local brain tissue and affect its function, where the extent of damage is related to the time of ischemia occurrence and the amount of residual blood flow. Stroke, also called cerebral apoplexy or cerebrovascular accident, is a group of diseases characterized by cerebral ischemia and hemorrhagic damage. It is associated with high fatality and disability rates in adults. Ischemic stroke is related to several risk factors, including hypertension, atherosclerosis, thrombosis and diabetes. In recent decades, numerous studies have been conducted on stroke, but the pathogenesis of ischemic stroke has not been fully elucidated, and as of yet no effective treatment is known. In this study, we aimed to explore the effects of ZFG-medicated serum treatment on cerebral I/R injury as well as mechanisms underlying such effects.

Studies have shown that angiogenesis is an important process involving self-repair and remodeling after cerebral ischemia injury, which is closely associated with the prognosis of ischemic cerebrovascular disease. Angiogenesis coordinates endothelial cell differentiation, proliferation, migration, and apoptosis, and may provide key neurovascular substrates for neural regeneration. Notch and Wnt signal pathways interact with each other to regulate the behavior of endothelial cells.

It is well known that VEGF is a pleiotropic angiogenic growth factor, which stimulates the proliferation of vascular endothelial cells. In addition, VEGF plays critical roles in neovascular remodeling following ischemic stroke. VEGF may also protect ischemic neurons from injury and promote brain plasticity. Our results indicated that ZFG-medicated serum treatment increased VEGF levels in BMECs under OGD/R condition, thus promoting angiogenesis (Fig. 4). The Notch signaling pathway plays a key role in angiogen-
Normal expressions of Notch receptor and Delta like (DLL) may regulate angiogenesis, regeneration, formation and branch formation of blood vessels. Studies indicate that the Notch pathway may be involved in pathophysiological processes following cerebral ischemia. Notch1 molecular target gene Hes1 protein was significantly increased following cerebral ischemia, indicating that Notch1 may regulate angiogenesis by up-regulating the expression of its target, the Hes1 protein, after cerebral ischemia. The Notch signaling pathway also contributes to angiogenesis by the up-regulation of VEGF, which may contribute to the improvement of neurological functions after cerebral ischemia. Our results presented that oxygen–glucose deprivation and reoxygenation stimulation significantly upregulated the expression of the Notch ligand DLL4, the receptor Notch-1 and the Notch target gene Hes-1 in BMECs. Meanwhile, we observed that ZFG-mediated serum obtained the Notch signaling inhibitor DAPT significantly reversed the effects of ZFG-medicated serum (Figs. 5A, 5B), suggesting that ZFG-medicated serum significantly inhibited OGD/R-induced Notch signaling activation.

Wnt signaling, which promotes cell proliferation and regulates angiogenesis and neurogenesis, is activated after cerebral ischemia. Our results presented that oxygen–glucose deprivation and reoxygenation stimulation significantly upregulated the expression of the Wnt ligand Wnt3a, β-catenin, and CyclinD1 under OGD/R conditions. The Notch signaling pathway and the Wnt signaling pathway may play an important regulatory role in the pathogenesis and development of ischemic stroke. Nrarp acts as a molecular link between Notch and Wnt signaling in endothelial cells to control the stability of new vessel connections. DLL4/Notch induced expression of Nrarp limits Notch signaling and promotes Wnt/β-catenin signaling in endothelial stalk cells. That is, DLL4/Notch signaling induces expression of Nrarp, where it differentially modulates Notch- and Wnt signaling activity to balance cell proliferation and maintain vessel stability.
bility in angiogenesis. Our results showed that ZFG-mediated serum decreased the expression of Nrarp protein induced by DLL4/Notch signaling, indicated that ZFG may promote angiogenesis by Notch and Wnt signaling pathways.

In conclusion, ZFG-mediated serum treatment can protect BMECs against OGD/R-induced injury by inducing angiogenesis via Notch and Wnt signaling pathways. Angiogenesis is an important mechanism of self-repair and remodeling after cerebral ischemia, which provides a new direction of functional improvement for ischemic stroke patients and displays much promise as the mechanism underlying the therapeutic action of traditional Chinese medicine used to treat cerebral ischemia.

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Conflicts of Interest The authors declare no conflict of interest.

REFERENCES


