RESEARCH PAPER

Acta Neurobiol Exp 2022, 82: 398–407
DOI: 10.55782/ane-2022-038

since 1928 ACTA NEUROBIOLOGIÆ EXPERIMENTALIS

Bone marrow stromal cells attenuate oxygen and glucose deprivation followed by re-oxygenation-induced brain microvascular endothelial cell injury by mediating the plasminogen activator/plasminogen activator receptor/stromal cell-derived factor-1α/C-X-C chemokine receptor type 4 pathway

Cui-Ni Fang[1], Tao Song[2], Xi Tao[2], Shu-Fen Liu[2], Zhen Feng[1]*

[1] Department of Rehabilitation Medicine, the First Affiliated Hospital of Nanchang University, Nanchang, China,
[2] Hunan Provincial People's Hospital, the First-affiliated Hospital of Hunan Normal University, Changsha, China,
** Email: fengzzhenf6623@163.com*

Ischemic stroke is a severe threat to the health of older individuals. Bone marrow mesenchymal stem cells (BMSCs) have been implicated in ischemic stroke. Urokinase-type plasminogen activator (uPA) and its specific receptor (uPAR) are associated with the pathological process of ischemic stroke. However, the relationship between BMSCs and uPA/uPAR in ischemic stroke remains unclear. For simulating the occurrence of an ischemic stroke *in vitro*, human cerebral microvascular endothelial cells (HBMECs) were subjected to oxygen and glucose deprivation followed by re-oxygenation (OGD/R) and were then cocultured with BMSCs. 3,4,5-dimethylthiazol-2,5-diphenyltetrazolium bromide and bromodeoxyuridine staining were used for measuring cell viability and proliferation. Flow cytometry was performed for assessing cell apoptosis. Endothelial cell tube formation was determined using angiogenesis assays. Alterations in the protein and gene expression in HBMECs were evaluated using western blot analysis and quantitative reverse transcription-polymerase chain reaction, respectively. OGD/R considerably inhibited the viability and proliferation of HBMECs by inducing apoptosis, which was reversed by BMSCs. Consistently, OGD/R-induced inhibition of angiogenesis was attenuated by BMSCs. In addition, BMSCs could protect HBMECs against OGD/R-induced injury by positively regulating the uPA/uPAR/stromal cell-derived factor-1α (SDF-1α)/C-X-C chemokine receptor type 4 (CXCR4) pathway, and uPA/uPAR could mediate the SDF-1α/CXCR4 pathway in OGD/R-treated HBMECs. Therefore, this study provides novel strategies to investigate the specific role of BMSCs in ameliorating OGD/R-induced vascular endothelial cell injury.

Key words: ischemic stroke, bone marrow mesenchymal stem cell, urokinase-type plasminogen activator, stromal cell-derived factor-1α

INTRODUCTION

Stroke is an acute cerebrovascular disease with a sudden onset and common features involving focal neurological deficits. Strokes, including those of the ischemic and hemorrhagic type, are known to have high mortality rates (Han et al., 2018). The pathogenesis of ischemic stroke is extremely complicated. Immediate restoration of the blood supply is the primary treatment for ischemic stroke; however, the outcomes are still not ideal (Zhao et al., 2018). Furthermore, a dysfunction

Received 18 November 2021, accepted 28 May 2022

in the brain microvessel endothelial cells (BMECs) has been confirmed to occur as a result of ischemic stroke (Jorgensen et al., 1993; Shao et al., 2017). Therefore, maintaining the function of BMECs can be considered a useful strategy for the treatment of ischemic stroke.

Study have demonstrated that inflammation-related tissue destruction in lipopolysaccharide-induced disseminated intravascular coagulation model rats showed improvement after pretreatment with BMSCs (Wang et al., 2018). In addition, BMSCs down-regulate the levels of inflammatory factors in H_2O_2-induced retinal ganglion cells and up-regulate the levels of antioxidant factors in these cells (Cui et al., 2017). However, the mechanism by which BMSCs regulate the progression of ischemic stroke-related cellular remains unclear.

The urokinase-type plasminogen activator (uPA) system is composed of uPA, its specific receptor (uPAR), and plasminogen activator inhibitor type-1 (Smith and Marshall, 2010). In addition, the combination of uPA and uPAR can activate cell signaling pathways through plasminogen-dependent and independent mechanisms to promote tissue remodeling (Alfano et al., 2005). A study has shown that adipose-derived stem cells can increase uPA levels by mediating vascular morphogenesis (Kachgal and Putnam, 2011). Stromal cell-derived factor-1 (SDF-1) is a chemokine and member of the C-X-C family; SDF-1α is the predominantly expressed subtype of SDF-1 (Son et al., 2006; Steingen et al., 2008). Xiang et al. (2019) found that regulating the activation of the SDF-1α/C-X-C chemokine receptor type 4 (CXCR4) pathway could protect neurons and endothelial cells against cerebral ischemia-induced injury. uPA can also regulate the progression of liver cancer by mediating the uPAR/SDF-1 pathway (Hsu et al., 2012). However, whether the SDF-1α/CXCR4 pathway participates in BMSC-mediated ischemic stroke remains unexplored.

Based on the above observations, we conducted experiments to verify the hypothesis that BMSCs participate in protecting human cerebral microvascular endothelial cells (HBMECs) subjected to hypoxic-ischemic brain injury, which may be associated with the uPA/uPAR-mediated SDF-1α/CXCR4 pathway.

METHODS

Cell culture and establishment of an oxygen and glucose deprivation/re-oxygenation (OGD/R) model

HBMECs (HBEC-5i and CRL-3245, ATCC) and BMSCs (PCS-500-012, ATCC) were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 100 mg/L penicillin, and streptomycin at 37°C in a 5% CO_2 incubator. To conduct the OGD treatment, the HBMECs were first placed in a deoxygenated glucose-free medium and then incubated in a hypoxic vessel for 4 h at 37°C under 95% N_2, and 5% CO_2. The cells were then transferred to DMEM supplemented with high glucose and 10% FBS under normoxic conditions (5% CO_2) at 37°C for 24 h.

pLKO.1 expression vectors encoding a short-hairpin RNA (shRNA) that target uPA, SDF-1α, or a non-targeted sequence (negative control) were obtained from GenScript Co., Ltd (Nanjing, China). HBMECs were then transfected with the vector using lipofectamine 2000 (Sigma Aldrich, St. Louis, MO, USA), after which the transfectants were selected by incubation in the presence of G418 (Sigma Aldrich, St. Louis, MO, USA). Quantitative reverse transcription-polymerase chain reaction (RT-qPCR) was used to verify the efficiency of transfection.

To promote the overexpression of SDF-1α, lipofectamine 2000 (Invitrogen) was used to transfect HBMECs with pcDNA3.1 or pcDNA3.1-SDF-1α (Genepharma) for 48 h.

After the OGD/R treatment, the HBMECs were incubated in six-well plates (Corning Inc., Corning, NY, USA) at a concentration of 100,000 cells per 2 ml at 37°C. After 12 h, the culture (complete) medium was removed and either 100,000 BMSCs in a fresh medium or the medium alone was added to each well. The HBMECs were cultured in a DMEM normal medium at 37°C with 5% CO_2. Subsequently, the cells were collected for further analysis.

Cell co-cultivation was conducted in a transwell co-cultivation system. Briefly, HBMECs and BMSCs were cultured in the upper and lower chambers of a Transwell chamber (Corning) with 5% CO_2 at 37°C for 48 h.

3,4,5-dimethylthiazol-2,5-diphenyltetrazolium bromide (MTT) assay

HBMECs (3×10^5 per well) were inoculated in the wells of a 96-well plate. MTT solution (20 µl) was added to the cells at 0, 12, 24, 48, or 72 h. Then, after removing the supernatant, 150 µl of dimethyl sulfoxide was added to each well. Finally, the absorbance was measured at 490 nm using a microplate reader.

Bromodeoxyuridine (BrdU) assays

HBMECs were added to the wells of a 96-well plate at a density of 5.0×10^3 cells/well. Cells were then fixed in 4% paraformaldehyde and incubated first with anti-BrdU antibody (1:200, Abcam) overnight at

4°C, followed by incubation with anti-rabbit immunoglobulin G secondary antibody (1:1000, Abcam) for 1 h at room temperature. The nuclei were stained with 4′,6-diamidino-2-phenylindole (Beyotime, Shanghai, China). Finally, the labeled cells were observed under a fluorescence microscope (Olympus BX53 Tokyo, Japan). The data were quantified using Image J (NIH).

Flow cytometry

HBMECs were sorted as previously described (Cossarizza et al., 2019). They were then digested with trypsin and resuspended in a binding buffer. Next, the cells were stained with 5 µl of FITC Annexin V (BD Biosciences, Franklin Lake, NJ, USA) and propidium iodide (BD Biosciences, Franklin Lake, NJ, USA) for 30 min at 37°C in the dark. Fluorescence-activated cell sorting (FACScan™; BD Biosciences, Franklin Lake, NJ, USA) was used to analyze the apoptosis rate using Image J software (BD Biosciences, Franklin Lake, NJ, USA).

RT-qPCR

Total RNA was extracted from HBMECs using TRIzol reagent (Thermo Fisher Scientific) and then reverse transcribed into complementary DNA using a PrimeScript RT reagent kit (TaKaRa, Otsu, Shiga, Japan). RT-qPCR was performed on a 7900HT system (Applied Biosystems, CA, USA) using an SYBR® Premix Ex Taq™ II kit (TaKaRa Bio, Otsu, Shiga, Japan). The PCR protocol included denaturation at 90°C for 15 min, followed by 40 cycles of denaturation at 90°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min. The primers used were purchased from GeneCreate Biological Engineering Co., Ltd (Wuhan, China). The following primers were used:
uPA forward, 5′-CTACTACGGCTCTGAAGTCACCAC-3′,
reverse, 5′-GTAGACGCCTGGCTTGTCCT-3′;
uPAR forward, 5′-GCCCAATCCTGGAGCTTGA-3′,
reverse, 5′-TCCCCTTGCAGCTGTAACACT-3′;
SDF-1α forward, 5′-AGAGCCAACGTCAAGCATCT-3′,
reverse, 5′-GGGCAGCCTTTCTCTTCTTC-3′;
CXCR4 forward, 5′-CCGTGGCAAACTGGTACTTT-3′,
reverse, 5′-GACGCCAACATAGACCACCT-3′;
β-actin forward, 5′-CCCTGGAGAAGAGCTACGAG-3′,
reverse 5′-CGTACAGGTCTTTGCGGATG-3′.

Western blot analysis

HBMECs were lysed in a radioimmunoprecipitation assay lysis buffer (KeyGEN, Nanjing, China). The protein concentrations in the lysates were determined using a BCA Assay kit (Solar Life Science, Beijing, China). Equal amounts of protein (30 µg) were then subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, after which the separated proteins were transferred onto polyvinylidene difluoride membranes (Thermo Fisher Scientific). The membranes were blocked with 5% skim milk for 1 h. After adding specific antibodies to uPA (ab169754, Abcam, UK), uPAR (ab218106, Abcam, UK), SDF-1α (ab155090, Abcam, UK), and CXCR4 (ab181020, Abcam, UK), the membranes were incubated overnight at 4°C, and then incubated with the secondary antibody for 2 h. Placed in a gel imager for development. The protein bands were analyzed using ImageLab software.

Matrigel assay

Approximately 50 µL of melted Matrigel (4°C) was added to each well in a 96-well cell culture plate. The cells were digested with trypsin and a cell suspension was prepared using a cell culture medium. The cells were seeded in 96-well cell culture plates precoated with Matrigel at 1500 cells/well and co-cultured with BMSC supernatants. After 5 h of culture, the culture plates were observed under a microscope and photographed, and the number of branches in the formed microvessels was counted.

Statistical analysis

All experimental data were processed using SPSS 18.0 statistical software. A one-way analysis of variance test was used to compare multiple groups. $P<0.05$ was considered statistically different.

RESULTS

BMSCs promote the proliferation and inhibited the apoptosis of HBMECs

To investigate the function of BMSCs in ischemic stroke, HBMECs were subjected to OGD/R and then co-cultured with BMSCs. As shown in Fig. 1A, OGD/R considerably decreased the viability of HBMECs, which was reversed by BMSCs. In addition, OGD/R substantially inhibited the proliferation of HBMECs; however, this phenomenon was partially restored by BMSCs (Fig. 1B). OGD/R-induced apoptosis in HBMECs was substantially inhibited in the presence of BMSCs (Fig. 1C). Furthermore, the angiogenesis of HBMECs was substantially re-

duced by OGD/R, which increased after treatment with BMSCs (Fig. 1D). In summary, the BMSCs promoted the proliferation and inhibited the apoptosis of HBMECs.

BMSCs attenuate OGD/R-induced HBMEC injury by the upregulation of uPA/uPAR pathway

To determine the relationship between BMSCs and the uPA/uPAR axis, HBMECs were transfected with sh-uPA. As shown in Fig. 2A and 2B, the uPA and uPAR levels in HBMECs were considerably inhibited by OGD/R; however, they were considerably increased after being co-cultured with BMSCs. In addition, the knockdown of uPA attenuated the effect of BMSCs on uPA and uPAR levels (Fig. 2C and 2D). The proliferative, anti-apoptotic, and angiogenesis effects of BMSCs on OGD/R-treated HBMECs were reversed in the presence of sh-uPA (Fig. 2E and 2H). Thus, BMSCs attenuated OGD/R-induced HBMEC injury by upregulating uPA/uPAR pathway.

uPA/uPAR can protect HBMECs against OGD/R-induced injury by mediating the SDF-1α/CXCR4 pathway

We further studied the relationship between uPA/uPAR and the SDF-1α/CXCR4 pathway. The expression levels of SDF-1α and CXCR4 in HBMECs were reduced after OGD/R but could be restored to a considerable extent by BMSCs. (Fig. 3A, B). However, knockdown of uPA reversed these phenomena (Fig. 3A, B). To deter-

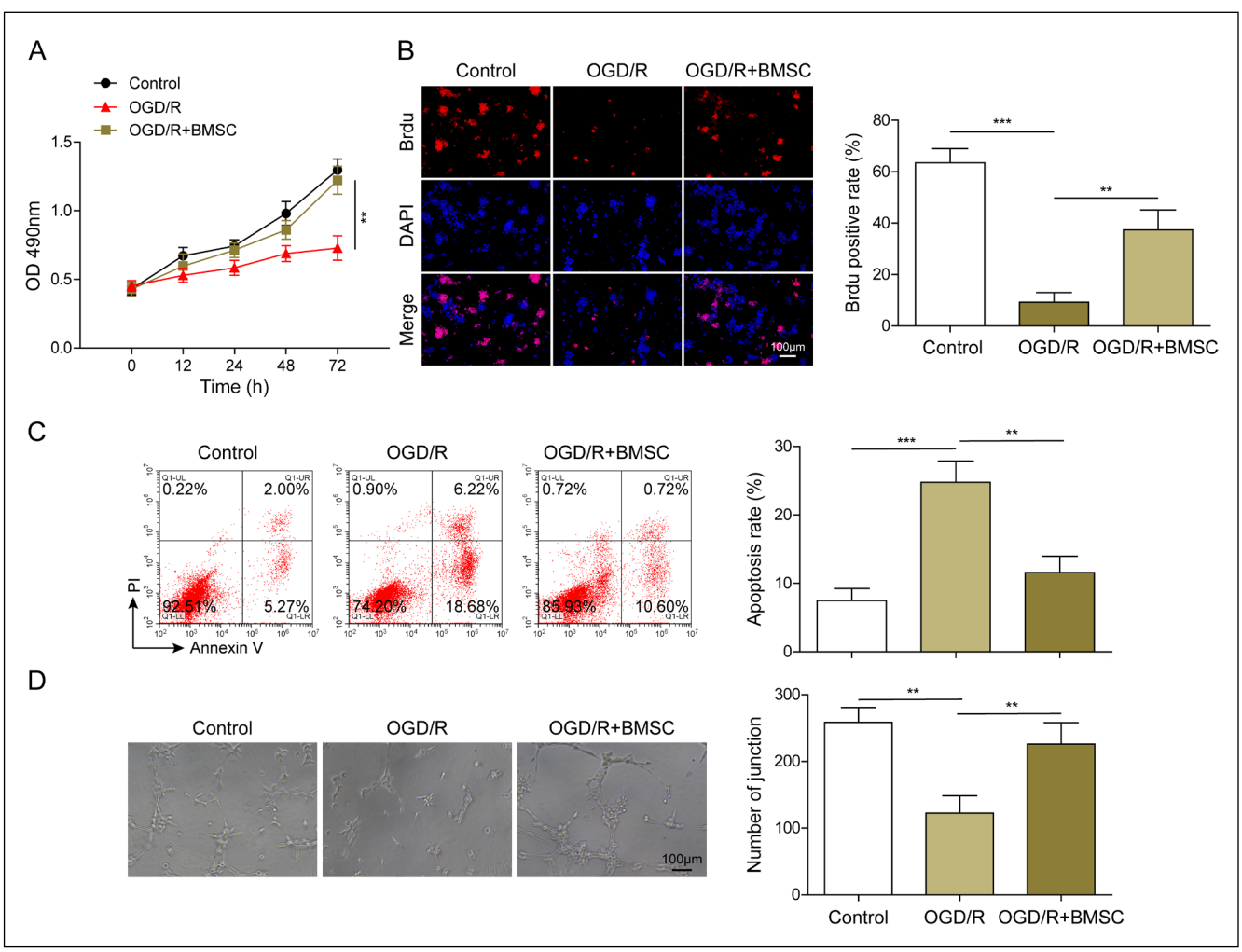

Fig. 1. BMSCs inhibit the apoptosis and promote the proliferation of OGD/R-treated HBMECs. HBMECs were treated with OGD/R or OGD/R + BMSCs. (A) The viability of HBMECs was determined using MTT assays. (B) BrdU staining was used to detect the proliferation of HBMECs. (C) Flow cytometry was used to detect the apoptosis of HBMECs. (D) *In vitro* angiogenesis was used to evaluate the angiogenic ability of HBMECs. n=3, $^{**}P<0.01$, $^{***}P<0.001$.

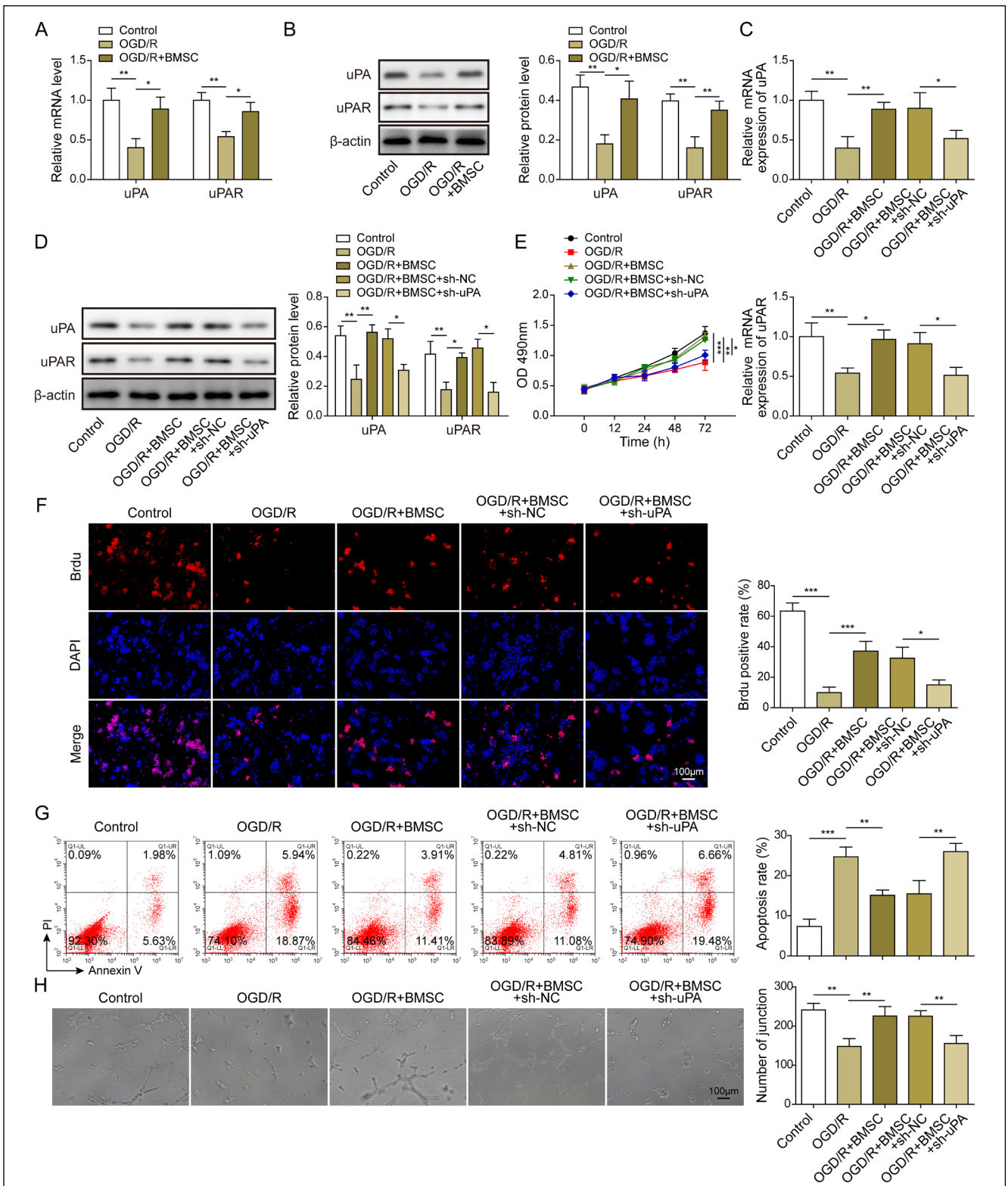

Fig. 2. BMSCs improve OGD/R-induced HBMECs damage by upregulating the uPA/uPAR expression. (A, B) RT-qPCR and western blot analyses were used to detect uPA and uPAR expression in HBMECs that were co-cultured with BMSCs. HBMECs were treated with OGD/R, OGD/R + BMSCs, OGD/R + BMSCs + sh-NC, or OGD/R + BMSCs + sh-uPA. (C) The expression of uPA and uPAR was detected by RT-qPCR and western blot analysis. (E) MTT assays were used to determine the viability of HBMECs. (F) The proliferation of HBMECs was determined by BrdU staining. (G) Flow cytometry was used to examine the apoptosis of HBMECs. (H) Angiogenesis was evaluated in *in vitro* angiogenesis experiments. n=3, **P*<0.05, ***P*<0.01, ****P*<0.001.

mine the role of the SDF-1α/CXCR4 pathway in this process, we knocked down SDF-1α by transfecting it with sh-SDF-1α. The results indicated that the expression levels of SDF-1α and CXCR4 were downregulated after the suppression of SDF-1α (Fig. 3C, D). In addition, BMSCs reversed the OGD/R-induced cell proliferation inhibition and apoptosis promotion effects, and the effect of the BMSCs was reversed substantially by sh-SDF-1α (Figs 3E-3G). BMSC-induced angiogenesis in OGD/R-treated HBMECs was reversed considerably by SDF-1α silencing (Fig. 3H). Therefore, uPA/uPAR protected HBMECs against OGD/R-induced injury by mediating the SDF-1α/CXCR4 pathway.

BMSCs attenuate OGD/R-induced HBMEC injury by upregulating the uPA/uPAR/SDF-1α/CXCR4 pathway

To confirm the mechanism by which BMSCs regulate OGD/R-induced HBMEC injury, rescue experiments were performed. The data demonstrated that the effect of sh-uPA on the expression levels of SDF-1α and CXCR4 in OGD/R and BMSCs co-treated with HBMECs were reversed considerably after SDF-1α was overexpressed. However, the expression of uPA and uPAR induced by OGD/R and BMSCs was only affected by sh-uPA, and overexpression of SDF-1α did not affect the levels of uPA and uPAR (Fig. 4A). Overexpression of SDF-1α remarkably upregulated the inhibitory effect of uPA knockdown on the viability and proliferation of HBMECs co-treated with OGD/R and BMSCs (Fig. 4B, C). Compared with the downregulation of uPA alone to promote apoptosis, the simultaneous upregulation of SDF-1α inhibited the apoptosis of OGD/R and BMSC-treated cells (Fig. 4D). Furthermore, the increase in SDF-1α expression reversed the effect of sh-uPA on HBMEC angiogenesis induced by the combination of BMSCs and OGD/R (Fig. 4E). Therefore, BMSCs attenuated OGD/R-induced HBMEC injury by upregulating the uPA/uPAR/SDF-1α/CXCR4 pathway.

DISCUSSION

In recent years, research on mesenchymal stem cells has gained much interest in terms of the treatment of various diseases and for their protective effect in the treatment of neonatal hypoxic-ischemic brain injury (Herz et al., 2018). A recent study has demonstrated that uPA can inhibit the progression and metastasis of liver cancer by mediating uPAR levels (Hsu et al., 2012). Therefore, we conducted *in vitro* experiments to demonstrate that BMSCs can improve the outcome in patients experiencing ischemic stroke through the uPA/uPAR-mediated SDF-1α/CXCR4 pathway.

HBMECs are the main components of the blood–brain barrier (Vatine et al., 2019). As with peripheral endothelial cells, adhesion molecules can be expressed on the surface of HBMECs to regulate the entry of white blood cells into the brain (Hunter et al., 2019; Nishihara et al., 2020). Therefore, maintaining the structural and functional integrity of HBMECs is crucial in preventing cells or harmful substances from penetrating the blood–brain barrier in the brain and protecting the brain homeostasis (Ma and Liu, 2019; Yamashita et al., 2020). Therefore, we simulated the occurrence of ischemic stroke *in vitro* by inducing hypoxia and hypoglycemia in a single layer of HBMECs to determine the effect and mechanism of BMSCs in improving the outcome of the stroke (Cui and Yang, 2013). The enhanced secretion of angiogenic cytokines after treatment of patients with ischemic stroke with BMSCs has a strong angiogenic effect on the remodeling of the microvascular system during neovascularization (Sasaki et al., 2009). Our results demonstrated that after HBMECs subjected to OGD/R were co-cultured with BMSCs, the activity, proliferation rate, and angiogenesis of HBMECs increased, whereas the apoptosis rate decreased. These results indicated that BMSCs play a protective role in OGD/R-induced HBMEC injury.

uPA is a serine protease that is cleaved by plasmin after binding with uPAR to generate an active form that catalyzes the conversion of plasminogen to plasmin (Qiu et al., 2007). Some previous studies showed that the concentration of uPA in ischemic tissue increased during the recovery phase of ischemic injury, and expression of the uPAR gene was induced in activated astrocytes (Wu et al., 2014; Zamanian et al., 2012). The combination of endogenous uPA and uPAR can improve the neurological functions in experimental models of ischemic stroke (Merino et al., 2017). The results of our study indicated that the expression of uPA and uPAR in HBMECs induced by OGD/R was downregulated, and that the expression of uPA and uPAR increased after treatment with BMSCs. Moreover, the knockdown of uPA reversed the protective effect of BMSCs on OGD/R-induced HBMEC injury. SDF-1α is a cytokine that can mediate the mobilization and homing of bone marrow mesenchymal stromal cells and progenitor cells in vascular injuries (Cheng et al., 2017), lymphocyte production, bone marrow production, and germ cell mobilization (Doitsidou et al., 2002). Studies on brain damage after stroke have shown that linagliptin can reduce damage through SDF-1α/CXCR4, which may be mediated by the regulation of neovascularization by endothelial progenitor cells (Dai et al., 2018; Sobrino et al., 2007; Wang et al., 2012). Our study demonstrated

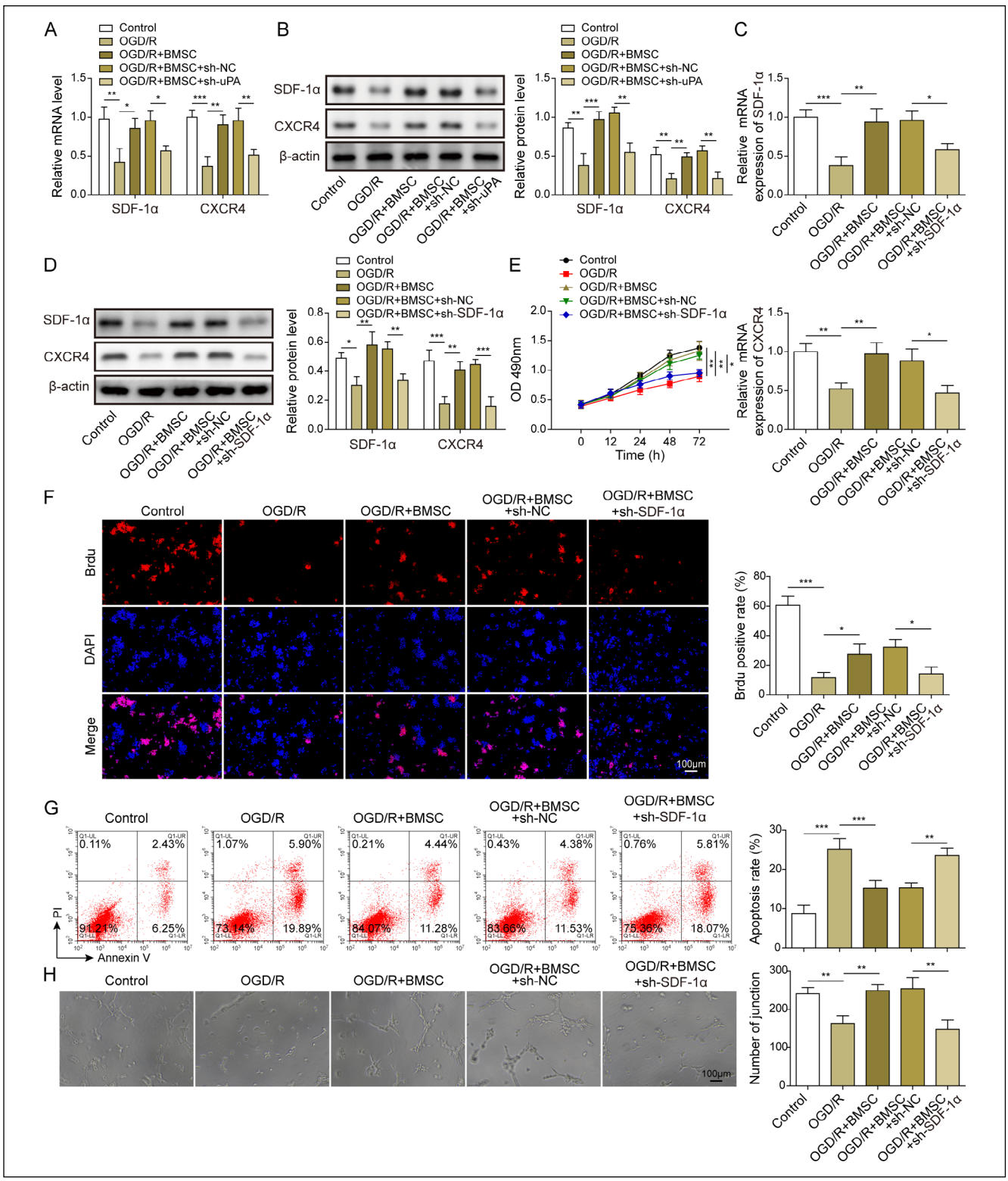

Fig. 3. uPA/uPAR attenuates OGD/R-induced HBMEC injury via mediating the SDF-1α/CXCR4 axis. (A, B) HBMECs were treated with OGD/R, OGD/R + BMSCs, OGD/R + BMSCs + sh-NC, or OGD/R + BMSCs + sh-uPA. RT-qPCR and Western blot analyses were used to detect the expression of SDF-1α and CXCR4 in HBMECs. (C, D) HBMECs were treated with OGD/R, OGD/R + BMSCs, OGD/R + BMSCs + sh-NC, or OGD/R + BMSCs + sh-SDF-1α. RT-qPCR and western blot analyses were used to detect the expression of SDF-1α and CXCR4 in HBMECs. (E) MTT assays were used to determine the viability of HBMECs. (F) The proliferation of HBMECs was determined using BrdU staining. (G) Flow cytometry was used to measure the apoptosis of HBMECs. (H) The angiogenesis ability of cells was evaluated using *in vitro* angiogenesis experiments. n=3, **P*<0.05, ***P*<0.01, ****P*<0.001.

that after the knockdown of uPA, the expression levels of SDF-1α and CXCR4 in HBMECs treated with OGD/R and BMSCs were reduced. In addition, after the knockdown of SDF-1α, the activity, proliferation, and angiogenesis of HBMECs treated with OGD/R and BMSCs were reduced, whereas apoptosis was increased. In the rescue experiment, the effects of sh-uPA and BMSCs on OGD/R-induced HBMEC injury were reversed after transfection with the SDF-1α overexpression vector. These results suggest that BMSCs upregulate the uPA/

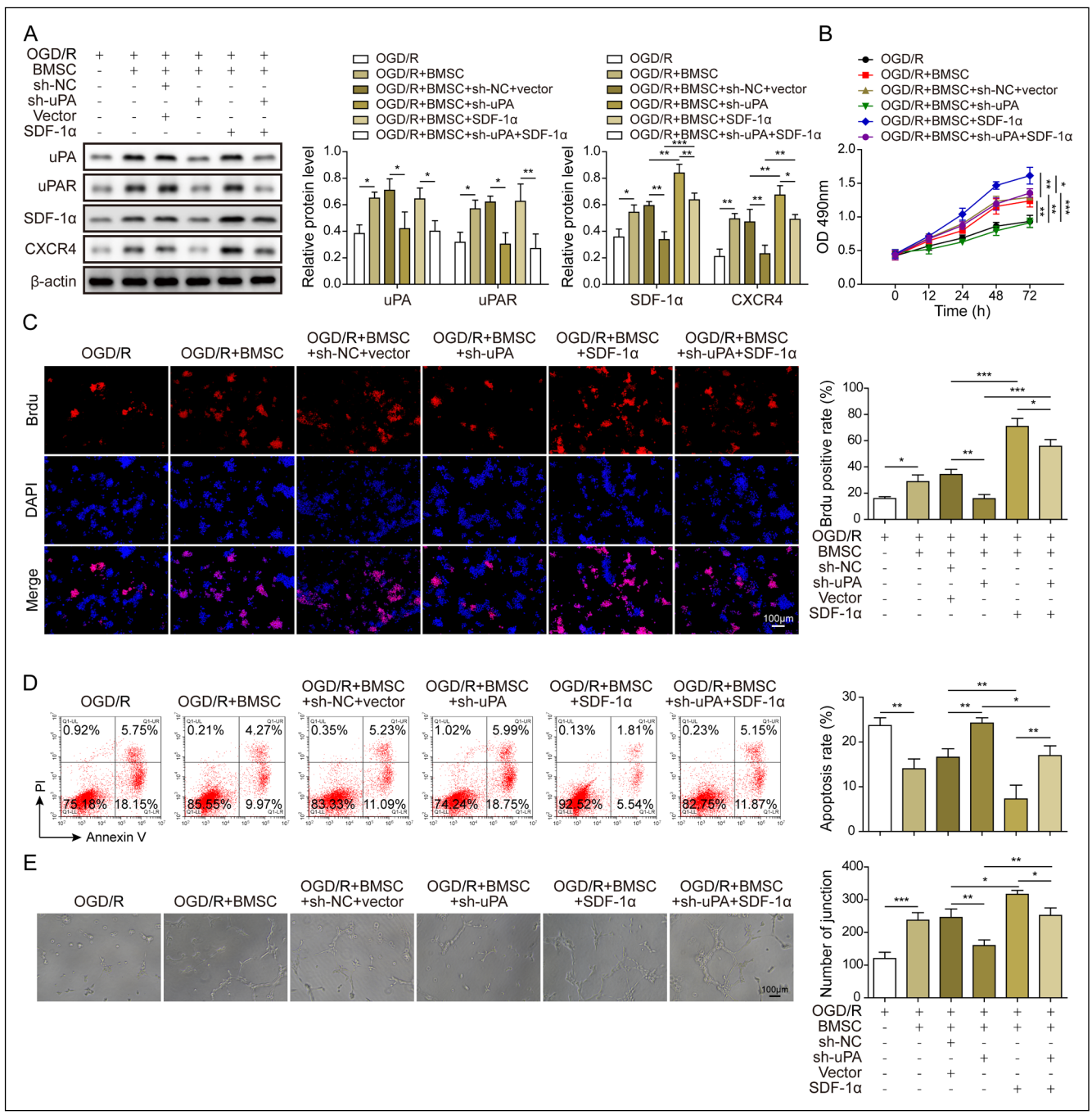

Fig. 4. BMSCs alleviate OGD/R-induced HBMEC injury by upregulating the uPA/uPAR/SDF-1α/CXCR4 pathway. HBMECs were treated with OGD/R, OGD/R + BMSCs, OGD/R + BMSCs + sh-NC + vector, OGD/R + BMSCs + sh-uPA, OGD/R + BMSCs + pcDNA3.1-SDF-1α, or OGD/R + BMSCs + sh-uPA + pcDNA3.1-SDF-1α. (A) Western blot analyses were used to detect the protein levels of SDF-1α and CXCR4 in HBMECs. (B) MTT assays were used to determine the cell viability of HBMECs. (C) The proliferation of HBMECs was determined by BrdU staining. (D) Flow cytometry was used to measure the apoptosis of HBMECs. (E) *In vitro* vascular tube formation experiments were used to detect angiogenesis of HBMECs. n=3, **P*<0.05, ***P*<0.01, ****P*<0.001.

uPAR-mediated SDF-1α/CXCR4 pathway to protect OGD/R-induced HBMEC injury.

Furthermore, the vascular endothelial growth factor (VEGF) can reportedly regulate the expression of the SDF-1α/CXCR4 axis (Yeh et al., 2021), and some micro RNAs (miR-612, miR-637, and miR-874) can regulate the expression of VEGF (Castanhole-Nunes et al., 2022). Thus, BMSCs may regulate the SDF-1α/CXCR4 axis by mediating the miR-612/VEGF, miR-637/VEGF, or the miR-874/VEGF axis. Our future research will attempt to confirm this hypothesis.

Indeed, It has been reported that bone marrow mesenchymal stromal cells can function similar to BMSCs in the treatment of ischemic stroke. For instance, exosomes secreted by bone marrow mesenchymal mesenchymal stromal cells can promote post-stroke functional recovery by enhancing neurogenesis and neural plasticity (Wei et al., 2022). Consequently, our future studies will focus on the function of bone marrow mesenchymal stromal cells in ischemic stroke.

CONCLUSION

In summary, our results demonstrated that BMSCs can regulate cell proliferation, apoptosis, and angiogenesis through the uPA/uPAR-mediated SDF-1α/CXCR4 pathway to improve the outcome of ischemic stroke and identify other therapeutic targets for the prevention and treatment of ischemic stroke in the future.

REFERENCE

Alfano D, Franco P, Vocca I, Gambi N, Pisa V, Mancini A, et al. (2005) The urokinase plasminogen activator and its receptor: role in cell growth and apoptosis. Thromb Haemost, 93: 205–211.

Castanhole-Nunes MMU, Tunissiolli NM, Oliveira A, Mattos MF, Galbiatti-Dias ALS, Kawasaki-Oyama RS, et al. (2022) MiR-612, miR-637, and miR-874 can regulate VEGFA expression in hepatocellular carcinoma cell lines. Genes 13(2).

Cheng X, Wang H, Zhang X, Zhao S, Zhou Z, Mu X, et al. (2017) The role of SDF-1/CXCR4/CXCR7 in neuronal regeneration after cerebral ischemia. Front Neurosci 11: 590.

Cossarizza A, Chang, HD, Radbruch A, Acs A, Adam D, Adam-Klages S, et al. (2019) Guidelines for the use of flow cytometry and cell sorting in immunological studies (second edition). Eur J Immunol, 49: 1457–1973.

Cui H, Yang L (2013) Analysis of microRNA expression detected by microarray of the cerebral cortex after hypoxic-ischemic brain injury. J Craniofac Surg 24: 2147–2152.

Cui Y, Xu N, Xu W, Xu G (2017) Mesenchymal stem cells attenuate hydrogen peroxide-induced oxidative stress and enhance neuroprotective effects in retinal ganglion cells. In Vitro Cell Dev Biol Anim, 53: 328–335.

Dai X, Zeng J, Yan X, Lin Q, Wang K, Chen J, et al. (2018) Sitagliptin-mediated preservation of endothelial progenitor cell function via augmenting autophagy enhances ischaemic angiogenesis in diabetes. J Cell Mol Med 22: 89–100.

Doitsidou M, Reichman-Fried M, Stebler J, Koprunner M, Dorries J, Meyer D, et al. (2002) Guidance of primordial germ cell migration by the chemokine SDF-1. Cell 111: 647–659.

Han B, Zhang Y, Zhang Y, Bai Y, Chen X, Huang R, et al. (2018) Novel insight into circular RNA HECTD1 in astrocyte activation via autophagy by targeting MIR142-TIPARP: implications for cerebral ischemic stroke. Autophagy, 14: 1164–1184.

Herz J, Koster C, Reinboth BS, Dzietko M, Hansen W, Sabir H, et al. (2018) Interaction between hypothermia and delayed mesenchymal stem cell therapy in neonatal hypoxic-ischemic brain injury. Brain Behav Immun 70: 118–130.

Hsu WH, Chen CN, Huang HI, Lai YL, Teng CY, Kuo WH (2012) Urokinase induces stromal cell-derived factor-1 expression in human hepatocellular carcinoma cells. J Cell Physiol, 227: 697–704.

Hunter LW, Jayachandran M, Miller VM (2019) Sex differences in the expression of cell adhesion molecules on microvesicles derived from cultured human brain microvascular endothelial cells treated with inflammatory and thrombotic stimuli. Biol Sex Differ, 10: 26.

Jorgensen MB, Finsen BR, Jensen MB, Castellano B, Diemer NH, Zimmer J (1993) Microglial and astroglial reactions to ischemic and kainic acid-induced lesions of the adult rat hippocampus. Exp Neurol, 120: 70–88.

Kachgal S, Putnam AJ (2011) Mesenchymal stem cells from adipose and bone marrow promote angiogenesis via distinct cytokine and protease expression mechanisms. Angiogenesis, 14: 47–59.

Ma X, Liu W (2019) Calcium signaling in brain microvascular endothelial cells and its roles in the function of the blood-brain barrier. Neuroreport 30: 1271–1277.

Merino P, Diaz A, Jeanneret V, Wu F, Torre E, Cheng L, et al. (2017) Urokinase-type plasminogen activator (uPA) binding to the uPA receptor (uPAR) promotes axonal regeneration in the central nervous system. J Biol Chem 292: 2741–2753.

Nishihara H, Gastfriend BD, Soldati S, Perriot S, Mathias A, Sano Y, et al. (2020) Advancing human induced pluripotent stem cell-derived blood-brain barrier models for studying immune cell interactions. FASEB J 34: 16693–16715.

Qiu D, Owen K, Gray K, Bass R, Ellis V (2007) Roles and regulation of membrane-associated serine proteases. Biochem Soc Trans 35: 583–587.

Sasaki M, Radtke C, Tan AM, Zhao P, Hamada H, Houkin K, et al. (2009) BDNF-hypersecreting human mesenchymal stem cells promote functional recovery, axonal sprouting, and protection of corticospinal neurons after spinal cord injury. J Neurosci 29: 14932–14941.

Shao H, Yang Y, Qi AP, Hong P, Zhu GX, Cao XY, et al. (2017) Gastrodin reduces the severity of status epilepticus in the rat pilocarpine model of temporal lobe epilepsy by inhibiting Nav1.6 sodium currents. Neurochem Res, 42: 360–374.

Smith HW, Marshall CJ (2010) Regulation of cell signalling by uPAR. Nat Rev Mol Cell Biol 11: 23–36.

Sobrino T, Hurtado O, Moro MA, Rodriguez-Yanez M, Castellanos M, Brea D, et al. (2007) The increase of circulating endothelial progenitor cells after acute ischemic stroke is associated with good outcome. Stroke, 38: 2759–2764.

Son B R, Marquez-Curtis LA, Kucia M, Wysoczynski M, Turner AR, Ratajczak J, et al. (2006) Migration of bone marrow and cord blood mesenchymal stem cells in vitro is regulated by stromal-derived factor-1-CXCR4 and hepatocyte growth factor-c-met axes and involves matrix metalloproteinases. Stem Cells 24: 1254–1264.

Steingen C, Brenig F, Baumgartner L, Schmidt J, Schmidt A, Bloch W (2008) Characterization of key mechanisms in transmigration and invasion of mesenchymal stem cells. J Mol Cell Cardiol 44: 1072–1084.

Vatine GD, Barrile R, Workman MJ, Sances S, Barriga BK, Rahnama M, et al. (2019) Human iPSC-Derived blood-brain barrier chips enable disease modeling and personalized medicine applications. Cell Stem Cell 24: 995–1005.

Wang B, Wu S, Ma Z, Wang T, Yang C (2018) BMSCs pre-treatment ameliorates inflammation-related tissue destruction in LPS-induced rat DIC model. Cell Death Dis 9: 1024.

Wang Y, Huang J, Li Y, Yang GY (2012) Roles of chemokine CXCL12 and its receptors in ischemic stroke. Curr Drug Targets, 13: 166–172.

Wei R, Zhang L, Hu W, Shang X, He Y, Zhang W (2022) Zeb2/Axin2-enriched BMSC-derived exosomes promote post-stroke functional recovery by enhancing neurogenesis and neural plasticity. J Mol Neurosci 72: 69–81.

Wu F, Catano M, Echeverry R, Torre E, Haile WB, An J, et al. (2014) Urokinase-type plasminogen activator promotes dendritic spine recovery and improves neurological outcome following ischemic stroke. J Neurosci 34: 14219–14232.

Xiang Y, Yao X, Wang X, Zhao H, Zou H, Wang L, et al. (2019) Houshiheisan promotes angiogenesis via HIF-1alpha/VEGF and SDF-1/CXCR4 pathways: in vivo and in vitro. Biosci Rep 39(10).

Yamashita M, Aoki H, Hashita T, Iwao T, Matsunaga T (2020) Inhibition of transforming growth factor beta signaling pathway promotes differentiation of human induced pluripotent stem cell-derived brain microvascular endothelial-like cells. Fluids Barriers CNS 17: 36.

Yeh JN, Yang RR, Wallace CG, Huang CR, Chu YC, Yip HK, et al. (2021) Impact of one versus two consecutive doses of endothelial cells (EPCs) and EPCs-derived condition medium on protecting myocardium from acute ischemia-reperfusion injury in rat. Cell Transplant 30: 9636897211007049.

Zamanian JL, Xu L, Foo LC, Nouri N, Zhou L, Giffard RG, et al. (2012) Genomic analysis of reactive astrogliosis. J Neurosci 32: 6391–6410.

Zhao M, Wang J, Xi X, Tan N, Zhang L (2018) SNHG12 promotes angiogenesis following ischemic stroke via regulating miR-150/VEGF pathway. Neuroscience 390: 231–240.