Article

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The Effect of *PGAM5* on Regulating Mitochondrial Dysfunction in Ischemic Stroke

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Background: Ischemic stroke is an acute cerebrovascular disease with high mortality rates and poor prognoses. The influence of ischemic stroke includes a heavy economic burden to patients and society, making the exploration of new therapeutic targets for preventing and treating ischemic stroke urgent. This study aimed to explore the effect of phosphoglycerate mutase family member 5 (*PGAM5*) on oxidative stress and mitochondrial dysfunction in ischemic stroke.

Methods: The model of ischemic neuronal brain injury was established through culturing purchased human neuroblastoma cells (SH-SY5Y) by oxygen-glucose deprivation/reoxygenation (OGD/R). There were six experimental groups, including the OGD/R model group (SH-cells of OGD/R model), OE-NC group (cells of OGD/R model transfected with scramble cDNA), OE-*PGAM5* group (cells of OGD/R model transfected with full-length sequence of *PGAM5*), si-NC group (cells of OGD/R model transfected with negative control small interference (si)RNA), si-*PGAM5* group (cells of OGD/R model transfected with siRNA for *PGAM5* knockdown), and a control group (cells cultured normally). Cell counting kit-8 (CCK-8) and flow cytometry were used to determine the activity and apoptosis of cells. Subsequently, the effects of *PGAM5* expression on oxidative stress and mitochondrial dysfunction were analyzed. Mitochondrial morphology was observed by transmission electron microscopy (TEM), and mitochondrial membrane potential (MMP) was determined by JC-1 fluorescent probe. The levels of reactive oxygen species (ROS) were measured by flow cytometry, and levels of malondialdehyde (MDA) and superoxide dismutase (SOD) were measured by enzyme-linked immunosorbent assay (ELISA) assay. The expression of light chain (LC)3-II/I and autophagy-related gene 5 (ATG5) proteins were measured, and the regulation of *PGAM5* expression on *PTEN*-induced putative protein kinase 1 (*PINK1*)/*Parkin* pathway was also explored.

Results: *PGAM5* overexpression in OGD/R cells decreased the cell viability ($p < 0.001$) while increasing cell apoptosis ($p < 0.01$) compared to the OGD/R group. Inhibition of *PGAM5* expression reversed the decreased cell viability ($p < 0.001$) and the increased cell apoptosis ($p < 0.01$). The JC-1 fluorescence showed that OGD/R treatment reduced mitochondrial membrane potential ($p < 0.001$) and TEM showed an obvious increase in phagosomes. In addition, OGD/R treatment enhanced oxidative stress (increased ROS, $p < 0.01$; increased MDA, $p < 0.001$; decreased SOD, $p < 0.001$), which could be further enhanced by overexpression of *PGAM5* (ROS, $p < 0.001$; MDA, $p < 0.001$; SOD, $p < 0.001$) while reversed by the inhibition of *PGAM5* (ROS, $p < 0.01$; MDA, $p < 0.001$; SOD, $p < 0.001$). The OGD/R-activated *PINK1*/*Parkin* pathway was inhibited by the knockdown of *PGAM5* ($p < 0.01$) but promoted by the overexpression of *PGAM5* ($p < 0.05$).

Conclusions: *PGAM5* stimulates oxidative stress and impairs mitochondrial function in ischemic stroke, and regulates the *PINK1*/*Parkin* signaling pathway. Therefore, *PGAM5* is likely to be a target for the therapy of ischemic stroke.

Keywords: *PGAM5*; oxidative stress; mitochondrial dysfunction; *PINK1*/*Parkin* pathway

Introduction

Ischemic stroke is an acute cerebrovascular disease caused by cerebral blood supply disorder and is characterized by strong concealment, sudden onset, high mortality, and poor prognosis. The timeliness of the therapy for ischemic stroke is a difficult problem for doctors consistently [1–4]. The increasing aging population has led to a rise in deaths and disabilities due to ischemic stroke, making it a significant annual contributor to the economic burden on both patients and society as it consistently ranks second among various diseases [5–7]. Therefore, it is of practical significance to explore new therapeutic targets for preventing and treating ischemic stroke.

Neuronal death including autophagy is increasingly activated during ischemic brain injury and contributes to stroke development [8–11]. Since mitochondria are proved as important sites for oxidative phosphorylation of adeno-

sine triphosphate (ATP) in cells, maintaining the dynamic balance between division and fusion, and providing sufficient energy for complex and orderly life activities [12–14]. It has been proved that the mitochondrial dysfunction played roles in cellular lipid homeostasis and many diseases [15], and abnormal mitochondrial function will cause axon and neuron dysfunction and consequently result in abnormal neuronal death [16]. Therefore, neurons must strictly control the quality of mitochondria, and mitophagy is one of the important strategies for mitochondrial quality control [17]. *PTEN*-induced putative protein kinase 1 (*PINK1*)/*Parkin* is a key signaling pathway regulating mitochondria function or dysfunction. When mitochondrial dysfunction occurs, abnormally degraded PINK1 will accumulate on the mitochondrial membrane, activate the activity of Parkin's E3 ligase, and initiate autophagy [18–20]. Therefore, the interaction between *PINK1* and *Parkin* is the basis of mitochondrial quality control [21]. On the other hand, the reactive oxygen species (ROS) function as key signaling molecule in the brain, directly or indirectly mediating many processes related to ischemic brain injury [22–24]. There is a close and complex interaction between autophagy and oxidative stress in cerebral ischemic injury [25]. For example, excessively high ROS after cerebral ischemia-reperfusion can trigger intracellular calcium overload, causing mitochondrial dysfunction and mitophagy [26]. Therefore, regulating oxidative stress and mitophagy is an effective strategy for treating ischemic stroke.

PGAM5 (phosphoglycerate mutase family member 5) and casein kinase II (*CK2*) have been reported to be associated with mitophagy [27,28]. B-cell lymphoma-2 like 1 (*BCL2L1*), rather than *BCL2*, interacts with *PGAM5* and inhibits *PGAM5* expression, thereby inhibiting hypoxia-induced mitophagy [29]. It is confirmed that PGAM5 regulated PINK1/Parkin-mediated mitochondrial phagocytosis and exerted neuroprotective effects on carbonyl cyanide m-chlorophenyl hydrazone (CCCP)-induced cell apoptosis [30].

This study hypothesized that *PGAM5* had effects on ischemic stroke by regulating mitochondrial dysfunction. To verify our hypothesis, we regulated *PGAM5* expression through cell transfection and investigated the induced effects on oxygen-glucose deprivation/reoxygenation (OGD/R) treated cells, including the effects on cell proliferation and apoptosis, the oxidative stress determined by ROS levels, malondialdehyde (MDA) and superoxide dismutase (SOD), the mitochondrial membrane potential, autophagy, and the PINK1/Parkin pathway. This study revealed how the *PGAM5* protects ischemic neurons and repairs nerve damage, providing new targets and directions for the diagnosis and treatment of ischemic stroke in clinical practice.

Table 1. Small interference (si)RNA sequences.

Name	Sequence (5′-3′)
si-NC sense	UUC UCC GAA CGU GUC ACG UTT
si-NC antisense	ACG UGA CAC GUU CGG AGA ATT
si-*PGAM5* sense	CUG UGC AGU AUU ACG AAG ATT
si-*PGAM5* antisense	UCU UCG UAA UAC UGC ACA GTT

si-*PGAM5*, siRNA against *PGAM5*; *PGAM5*, phosphoglycerate mutase family member 5; si-NC, negative control siRNA.

Materials and Methods

Establishment of OGD/R Model

The human neuroblastoma cell line of SH-SY5Y (iCell-h187) was obtained from iCell Bioscience Inc. (Shanghai, China) and cultured in a medium specifically designed for SH-SY5Y cells (iCell-h187-001b, iCell Bioscience Inc., Shanghai, China) in 5% CO_2 incubator at 37 °C. The OGD/R model was established by replacing the SH-SY5Y cell culture medium with a cell oxygen-glucose deprivation culture medium and adjusting the pH to 7.2–7.4. Then, the cells were cultured in a hypoxic culture box at 37 °C, 0.6% O_2, 94.5% N_2, and 5% CO_2 for 8 hours. Afterwards, the cell plate was quickly removed from the hypoxic culture box and cultured in a common culture medium for 24 hours. Untreated cells were simultaneously used to construct a control group. For all cell lines used in this study, the short tandem repeat (STR) analysis and mycoplasma test were performed to ensure all cell lines were unique and uncontaminated, respectively.

Cell Transfection

When SH-SY5Y cells reached the logarithmic phase, they were digested, collected, and adjusted to a density of 1×10^5/mL. Then, the cells were seeded into six-well plates and cultured at 37 °C and 5% CO_2 for 24 hours. The experimental groups were divided into OGD/R model group (SH-cells of OGD/R model), OE-NC group (cells of OGD/R model transfected with scramble cDNA), OE-*PGAM5* group (cells of OGD/R model transfected with full-length sequence of *PGAM5*), si-NC group (cells of OGD/R model transfected with negative control small interference (si)RNA), si-*PGAM5* group (cells of OGD/R model transfected with siRNA for *PGAM5* knockdown), and a control group (cells cultured normally). Lipofectamine™ 2000 (11668019, Invitrogen, Carlsbad, CA, USA) was used to transfect for 48 hours according to the instructions, and then the following experimental studies were performed. The sequences of small interference (si)RNA were listed in the Table 1.

Table 2. The sequences of primers.

Name of primer	Sequences of primers (5′-3′)
PGAM5-F	GCGGAAGAGGAACGTGGAA
PGAM5-R	GGAATGCCTGATGAGGAAGATG
PINK1-F	GGCGGAAACGGCTGTCTGA
PINK1-R	TGCGGCTTTCAAGGTGGG
Parkin-F	ATCGCAACAAATAGTCGG
Parkin-R	GTTCCTGAGGCTTCAAATAC
Actin-F	ACACTGTGCCCATCTACG
Actin-R	TGTCACGCACGATTTCC

PGAM5, phosphoglycerate mutase family member 5; *PINK1*, *PTEN*-induced putative protein kinase 1; F, forward; R, reverse.

Cell Viability Detection

The cells were inoculated in 96-well plates, subjected to oxidative deprivation, and replaced with a normal culture medium. All groups except for the control group received the addition of Cell counting kit-8 (CCK-8) detection solution (MA0218-5, Meilunebio, Dalian, China). The control group received normal culture solution and was placed in the incubator in the dark for two hours. The absorbance was detected at 450 nm by microplate reader (CMax Plus, Molecular Devices, Shanghai, China) to calculate cell viability.

Cell Apoptosis Detection

After 48 hours of transfection, cells from each group were collected, centrifuged at 1500 rpm for 5 minutes to remove the supernatant, and then washed with 5 mL phosphate-buffered saline (PBS; G4202, Serveicebio, Wuhan, China). The cells were resuspended and mixed with Annexin V-FITC and propidium iodide (PI) (AP101, MultiSciences, Hangzhou, China) at room temperature for 20 minutes in the dark. The rate of apoptosis was then detected by flow cytometry (CytoFLEX, Beckman Coulter, Brea, CA, USA).

Quantitative Real-Time Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from the cells according to the instructions on the TRIzol kit (DP424, Tiangen, Beijing, China), and reverse transcribed into cDNA according to the reverse transcription kit (KR116, Tiangen, Beijing, China) instructions. Gene amplification was completed using the premixed qPCR reagent which was based on the SYBR Green method (FP207, Tiangen, Beijing, China) and the qPCR instrument (LightCycler96, Roche, Shanghai, China). The results were calculated by $2^{-\Delta\Delta Ct}$ method and the sequences of primers used in this study were listed in Table 2.

Western Blot

After the centrifugation of cell lysis, the supernatant was collected to determine the protein concentration using a BCA kit (P0010S, Beyotime Biotechnology Co., Ltd., Shanghai, China). The protein was separated by SDS-PAGE (10%) electrophoresis and transferred to a nitrocellulose membrane. The membrane was subsequently sealed at room temperature for 15 minutes and incubated with primary antibody, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:1000 dilution, Cat No. TA-08, ZSGB Biotechnology Co., Ltd., Beijing, China), PGAM5 (1:5000 dilution, Cat. No. 28445-1-AP, Proteintech, Wuhan, China), light chain (LC)3-II/I (1:2000 dilution, Cat. No. 14600-1-AP, Proteintech, Wuhan, China), autophagy-related gene 5 (ATG5; 1:1000 dilution, Cat. No. 10181-2-AP, Proteintech, Wuhan, China), PINK1 (1:1000 dilution, Cat. No. 23274-1-AP, Proteintech, Wuhan, China) and Parkin (1:2000 dilution, Cat. No. 14060-1-AP, Proteintech, Wuhan, China) at 4 °C overnight. After the membrane was incubated with secondary antibodies (1:2000 dilution, ZB-2305 & ZB-2301, ZSGB Biotechnology Co., Ltd., Beijing, China) at room temperature for 1 hour, the protein bands were imaged by electrochemiluminescence (ECL; 34075, Thermo Scientific, Shanghai, China) reagent and under the chemiluminescence instrument (Tanon-4600, Yuanpinghao Biotechnology Co., Ltd., Beijing, China). The grey scale values were calculated using ImageJ software (1.48, National Institutes of Health, Rockville, Maryland, USA). The GAPDH was used as an internal loading control and the protein expression levels were finally normalized by the expression level in the control group.

ROS Detection

After adding DCFH-DA (S0033S, Beyotime Biotechnology Co., Ltd., Shanghai, China) and incubating at 37 °C for 20 minutes, the cells were washed, collected, and detected for ROS using flow cytometry (CytoFLEX, Beckman Coulter, Brea, CA, USA).

Transmission Electron Microscope (TEM)

The cells were washed twice with PBS before being scraped and placed in PBS to form a cell suspension. The cells were centrifuged at 800–1000 rpm to obtain cell precipitates. The supernatant was discarded and then resuspended with an electron microscope fixative. The dynamic balance of mitochondrial fission and polymerization was observed by TEM (HT7800, HITACHI, Tokyo, Japan).

Mitochondrial Membrane Potential (MMP) Detection

The JC-1 fluorescent probe (C2006, Beyotime Biotechnology Co., Ltd., Shanghai, China) was used to detect the changes in mitochondrial membrane potential of SH-SY5Y cells. The fluorescence intensity of JC-1 monomer/aggregate (green fluorescence as monomer, red

fluorescence as aggregate) was found using a fluorescence microscope (CKX53, Olympus, Tokyo, Japan) according to the instructions of the JC-1 detection kit and quantified using Image J software (1.48, National Institutes of Health, Rockville, Maryland, USA). The results of the red/green fluorescence ratio were used to evaluate the mitochondrial membrane potential.

ELISA (Enzyme-Linked Immunosorbent Assay)

According to the manufacturer's instruction, cells were centrifugated at 3000 rpm for 10 min and the supernatant was collected for measuring the contents of SOD (A001-1, Jiancheng, Nanjing, China) and MDA (A003-1, Jiancheng, Nanjing, China). The optical density was measured at a wavelength of 460 nm using an ELISA reader (Varioskan Lux, Thermo Fisher, Shanghai, China).

Statistical Analysis

The data analyses were performed using SPSS 22.0 software (IBM Corp., Chicago, IL, USA). Data obtained by multiple repeated experiments was presented as mean ± standard deviation (SD). The statistical significance of the differences was compared using Student's *t*-test or one-way analysis of variance (ANOVA) analysis, followed by Bonferroni's post hoc analysis. $p < 0.05$ was set as the threshold for statistical significance.

Results

Overexpression of PGAM5 in the OGD/R Model

We measured the expression of *PGAM5* mRNA and protein in cells treated with OGD/R. In Fig. 1A, the *PGAM5* mRNA expression was significantly higher in the OGD/R group compared to the control group ($p < 0.01$). Similarly, the PGAM5 protein level displayed a similar increase, as shown in Fig. 1B ($p < 0.001$).

Knockdown of PGAM5 Promotes Proliferation and Suppresses Apoptosis of OGD/R-Injured Cells

Next, this study measured the effect of *PGAM5* knockdown on the activity and apoptosis of OGD/R-damaged cells. Fig. 2A showed that siRNA against *PGAM5* was successfully transfected into OGD/R-damaged cells, significantly down-regulating *PGAM5* expression in the si-*PGAM5* group ($p < 0.01$). Compared with the control group, OGD/R treatment significantly decreased cell proliferation and activity (Fig. 2B, $p < 0.001$), and increased cell apoptosis (Fig. 2C,D, $p < 0.001$). The knockdown of *PGAM5* ($p < 0.01$) significantly enhanced cell activity (Fig. 2B, $p < 0.001$) and inhibited apoptosis (Fig. 2C,D, $p < 0.01$).

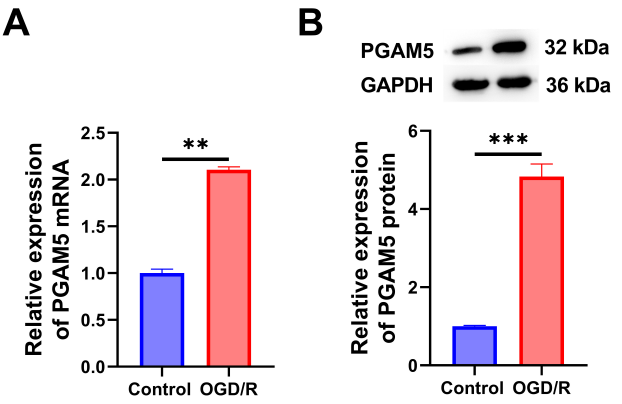

Fig. 1. *PGAM5* was upregulated in the oxygen-glucose deprivation/reoxygenation (OGD/R) cell model. (A) The expression of *PGAM5* mRNA in the control group and the OGD/R group. (B) The expression of PGAM5 protein in the control group and the OGD/R group. N = 3. OGD/R model group, SH-cells of OGD/R model); control group, cells cultured normally. $^{**}p < 0.01$, $^{***}p < 0.001$. *PGAM5*, phosphoglycerate mutase family member 5; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase.

Overexpression of PGAM5 Suppresses Proliferation and Promotes Apoptosis of OGD/R-Injured Cells

Furthermore, the effects of *PGAM5* overexpression on the activity and apoptosis of OGD/R-damaged cells were also evaluated. Fig. 2A showed that *PGAM5* plasmids were successfully transfected into OGD/R-damaged cells, significantly up-regulating *PGAM5* expression in the OE-*PGAM5* group ($p < 0.001$). Compared with the decreased cell proliferation (Fig. 2B, $p < 0.001$) and increased cell apoptosis (Fig. 2C,D, $p < 0.001$) induced by OGD/R treatment, the OE-*PGAM5* group showed stronger cytotoxicity, resulting in the further decreased cell viability ($p < 0.001$) and the further increased cell apoptosis ($p < 0.01$).

PGAM5 Knockdown/Overexpression Reduces/Induces Oxidative Stress after OGD/R Injury

OGD/R treatment enhanced the oxidative stress response of cells, accompanied by an increase in ROS (Fig. 3A, $p < 0.01$) and MDA levels (Fig. 3B, $p < 0.001$), as well as a decrease in SOD levels (Fig. 3C, $p < 0.001$). Overexpression of *PGAM5* ($p < 0.001$) showed a stronger oxidative stress response than OGD/R, increasing ROS and MDA levels ($p < 0.001$) and decreasing SOD levels ($p < 0.001$). In contrast, *PGAM5* knockdown ($p < 0.01$) effectively reduced ROS ($p < 0.01$) and MDA levels ($p < 0.001$) and increased SOD levels ($p < 0.001$).

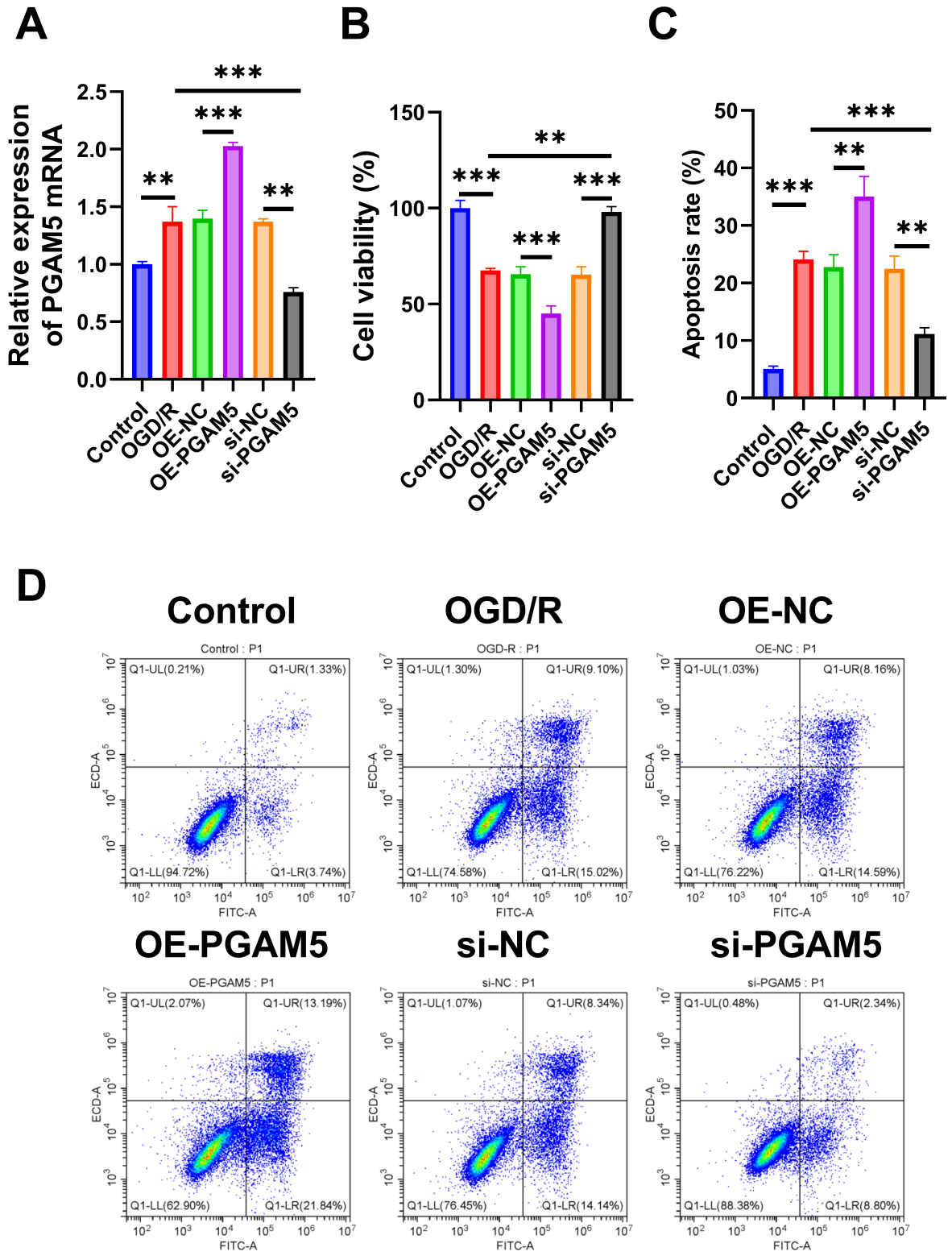

Fig. 2. *PGAM5* knockdown/overexpression promotes/suppresses cell viability and suppresses/promotes apoptosis after OGD/R injury. (A) Transfection of *PGAM5* plasmids into cells. (B) Cell viability in different groups. (C,D) Apoptosis rate in different groups. N = 3. OGD/R model group, SH-cells of OGD/R model; OE-NC group, cells of OGD/R model transfected with scramble cDNA; OE-*PGAM5* group, cells of OGD/R model transfected with full-length sequence of *PGAM5*; si-NC group, cells of OGD/R model transfected with negative control small interference (si)RNA; si-*PGAM5* group, cells of OGD/R model transfected with siRNA for *PGAM5* knockdown; control group, cells cultured normally. $^{**}p < 0.01$, $^{***}p < 0.001$.

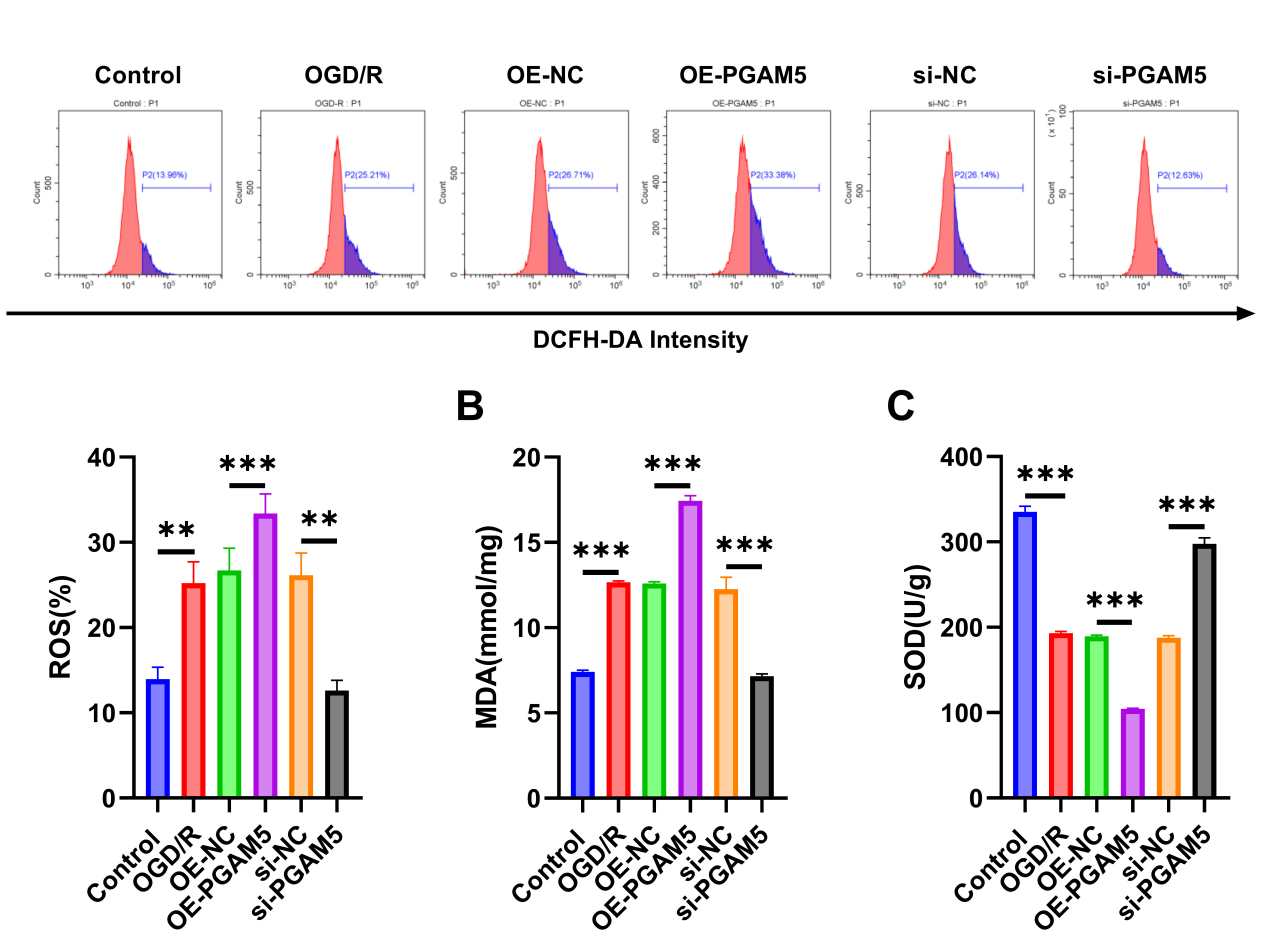

Fig. 3. *PGAM5* knockdown/overexpression reduces/induces oxidative stress after OGD/R injury. ROS levels with flow cytometry images (A), MDA (B), and SOD (C) in different groups. N = 3. OGD/R model group, SH-cells of OGD/R model; OE-NC group, cells of OGD/R model transfected with scramble cDNA; OE-*PGAM5* group, cells of OGD/R model transfected with full-length sequence of *PGAM5*; si-NC group, cells of OGD/R model transfected with negative control small interference (si)RNA; si-*PGAM5* group, cells of OGD/R model transfected with siRNA for *PGAM5* knockdown; control group, cells cultured normally. $^{**}p < 0.01$, $^{***}p < 0.001$. ROS, reactive oxygen species; MDA, malondialdehyde; SOD, superoxide dismutase.

PGAM5 Knockdown/Overexpression Restores/Aggregates the Decrease of Mitochondrial Membrane Potential after OGD/R Injury

The quantitative analysis in Fig. 4A and the fluorescence image in Fig. 4B both showed that OGD/R treatment significantly reduced MMP ($p < 0.001$). *PGAM5* overexpression ($p < 0.001$) resulted in a more significant loss of MMP ($p < 0.001$), while the successful si-*PGAM5* transfection ($p < 0.01$) restored MMP ($p < 0.001$) and approached the level of the control group.

PGAM5 Regulates Mitochondrial Autophagy after OGD/R Injury

As shown in Fig. 5A, the mitochondria in the control group were clear and intact, with only a small amount of autophagosomes formed. Both the OGD/R group and OE-*PGAM5* group showed mitochondrial ridge rupture, vacuolar-like changes, and increased autophagosome formation. Compared with the OGD/R group and OE-*PGAM5* group, the si-*PGAM5* group showed reduced mitochondrial swelling, ridge rupture, vacuolar changes, and autophagosome formation. Furthermore, the OGD/R group significantly increased the protein levels of LC3-II/I ($p < 0.01$) and ATG5 ($p < 0.01$) as shown in Fig. 5B–D. The OE-*PGAM5* group ($p < 0.001$) established more significant increases in LC3-II/I ($p < 0.01$) and ATG5 ($p < 0.001$) protein levels which was reversed ($p < 0.001$ for LC3-II/I; $p < 0.01$ for ATG5) by si-*PGAM5* transfection ($p < 0.001$).

PGAM5 Significantly Promotes PINK1/Parkin Signaling Pathway Activation during OGD/R Injury

As shown in Fig. 6A–E, compared with the control group, OGD/R injury significantly increased the *PINK1* and *Parkin* mRNA ($p < 0.001$) levels with a smaller increase in protein levels ($p < 0.01$). *PGAM5* overexpression ($p < 0.001$) showed stronger activation of the *PINK1*/*Park* path-

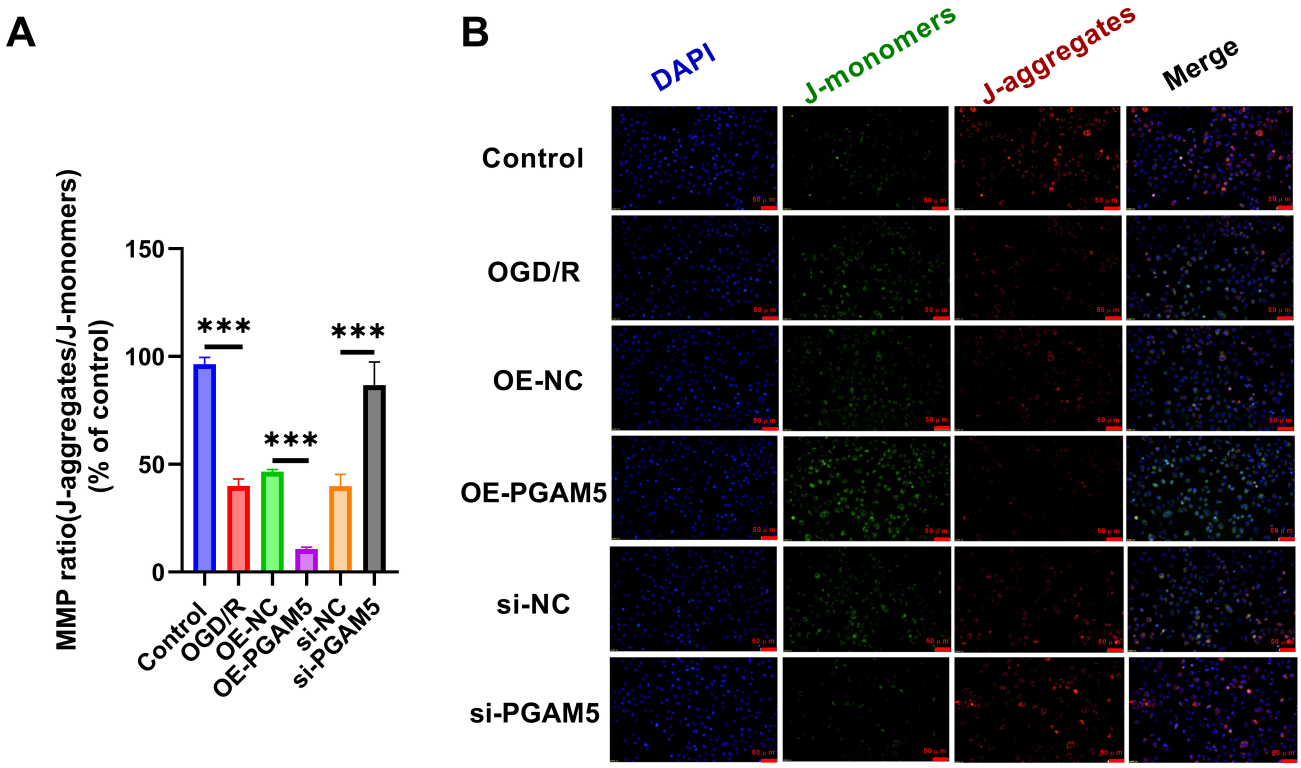

Fig. 4. *PGAM5* knockdown/overexpression restores/aggregates the decrease of mitochondrial membrane potential after OGD/R injury. (A) Fluorescence quantification of mitochondrial membrane potential (MMP). (B) JC-1 fluorescence image for detecting mitochondrial membrane potential. Scale bar = 50 µm. N = 3. OGD/R model group, SH-cells of OGD/R model; OE-NC group, cells of OGD/R model transfected with scramble cDNA; OE-*PGAM5* group, cells of OGD/R model transfected with full-length sequence of *PGAM5*; si-NC group, cells of OGD/R model transfected with negative control small interference (si)RNA; si-*PGAM5* group, cells of OGD/R model transfected with siRNA for *PGAM5* knockdown; control group, cells cultured normally. ***$p < 0.001$.

way than the OGD/R group, in which the levels of *PINK1* mRNA ($p < 0.001$), Parkin *mRNA* ($p < 0.001$), PINK1 protein ($p < 0.01$), and Parkin protein ($p < 0.05$) increased. Si-*PGAM5* transfection ($p < 0.01$) showed an inhibitory effect on the *PINK1*/*Parkin* pathway, accompanied by a decrease in *PINK1* mRNA ($p < 0.001$), PINK1 protein ($p < 0.01$), *Parkin* mRNA ($p < 0.001$), and Parkin protein ($p < 0.01$).

Discussion

There is a close and complex interaction between autophagy and oxidative stress in ischemic brain injury. Thoroughly reviewing the regulatory effects of oxidative stress and autophagy on cerebral ischemia-reperfusion is of great theoretical and practical significance for a comprehensive understanding in the pathophysiological mechanisms of ischemic brain injury and the development of new drugs. Therefore, this study first explored the regulatory effect of *PGAM5* on oxidative stress, dysfunction, autophagy in mitochondria after ischemic stroke.

In this study, an OGD/R model was successfully constructed to simulate ischemia-reperfusion *in vitro*. It was found that OGD/R treatment significantly increased both the mRNA and protein expressions of *PGAM5* in SH-SY5Y cells, indicating that *PGAM5* overexpression is associated with poor prognosis of ischemic stroke. *PGAM5* is associated with mitophagy [31,32] and the B-cell lymphoma-2 like 1 (*BCL2L1*)-*PGAM5*-*FUNDC1* axis plays an important role in mitophagy and oxidative stress [29,33]. However, the mechanism to regulate oxidative stress and mitochondrial dysfunction induced by autophagy after ischemic stroke remains unclear. Therefore, in this study, *PGAM5* overexpression and knockdown plasmids were successfully transfected into OGD/R-injured SH-SY5Y cells, and the effects of *PGAM5* on cell activity and biological characteristics were investigated. We found that overexpression of *PGAM5* aggravates cell injury and apoptosis, while *PGAM5* knockdown protects cells from OGD/R injury and contributes to the treatment of ischemic stroke. Gao C. *et al.* [34] confirmed that LFHP-1c, an inhibitor of *PGAM5*, not only reduced *PGAM5* enzyme activity but also significantly inhibited rBMEC apoptosis in rat brains following OGD/R-induced injury. This effect led to an improvement in ischemia-induced blood-brain barrier damage and supported the efficacy of our results in the treatment of ischemic stroke.

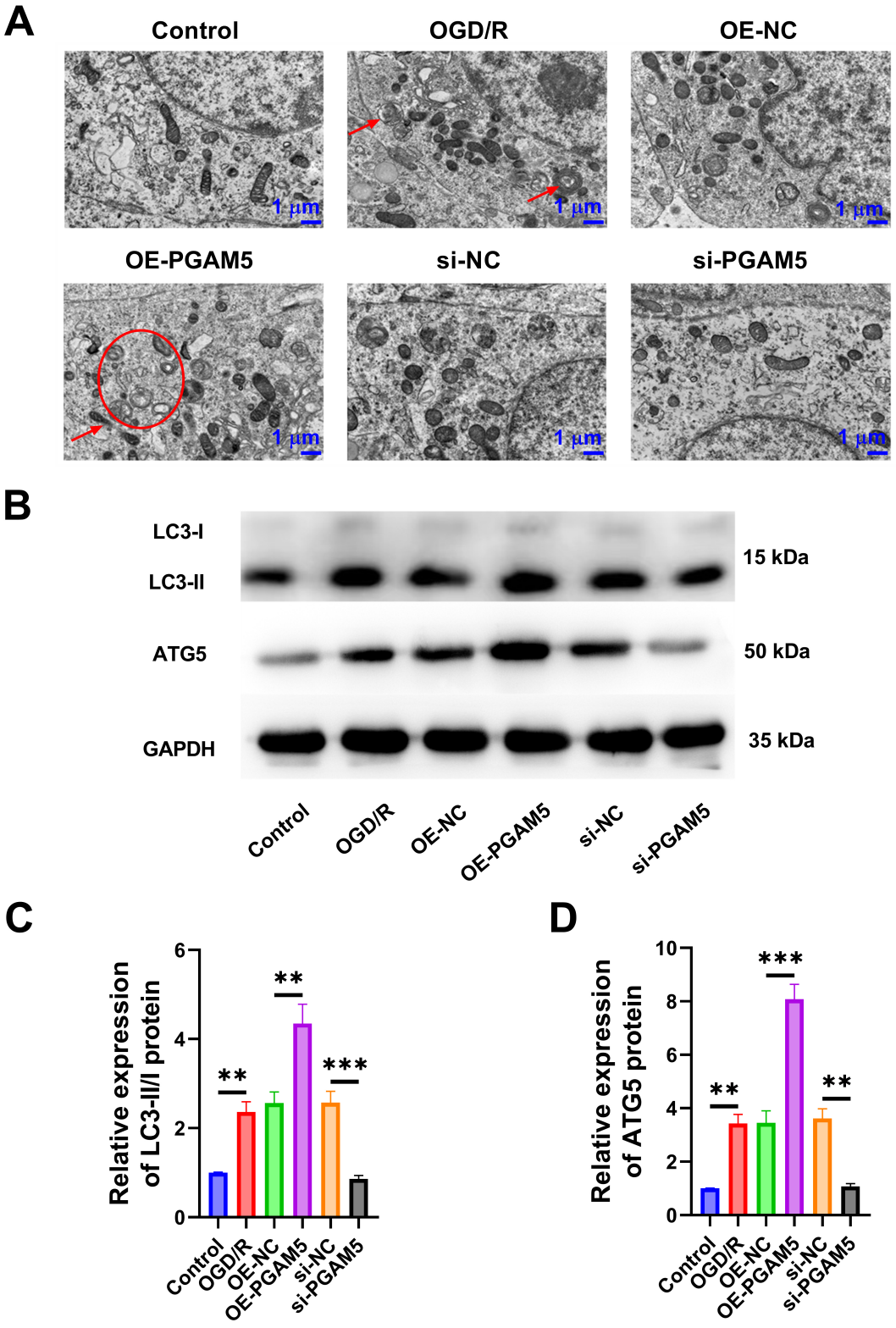

Fig. 5. *PGAM5* regulates mitochondrial autophagy after OGD/R injury. (A) Observation of mitochondrial autophagy by transmission electron microscopy (TEM). Scale bar = 1 µm. Red arrow indicates the ruptured mitochondrial ridge; red circle specifies the autophagosome formation. (B–D) Light chain (LC)3-II/I and autophagy-related gene 5 (ATG5) expression was measured by western blot. N = 3. Control, SH-SY5Y cells cultured normally; OGD/R, SH-SY5Y cells of OGD/R model; OE-NC, SH-SY5Y cells of OGD/R model transfected with scramble cDNA; OE-*PGAM5*, SH-SY5Y cells of OGD/R model transfected with full-length sequence of *PGAM5*; si-NC, SH-SY5Y cells of OGD/R model transfected with negative control small interference (si)RNA; si-*PGAM5*, SH-SY5Y cells of OGD/R model transfected with siRNA for *PGAM5* knockdown. $^{**}p < 0.01$, $^{***}p < 0.001$.

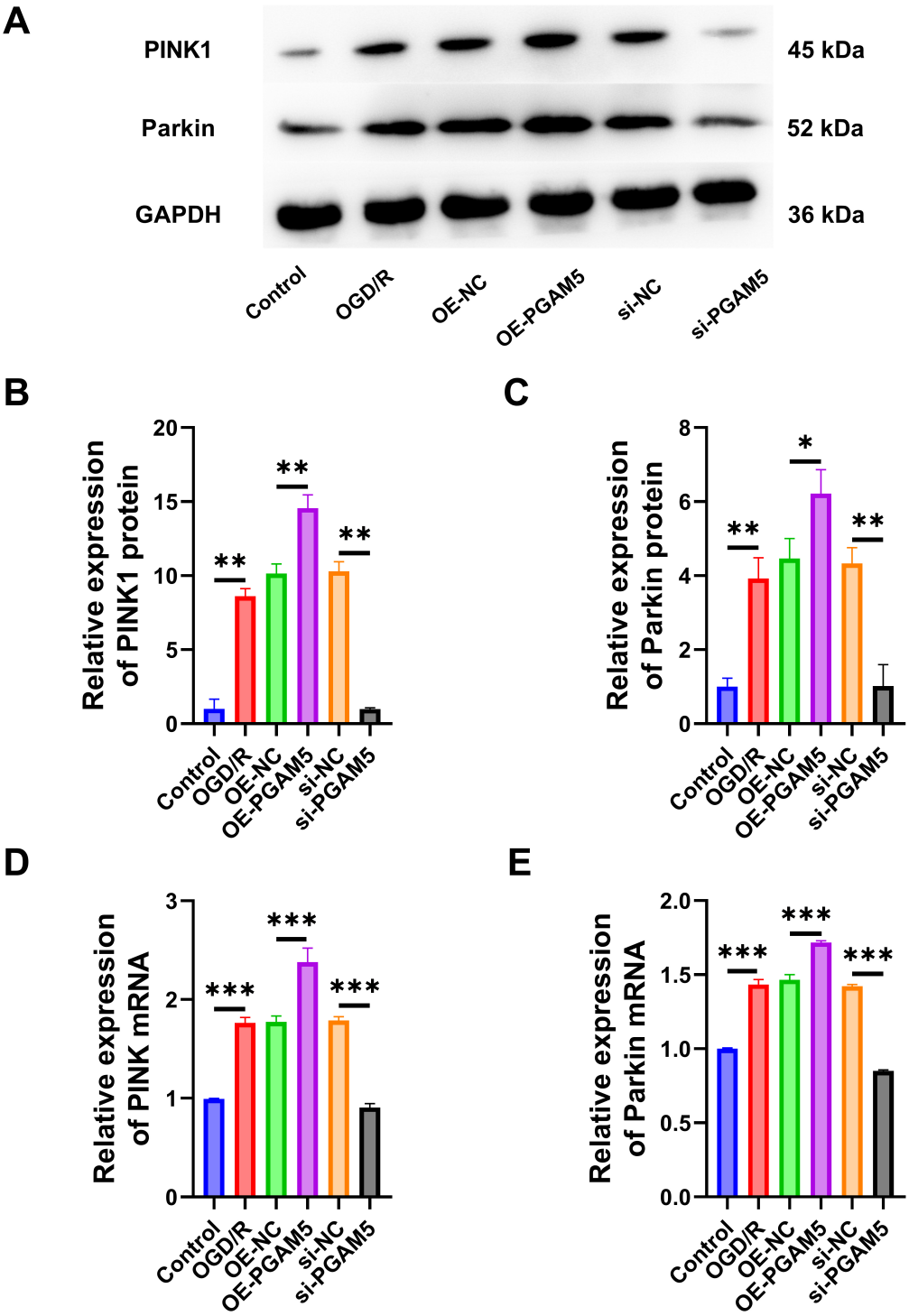

Fig. 6. *PGAM5* significantly promotes the *PINK1*/*Parkin* signaling pathway activation during OGD/R injury. (A–C) PINK1 and Parkin protein expression was detected by western blot. *PINK1* mRNA level (D) and *Parkin* mRNA level (E) were measured by qRT-PCR. N = 3. OGD/R model group, SH-cells of OGD/R model; OE-NC group, cells of OGD/R model transfected with scramble cDNA; OE-*PGAM5* group, cells of OGD/R model transfected with full-length sequence of *PGAM5*; si-NC group, cells of OGD/R model transfected with negative control small interference (si)RNA; si-*PGAM5* group, cells of OGD/R model transfected with siRNA for *PGAM5* knockdown; control group, cells cultured normally. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. *PINK1*, *PTEN*-induced putative protein kinase 1.

Oxidative stress leads to increase ROS, which directly or indirectly mediates many processes of ischemic brain injury [35]. In addition, non-dependent iNOS is highly expressed in nerve cells and inflammation, leading to increase NO production. This increase in NO production interferes with the activity of superoxide dismutase (SOD) and contributes to cerebral ischemic injury [36,37]. In this study, OGD/R injury-induced oxidative stress significantly increased ROS and MDA levels, and decreased SOD levels, which indicates that oxidative stress is one of the factors leading to ischemic stroke disease. *PGAM5* knockdown inhibited the oxidative stress response, significantly reduced ROS and MDA levels, and increased SOD expression.

PINK1/*Parkin* pathway is one option to induce mitophagy [38,39], and ROS may act as a trigger for *PINK1*/*Parkin*-dependent mitochondrial phagocytosis by inducing Parkin translocation to mitochondria [40–42]. Zeb A. *et al*. [43] proved that the accumulated *PGAM5* interfered with the processing of *PINK1* in mitochondria, resulting in the accumulation of *PINK1* on the mitochondrial outer membrane, which further induced mitophagy. This study further confirmed that the overexpression of *PGAM5* exacerbated mitochondrial autophagy, which may be due to the promotion of ROS generation by *PGAM5* overexpression. In this study, TEM results showed that overexpression of *PGAM5* exacerbates mitochondrial damage and increases autophagosomes. *PGAM5* overexpression also significantly reduces MMP and promotes the expression of LC3-II/I and ATG5 proteins. We inferred that the cause of the above results may be the activation of the *PINK1*/*Parkin* pathway. Therefore, this study further validated the regulatory effect of *PGAM5* on the *PINK1*/*Parkin* pathway. The results showed that overexpression of *PGAM5* significantly promotes the mRNA and protein expressions of *PINK1* and *Parkin*, which could be reversed by *PGAM5* knockdown. Therefore, knocking down *PGAM5* could repair mitochondrial damage and restore MMP by inhibiting the *PINK1*/*Parkin* pathway, effectively reversing the oxidative stress response and mitochondrial dysfunction caused by OGD/R. The findings of this study provide new targets for developing therapeutic strategies for ischemic stroke. However, additional cell lines would be needed for further verification. Moreover, for clinical usage, effective antibodies targeting *PGAM5* and more clinical trials are required.

Conclusions

This study first explored the effect of *PGAM5* on oxidative stress and mitochondrial dysfunction in the cellular model of ischemic stroke and its potential association with the *PINK1*/*Parkin* pathway. This study demonstrated the protective effect on ischemic nerve cells, the inhibition of oxidative stress, and the mitigation of mitochondrial dysfunction through *PGAM5* knockdown. This process also revealed the potential involvement of the *PINK1*/*Parkin* pathway as an underlying mechanism. The results of this study suggest that *PGAM5* could be potentially used as an effective therapeutic target for ischemic stroke.

Availability of Data and Materials

Data to support the findings of this study are available on reasonable request from the corresponding author.

Author Contributions

CLM and LY contributed to the concept and designed the research study. CLM and LZ performed the research. LY and CL provided help and advice on the experiments. QG and GW contributed to the analysis and interpretation of the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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