SH2B1 protects against OGD/R-induced apoptosis in PC12 cells via activation of the JAK2/STAT3 signaling pathway

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Abstract. Apoptosis acts as the primary pathogenesis of cerebral ischemia/reperfusion (I/R) injury. Prior studies have revealed the effects of src homology 2 (SH2)B adaptor protein 1 (SH2B1) in myocardial infarction; however, involvement of SH2B1 in cerebral I/R injury and the underlying mechanisms remain to be investigated. In the present study, neural-like PC12 cells underwent 6 h of oxygen-glucose deprivation (OGD) followed by 24 h of reoxygenation (OGD/R). PC12 cells were pre-transfected with an adenovirus encoding for SH2B1 or GFP prior to exposure to OGD/R. Cell viability, LDH release and the apoptotic cascade were investigated. Reverse transcription-quantitative polymerase chain reaction and western blotting were employed to analyze mRNA and protein expression levels, respectively. The results of the present study revealed that OGD/R reduced SH2B1 expression in PC12 cells, accompanied by suppressed cell viability and enhanced cell death. Adenovirus-mediated SH2B1 overexpression, however, resulted in increased viability, reduced LDH release and a reduction in the expression levels of proteins associated with the apoptotic cascade in PC12 cells under the OGD/R condition. A mechanistic explanation may be that the positive effects of SH2B1 on neurons were in part derived from the activation of the JAK2/STAT3 signaling pathway.

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Furthermore, abolishment of JAK2/STAT3 signaling using a pharmacological inhibitor suppressed the inhibitory effects of SH2B1 under the OGD/R condition. The results of the present study suggested that SH2B1 may protect PC12 cells from OGD/R injury partially by the JAK2/STAT3-dependent inhibition of apoptosis and may provide a novel therapeutic target for the treatment of cerebral I/R injury.

Introduction

Ischemia/reperfusion (I/R) injury is an important factor in determining the resistance of the beneficial effects of revascularization on neurons in the treatment of ischemic stroke (1,2). Minimizing the severe outcomes of I/R is of clinical importance due to the effects associated with hemorrhagic transformation and disruption of the blood-brain barrier (3). A large body of evidence has demonstrated that apoptosis, with an increase in the apoptosis regulator Bcl-2 (Bcl-2)-associated-X (Bax)/Bcl-2 ratio and caspase-3, functions as an important part of the pathogenesis of cerebral I/R injury (4,5). The advantages of inhibiting apoptosis have been demonstrated; however, the precise mechanisms underlying these dynamic regulations in cerebral I/R injury require further investigation.

The src homology 2 (SH2) B adaptor protein 1 (SH2B1) belongs to the SH2 domain-containing adaptor protein family, and is a crucial metabolic mediator in the regulation of energy, insulin resistance and glucose homeostasis (6). Additionally, upon stimulation, SH2B1 exerts a series of pathophysiological functions in cellular proliferation, apoptosis, myogenesis, cardiac hypertrophy and myocardial infarction that are dependent on binding to particular protein tyrosine kinases (7-10), including both cytoplasmic tyrosine kinases, such as Janus kinases (JAK)1-3 and receptor tyrosine kinases against nerve growth factor, insulin and platelet-derived growth factor. Among such kinases, the activation of JAK2, as the first reported binding target of SH2B1, may be potentiated by SH2B1 to induce downstream effectors, including the signal transducer and activator of transcription 3 (STAT3) (7,11); however, the fundamental details of SH2B1 and the JAK2/STAT3 signaling pathway and corresponding regulation during cerebral I/R injury have not been determined.

The activity of the JAK2/STAT3 signaling pathway appears to exhibit different consequences in response to a

variety of pathological stresses, in particular, anti-apoptotic properties against I/R injury (12-14). Previous studies have indicated the importance of SH2B1 in promoting cardiac hypertrophy via the activation of the JAK2/STAT3 signaling pathway (7,11), which is supported by reports of pharmacological inhibition of JAK2 that negates cardiac aberration in SH2B1 transgenic mice (7,11). However, further investigation into whether the anti-apoptotic effects mediated by the JAK2/STAT3 signaling pathway via SH2B1 serves a role in neuroprotection in cerebral I/R injury is required. The present study demonstrated that SH2B1 overexpression attenuated neuronal apoptosis during 6 h of oxygen-glucose deprivation (OGD) followed by 24 h of reoxygenation (OGD/R) injury, which may be associated with the activation of the JAK2/STAT3 signaling pathway.

Materials and methods

PC12 cell culture. PC12 cells were obtained from the American Type Cell Culture Collection (ATCC; Manassas, VA, USA) and were maintained in Dulbecco's modified Eagle's medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) and antibiotics (100 U/ml penicillin and streptomycin) in a humidified incubator at 37°C with an atmosphere of 5% CO_2 and 95% air (15). Cells were supplied with fresh medium three times per week and were passaged at a 1:3 ratio twice per week.

Adenoviral vector establishment. Adenoviral vectors expressing SH2B1 (Ad-SH2B1) or green fluorescent protein (Ad-GFP) were synthesized and provided by Shanghai GeneChem, Inc. (Shanghai, China). Sequences were obtained from https://blast.ncbi.nlm.nih.gov/Blast.cgi (Accession no. 89817). In brief, the entire coding region of target gene SH2B1 was cloned into shuttle vector GV135 (Shanghai GeneChem, Inc.). The co-transfection of 293T cells (ATCC) with a SH2B1 recombinant shuttle vector and an auxiliary packaging plasmid (Microbix Biosystems Inc., Toronto, ON, Canada) was performed using an AdMax Adenovirus Packaging system (AdMax Local, Los Angeles, CA, USA) according to the manufacturer's protocol (16). By applying Cre/loxP enzymes, the recombinant adenovirus Ad-SH2B1 and the Ad-GFP negative control were obtained. Subsequently, adenoviruses were amplified, purified by an Adeno-X™ Virus Purification kit (BD Biosciences, Franklin Lakes, NJ, USA) according to the manufacturer's protocols, and routinely titrated to $1x10^{10}$ PFU/ml.

Adenoviral transduction and experimental design. For viral transfection, PC12 cells ($1x10^5$ cells per 24-well plate) were incubated with Ad-SH2B1 or Ad-GFP for 4 h at 37°C, prior to glucose deprivation as described below, at different multiplicities of infections as calculated via: Viral titer x viral volume/cell number (0, 10, 20, 40 and 80 MOI), and finally, 20 MOI was selected for the following experiments due to ~95% transfection efficiency with no significant effects on cell viability as determined by flow cytometry described below. After 4 h of incubation at 37°C, medium was removed, and PC12 cells were further incubated at 37°C with complete

medium (DMEM+FBS) for 48 h prior to OGD/R treatment (17). Each experiment was repeated in triplicate.

To determine the association between SH2B1 and OGD/R-induced neuronal impairment, PC12 cells were randomly allocated to four groups: i) Control group (untransfected, no OGD/R); ii) OGD/R group; iii) Ad-SH2B1 transfection plus OGD/R (Ad-SH2B1 + OGD/R); and iv) Ad-GFP transfection plus OGD/R (Ad-GFP + OGD/R). To further detect the underlying mechanisms of the anti-OGD/R effects conferred by SH2B1 in PC12 cells, an additional experiment was conducted in which Ad-SH2B1 or Ad-GFP was transduced into PC12 cells as aforementioned in the absence or presence of a JAK2 inhibitor (AG490, 10 μM, Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). These cells were then subjected to OGD/R treatment as described (17). The inhibitor was added into the medium 30 min at 37°C prior to the OGD procedure (17).

OGD/R establishment. To mimic the I/R procedure *in vitro*, oxygen deprivation was induced in an anaerobic chamber containing 95% N_2 and 5% CO_2 at 37°C, and glucose deprivation was concurrently induced by maintaining cells in a glucose-free Hanks' Balanced Salt Solution (Invitrogen; Thermo Fisher Scientific, Inc.) for 6 h at 37°C. Subsequently, PC12 cells from the OGD/R-treated groups were removed from the anoxia incubator and cultured under normal conditions as aforementioned to induce reoxygenation for an additional 24 h (17,18).

Evaluation of cell injury. Cell injury was assessed by measuring the content of lactate dehydrogenase (LDH) in the culture medium using an LDH diagnostic kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's protocol (17). LDH activity was then calculated using a microplate spectrophotometer (Shimadzu Corporation, Kyoto, Japan) at a wavelength of 440 nm. Alternations in absorbance were expressed as concentration units per liter (17).

Cell viability assay. Cell viability was measured using a Cell Counting kit-8 (CCK-8) assay according to the manufacturer's protocol (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) (15). Briefly, PC12 cells from all four groups were seeded into 96-well plates ($1x10^4$ cells/well) and were washed with PBS.CCK-8 solution (10 μl) was then added to each well for an additional 4 h at 37°C. The absorbance at 450 nm was determined with a microplate reader (Biotek Instruments, Inc., Winoski, VT, USA). Cell viability was expressed as a percentage of the control group cells.

Flow cytometric analysis of cell apoptosis. The apoptotic rate was detected by flow cytometry with an apoptosis detection kit (BD Pharmingen; BD Biosciences) (15). Briefly, following the corresponding interferences, cells were digested by 0.25% trypsin, collected from 24-well culture plates, washed twice with PBS and centrifuged at 20,000 x g at 4°C for 5 min. Then, PC12 cells from all four groups were incubated with 5 μl of 7-aminoactinomycin D (7-AAD) dye at room temperature for 15 min. Subsequently, 1 μl Annexin V-allophycocyanin (APC) was added into the cell suspension, and maintained

for 15 min in the dark. Finally, cells were analyzed by fluorescence-activated cell sorting via a flow cytometric analysis (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and CytExpert 1.0 software (Beckman Coulter, Inc., Brea, CA, USA). Cells that stained positively for Annexin V-APC or 7-AAD were considered as apoptotic cells.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). RT-qPCR was performed to detect mRNA expression as previously described (15,17,18). Briefly, total RNA from all four PC12 cells group was extracted and purified with TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.), and reverse-transcribed into cDNA with a commercial PrimeScript™ II 1st strand cDNA synthesis kit (Takara Bio, Inc., Otsu, Japan) according to the manufacturer's protocol. RT-qPCR was performed with an ABI Prism 7500 Sequence Detection system (Applied Biosystems; Thermo Fisher Scientific, Inc.) in the presence of primers and SYBR® Green Supermix (Bio-Rad Laboratories, Inc.). The following thermocycling conditions were used for the PCR: Initial denaturation at 50°C for 2 min; 40 cycles of denaturation at 95°C for 30 sec; annealing at 56°C for 30 sec; and extension at 72°C for 30 sec. The mRNA expression levels of targeted genes were normalized to the reference gene β-actin, and calculated using the comparative quantification method ($2^{-\Delta\Delta Cq}$) (19). The primer sequences used in the present study are listed as follows: SH2B1, forward 5'-CCCGTCTTAGCATTCCCTGT-3' and reverse, 5'-GGCTTAGGCACTCTTGGATGT-3'; β-actin, forward 5'-CACGATGGAGGGGCCGGACTCATC-3' and reverse, 5'-TAAAGACCTCTATGCCAACACAGT-3'.

Western blot analysis. Western blotting was performed to measure protein expression as previously described (15,17,18). In brief, PC12 cells of all four groups were lysed with ice-cold radioimmunoprecipitation assay buffer (Beyotime Institute of Biotechnology, Jiangsu, China), supplemented with 1 mmol/l PMFS, 1 µg/ml leupeptin and 1 µg/ml pepstatin as the protease inhibitors (Beyotime Institute of Biotechnology). Following centrifugation at 20,000 x g at 4°C for 5 min, total extracted protein was collected from the supernatant, and the protein concentration was measured with a commercial Bicinchoninic Acid protein assay kit (Beyotime Institute of Biotechnology). A total of 50 µg protein lysate was loaded in each lane and separated by 8-12% SDS-PAGE. Targeted proteins were then transferred onto nitrocellulose membranes (EMD Millipore, Billerica, MA, USA) via an electroblotting technique. Non-specific bindings were blocked with 5% non-fat milk dissolved in tris-buffered saline with 0.1% Tween-20 (TBST) for 2 h at room temperature. Following three washes with TBST, membranes were incubated with primary antibodies overnight at 4°C against SH2B1 (1:800; sc-136065; Santa Cruz Biotechnolgy, Inc., CA, USA), Bax (1:500; ab32503; Abcam, Cambridge, UK), Bcl-2 (1:600; cat. no. 2876S; Cell Signaling Technology, Inc., Danvers, MA, USA), cleaved caspase-3 (1:400; cat. no. 9661L; Cell Signaling Technology, Inc.), cleaved caspase-9 (1:500; cat. no. 9507S; Cell Signaling Technology, Inc.), total (t)-JAK2 (1:500; cat. no. 3230S; Cell Signaling Technology, Inc.), phosphorylated (p)-JAK2 (1:800; cat. no. 3776S; Cell Signaling Technology, Inc.), t-STAT3 (1:800 dilution; 4904P; Cell Signaling Technology, Inc.) and

p-STAT3 (1:600; cat. no. 9134S; Cell Signaling Technology, Inc.). GAPDH (1:1,000; ab181602; Abcam) was utilized as an internal reference control. Subsequently, the membranes washed three times with TBST and were incubated with a horseradish peroxidase-conjugated rabbit anti-rat IgG secondary antibody (1:1,000; BA1058; Wuhan Boster Biological Technology Ltd., Wuhan, China). The protein bands were visualized by an enhanced chemiluminescence reagent (Thermo Fisher Scientific, Inc.), and the signal intensities were calculated by BandScan 5.0 software (Glyko, Inc., Novato, CA, USA).

Statistical analysis. Data were presented as the mean ± standard deviation. All statistical analyses were performed using standard SPSS software (version 19.0, IBC Corp., Armonk, NY, USA). A Student's t-test was utilized for between-group comparisons. Multiple comparisons were conducted with one-way analysis of variance, followed by a Student-Newman-Keuls-q-test. P<0.05 was considered to indicate a statistically significant difference.

Results

OGD/R is associated with the downregulation of SH2B1 expression. To investigate the association between SH2B1 and cerebral I/R injury, mRNA and protein expression levels of SH2B1 were detected in PC12 cells following OGD/R. Compared with the control group, SH2B1 expression at both the mRNA and protein levels was significantly suppressed in OGD/R-treated untransfected PC12 cells (P<0.05; Fig. 1). Ad-SH2B1 transfection significantly increased SH2B1 expression (Ad-SH2B1 + OGD/R group vs. OGD/R group; P<0.05), indicating efficient transduction of the adenovirus. In addition, SH2B1 expression was not significant altered between the Ad-GFP + OGD/R and OGD/R groups (P>0.05).

Upregulation of SH2B1 increases cell viability and reduces LDH release. To determine whether neuronal SH2B1 upregulation alleviated OGD/R-induced injury, cell viability and LDH (necrotic biomarker) release in PC12 cells were measured by CCK-8 and ELISA, respectively. Compared with the control group, OGD/R treatment induced neuronal cell death as observed by the decrease in cell viability (Fig. 2A); however, LDH activity was increased (P<0.05; Fig. 2B). Conversely, these effects were significantly reversed by SH2B1 upregulation under the OGD/R condition (Ad-SH2B1 + OGD/R group vs. OGD/R group; P<0.05). The aforementioned parameters demonstrated no significant differences between the OGD/R and Ad-GFP + OGD/R groups (P>0.05). These data indicated that SH2B1 may serve neuroprotective roles in cerebral I/R injury.

Upregulation of SH2B1 reduces OGD/R-induced apoptosis. To investigate the potential mechanisms of the neuroprotective effects of SH2B1 in OGD/R, the apoptotic rate and apoptotic mediators, including Bax, Bcl-2 and cleaved caspase-9/3 were detected by flow cytometry and western blotting, respectively. In line with the flow cytometry results, the expression levels of Bax and cleaved caspase-9/3 revealed similar pattern of alternations. As presented in Fig. 3, OGD/R-induced untransfected
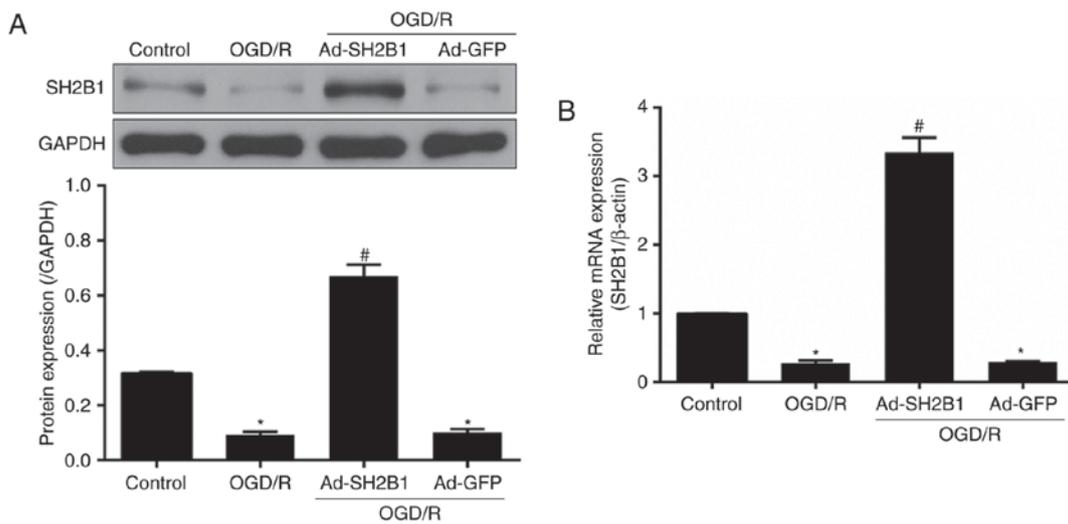
Figure 1. OGD/R is associated with the downregulation of SH2B1 expression. (A) Protein level of SH2B1 was detected by western blotting and quantification of the representative bands was normalized to GADPH. (B) mRNA expression levels of SH2B1 were measured by reverse transcription-quantitative polymerase chain reaction analysis. Data are presented as the mean ± standard deviation. n=3 for each condition. *$P<0.05$ vs. control group; #$P<0.05$ vs. OGD/R group. Ad, adenovirus vector; GFP, green fluorescent protein; OGD/R, oxygen-glucose deprivation and reoxygenation; SH2B1, src homology 2 B adaptor protein 1.
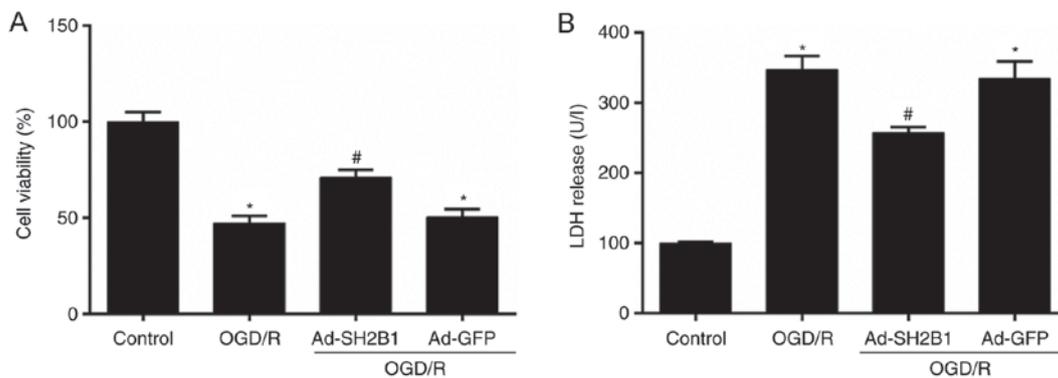

Figure 2. Upregulation of SH2B1 promotes cell viability and suppresses LDH release. (A) Cell viability and (B) LDH release into the culture medium were assessed by Cell Counting Kit-8 and ELISA, respectively. Data are presented as the mean ± standard deviation. The experimental procedure was performed independently three times. *$P<0.05$ vs. control group; #$P<0.05$ vs. OGD/R group. Ad, adenovirus vector; GFP, green fluorescent protein; OGD/R, oxygen-glucose deprivation and reoxygenation; SH2B1, src homology 2 B adaptor protein 1.

PC12 cells demonstrated an aggravated apoptotic rate, and upregulated Bax and cleaved caspase-9/3 expression levels; however, Bcl-2 anti-apoptotic effector expression levels were lower compared with in the control group (P<0.05). When OGD/R-inducted cells were pre-treated with Ad-SH2B1, the aforementioned parameters were significantly inversed (Ad-SH2B1 + OGD/R group vs. OGD/R group, P<0.05). Additionally, no significant differences were observed between the Ad-GFP + OGD/R and OGD/R groups (P>0.05). These results suggested that the effects of SH2B1 on OGD/R-induced PC12 cells may be associated with the deactivation of the apoptotic cascade.

Upregulation of SH2B1 activates the JAK2/STAT3 signaling pathway in OGD/R injury. The JAK2/STAT3 signaling pathway was extensively investigated as a major anti-apoptotic mediator in OGD/R injury (12,13). To investigate whether the JAK2-STAT3 axis was involved in the favorable effects of SH2B1 on OGD/R, the expression levels of JAK2 and STAT3

in PC12 cells were analyzed. As presented in Fig. 4, following OGD/R induction, protein expression levels of p-JAK2 and p-STAT3 were significantly reduced (OGD/R group vs. control group, P<0.05); however, Ad-SH2B1 delivery into OGD/R-treated PC12 cells significantly increased p-JAK2 and p-STAT3 expression levels compared with in the OGD/R group (P<0.05). Ad-GFP transfection resulted in no significant alterations in the expression levels of p-JAK2 and p-STAT3 (Ad-GFP + OGD/R group vs. OGD/R group, P>0.05). In addition, t-JAK2 and t-STAT3 revealed no significant differences in expression levels among the four groups (P>0.05).

Inhibitory roles of SH2B1 in OGD/R are dependent on the JAK2/STAT3 signaling pathway. To further examine whether the inhibitor effects of SH2B1 on OGD/R injury were dependent upon the activation of the JAK2/STAT3 signaling pathway, virus-transfected and OGD/R-treated PC12 cells were exposed to the JAK2 inhibitor AG490. As presented in Fig. 5, the expression levels of p-JAK2/STAT3 (Fig. 5A), cell
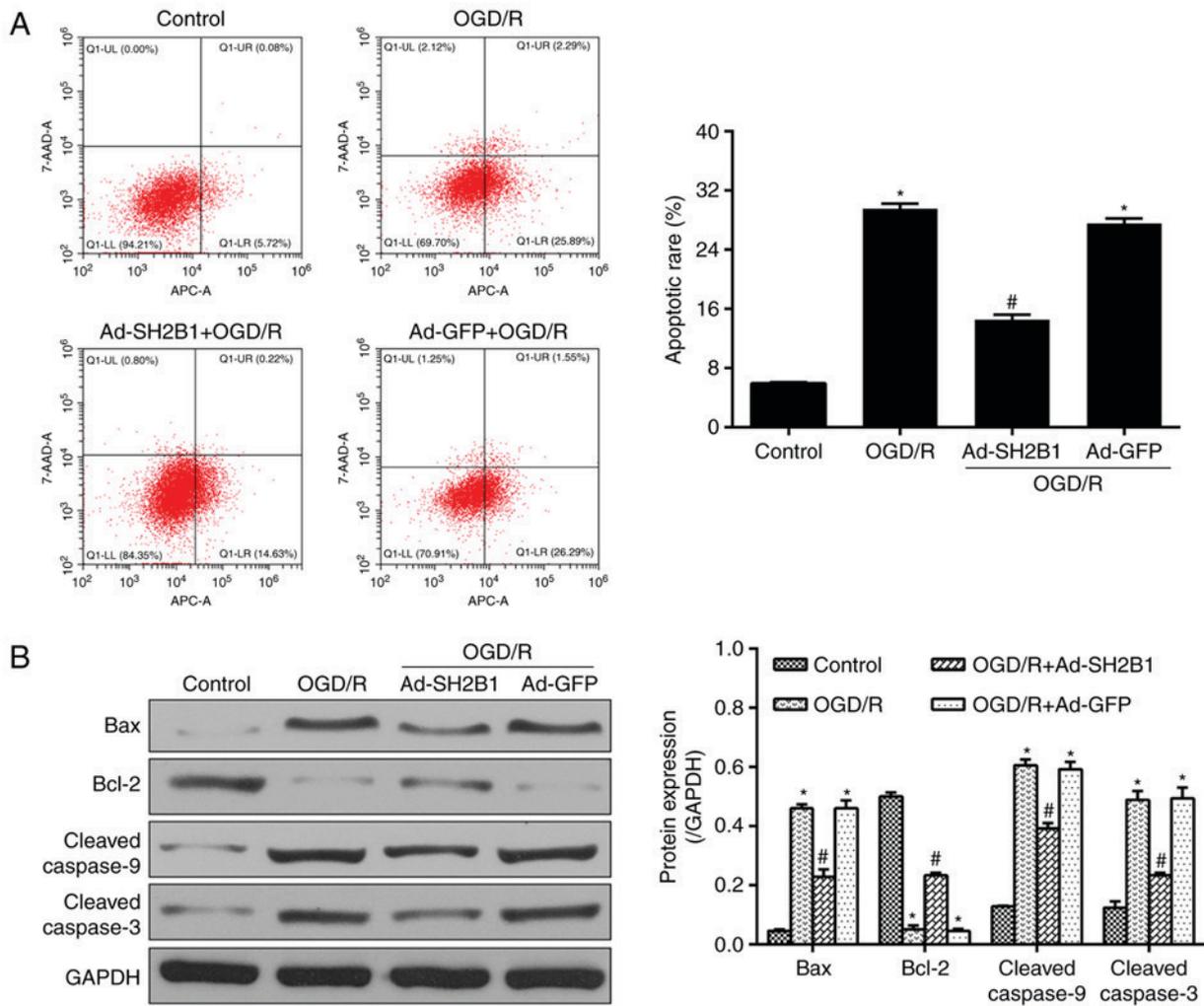
Figure 3. Upregulation of SH2B1 abolishes OGD/R-induced apoptosis. (A) Flow cytometry analysis and quantitative data for apoptotic rates in PC12 cells following OgD/R. (B) Representative protein bands and quantified evaluation of apoptosis-associated mediators including Bax, Bcl-2 and cleaved caspase-9/3. Data are presented as the mean ± standard deviation. n=3 per group. *P<0.05 vs. control group; #P<0.05 vs. OGD/R group. 7-AAD, 7-aminoactinomycin D; Ad, adenovirus vector; Bcl-2, B-cell lymphoma-2; Bax, Bcl-2-associated-X; GFP, green fluorescent protein; OGD/R, oxygen-glucose deprivation and reoxygenation; SH2B1, src homology 2 B adaptor protein 1; Ad-SH2B1 + OGD/R, Ad-SH2B1 transfection plus OGD/R; Ad-GFP + OGD/R, Ad-GFP transfection plus OGD/R.
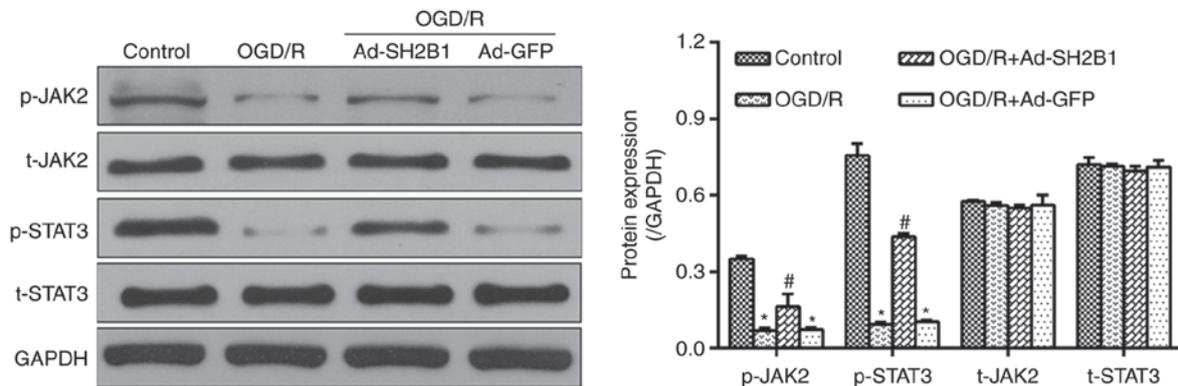

Figure 4. Upregulation of SH2B1 activates the JAK2/STAT3 signaling pathway in OGD/R injury. Representative western blotting bands for p-JAK2, JAK2, p-STAT3 and STAT3 were observed by western blot analysis. The histogram demonstrates the statistical measurements for the relative intensities normalized to GADPH. Data are presented as the mean ± standard deviation of three independent experiments. *P<0.05 vs. control; #P<0.05 vs. OGD/R. Ad, adenovirus vector; GFP, green fluorescent protein; JAK2, Janus kinase 2; OGD/R, oxygen-glucose deprivation and reoxygenation; p, phosphorylated; SH2B1, src homology 2 B adaptor protein 1; STAT3, signal transducer and activator of transcription 3; t, total.

viability and LDH activity (Fig. 5B), as well as the apoptotic rate (Fig. 5C) were significantly different between the Ad-GFP and Ad-SH2B1 groups under the OGD/R + PBS condition; however, significantly differences were also observed
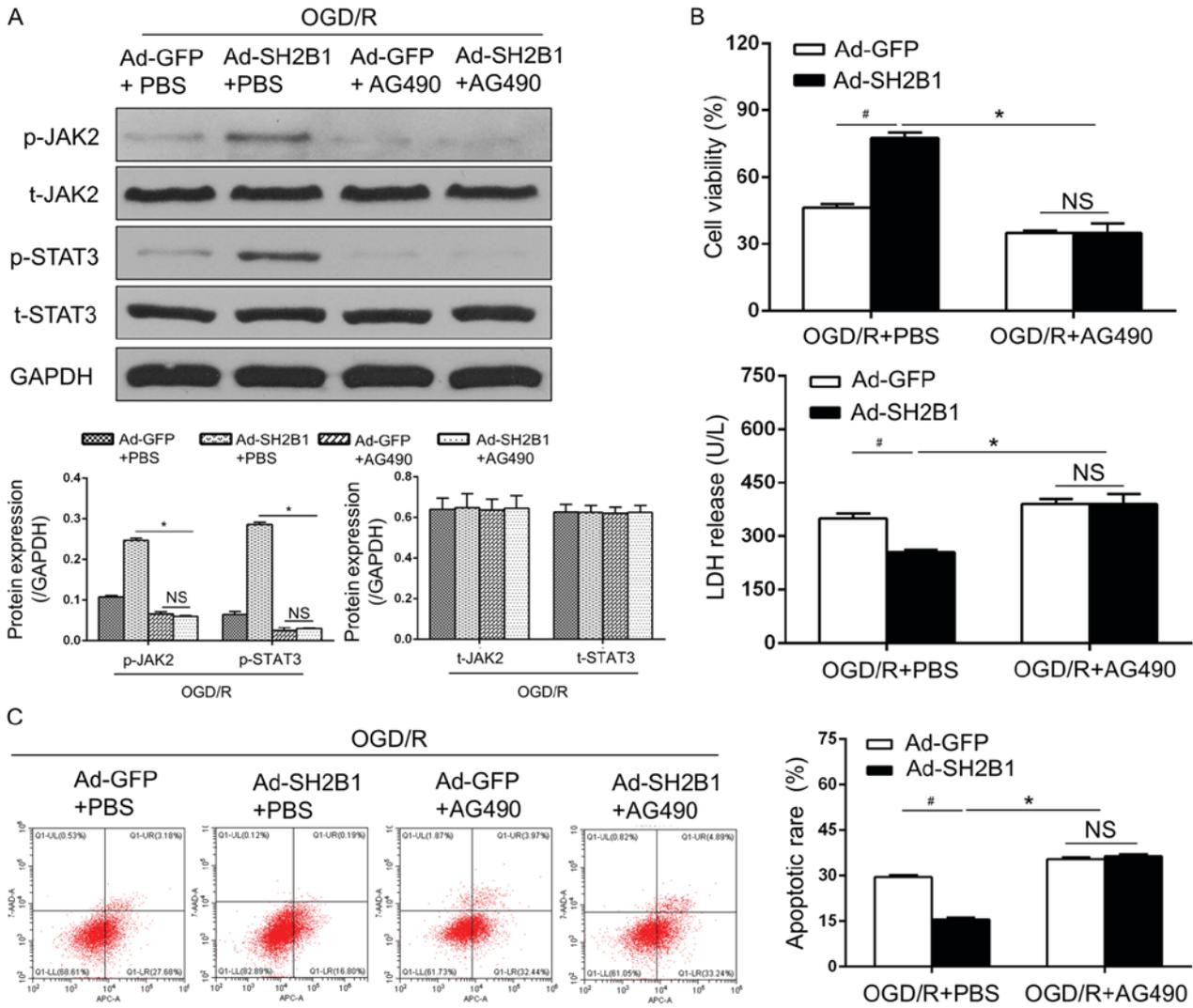
Figure 5. Inhibitory roles of SH2B1 against OGD/R are dependent on the JAK2/STAT3 signaling pathway. (A) Protein expression levels of JAK2/STAT3 signaling pathway-associated proteins were measured by western blot analysis. (B) Cell Counting Kit-8 assay was used to evaluate cell viability, and LDH release into the culture medium was detected by ELISA. (C) Flow cytometry images and quantification of apoptosis in each group. Data from three independent experiments are presented as the mean ± standard deviation. #P<0.05 vs. OGD/R + Ad-GFP + PBS group. *P<0.05 vs. OGD/R + Ad-SH2B1 + PBS group. 7-AAD, 7-aminoactinomycin D; Ad, adenovirus vector; APC, allophycocyanin; GFP, green fluorescent protein; JAK2, Janus kinase 2; AG490, JAK2 inhibitor; NS, no significance; OGD/R, oxygen-glucose deprivation and reoxygenation; p, phosphorylated; SH2B1, src homology 2 B adaptor protein 1; STAT3, signal transducer and activator of transcription 3; t, total.

between the Ad-SH2B1 + OGD/R + PBS group compared with the presence of AG490. Treatment with AG490, however, abrogated the effects of SH2B1 overexpression on p-JAK2/STAT3 expression levels, viability and cell death relative to OGD/R + PBS + Ad-SH2B1-treated PC12 cells (P<0.05). Overall, these data demonstrated that the protective effects of SH2B1 against OGD/R may be largely dependent upon the activation of the JAK2/STAT3 signaling pathway and the suppression of apoptosis.

Discussion

The present study provided evidence that selective overexpression of SH2B1 may exhibit anti-I/R effects, as it attenuated neuronal apoptosis *in vitro*. This conclusion was upheld with a series of novel experiments: Ad-SH2B1 transfection followed by OGD/R resulted in SH2B1 overexpression in neural-like PC12 cells; SH2B1 overexpression prior to the OGD/R

procedure significantly enhanced cell viability but repressed LDH release, which accompanied by a reduction in the expression of apoptosis-associated cascade. SH2B1 overexpression activated the JAK2/STAT3 survival pathways, while inhibition of the JAK2/STAT3 axis reversed neuron-protective roles of SH2B1 during OGD/R. To the best of the authors knowledge, the present study is the first to report the association between the anti-I/R effects of SH2B1 on apoptosis and the JAK2/STAT3 signaling pathway.

Timely and successful revascularization serves as the cornerstone for the treatment of ischemic stroke (1,2). Additionally, ischemic brain tissue is susceptible to secondary reperfusion injury that minimizes benefits of blood re-establishment itself (1,2). Considerable advances in the investigation of the molecular mechanisms of cerebral I/R injury have been made; however, efficient treatment methods to abolish the detrimental outcomes or cerebral I/R are required (1,2). Numerous studies have verified that the

activities of the apoptotic cascade, which induces particular mediators, including Bax, Bcl-2 and cleaved caspase-3, serve as the central etiology in the pathogenesis of cerebral I/R injury (4,12). Therefore, more extensive studies focusing on the interventions of prolonged apoptosis suggest that the repression of apoptosis may be a promising way to reduce the severity of I/R injuries (4,12); however, at present, there are limitations in reducing I/R-associated impairments (1-4). Consistently, the results of the present study revealed that PC12 cells subjected to SH2B1 overexpression and OGD/R were less vulnerable to apoptosis, as manifested by reduced expression levels of pro-apoptotic markers, including Bax and cleaved caspase-3, and an increase in the anti-apoptotic marker Bcl-2. In parallel, the apoptotic index and LDH release (necrotic marker) were reduced, and cell viability was improved following Ad-SH2B1 transduction. Thus, the findings of the present study provide compelling insights into the beneficial effects exerted by SH2B1 on neuronal cells in I/R injury.

As the pivotal cell survival signaling pathway in cerebral I/R prevention, the JAK2/STAT3 signaling pathway emerges as a powerful effector that resists cell death and apoptosis (12-14). Induction of JAK2 and STAT3 are primarily correlated with its protein expression and phosphorylation activities. Therefore, regulating the JAK2/STAT3 signaling pathway may facilitate the understanding for the management of cerebral I/R (12,13); however, whether a JAK2/STAT3-mediated mechanism is involved in the anti-apoptotic effects of SH2B1 remain to be determined in cerebral I/R. In the present study, p-JAK2 and p-STAT3 expression levels were profoundly increased following SH2B1 overexpression under the OGD/R condition. Furthermore, abolishment of the JAK2/STAT3 signaling pathway by pharmacological inhibitor, AG490, contributed to apoptosis and OGD/R injury in the presence or absence of SH2B1 overexpression in the present study. These data suggested that the beneficial neurological effects of SH2B1 against I/R may be particularly dependent on the JAK2/STAT3 signaling pathway and associated apoptosis-inhibiting mechanisms.

SH2B1, known as an adapter protein, exerts its versatile biological potencies primarily by binding to kinases, including JAK2 (6-9). Emerging data revealed that SH2B1 shares many critical roles in regulating the migration, apoptosis and proliferation of cancer cells (20). At present, the involvement of SH2B1 in cardiovascular insults have also drawn significant attention in cardiac hypertrophy and myocardial infarction (7,10). Recent reports from Wu *et al* (7) and others (10) have systematically demonstrated that SH2B1 directly binds to JAK2 in cardiomyocytes, thus aggravating hypertrophy in a JAK2/STAT3-dependent manner. Providing that the JAK2/STAT3 axis contributes outstanding functions in the attenuation of cerebral I/R injury (12-14), it appears to be reasonable to propose that SH2B1 may participate in cerebral I/R by activating the JAK2/STAT3 signaling pathway. Additionally, the activation of the SH2B1-JAK2-STAT3 axis exclusively alleviated OGD/R injury in PC12 cells as suggested by the data of the present study. Furthermore, compared with the diverse roles of SH2B1 in cerebral I/R and cardiac hypertrophy, SH2B1 may confer pleiotropic roles, potentially in a context-specific manner; however, further investigations are required.

SH2B1 couples upstream stimulators of tyrosine kinases with downstream effectors by creating multi-protein complexes, thereby regulating the catalytic actions of bound enzymes that exhibit a stimulus-specific pattern (7-10). For instance, SH2B1 overexpression appears to be sufficient to induce the formation of the SH2B1/JAK2 complex thereby elevating protein kinase B (AKT) activities in pancreatic b-cells *in vivo* and *in vitro* in diabetic models (9). Furthermore, the dual functionalities of SH2B1 constituting the aggravation or inhibition of pathological events have been verified recently; Blandino-Rosano *et al* (21) identified that upregulation of the SH2B1/JAK2 complex is responsible for a positive feedback mechanism in inducing pancreatic β-cell survival. Another study confirmed that SH2B1 enhances insulin and leptin signaling by activating phosphoinositide 3-kinase-AKT/mitogen-activated kinases and JAK2, respectively (22). Chen *et al* (20) indicated that re-establishment of SH2B1 reverses the impeding roles of microRNA-326-3p on proliferation and metastasis potentially via the activation of the JAK2/Ras-related C3 botulinum toxin substrate 1 signaling pathway. Furthermore, evidence of the positive associations between SH2B1 and cardiac remodeling have also demonstrated these similarities (7,11). Conversely, other investigations have affirmed that global deletion of SH2B1 leads to severe obesity and glucose intolerance, while restoration of SH2B1 may correct metabolic disorders in mice (8). In support of these findings, the present study directly rendered the notion that SH2B1 overexpression via adenoviral vectors was effective in protecting PC12 cells against OGD/R injury. Therefore, the aforementioned discrepancies may harbor deeper insights and increased complexity of the SH2B1-associated mechanism involved in variety of pathological conditions. A recent study revealed another epigenetic mechanism of SH2B1 on progressing myogenesis by erasing histone H3 lysine 9 (H3K9) trimethylation (me3) and inducing H3K4me3 on the promoters/enhancers of corresponding genes (23). Providing the association between SH2B1 and epigenetic modification (microRNA or histone methylation) and their participations in I/R injury, further investigation is required to improve understanding of the potential regulatory networks associated with SH2B1 during the pathogenesis of cerebral I/R injury.

In conclusion, the present study revealed that SH2B1 is an intrinsic positive mediator for I/R-induced neuronal apoptosis and that the SH2B1-JAK2-STAT3 axis may be considered as a compelling therapeutic target for the prevention of cerebral I/R. Notably, further studies are required to examine whether SHB21 directly affects other signaling pathways against cerebral I/R injury apart from the JAK2/STAT3 pathway.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

JY and LZ made substantial contributions to the conception and design of the present study. YS and NW performed the experiments including cell culture, adenoviral transfection, OGD/R establishment, apoptotic detection and western blotting assay. QS, ZC and YW performed data interpretation and statistical analysis, and were involved in drafting the manuscript or revising it critically for important intellectual content. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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