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S100A12 promotes inflammation and apoptosis in ischemia/reperfusion injury via ERK signaling *in vitro* study using PC12 cells

Xiang Zhang | Rui Shen | Zhongwen Shu | Quanbin Zhang | Zuoquan Chen

Department of Neurosurgery, Shanghai Tenth People's Hospital, Tongji University, Shanghai, China

Abbreviations:
ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain; BBB, blood-brain barrier; CAAF1s, calcium-binding protein in amniotic fluid-1; EN-RAGE, extracellular newly identified receptor for advanced glycation end products; I/R, ischemia/reperfusion; NLRP3, NOD-like receptor family, pyrin domain-containing 3; OGD/R, oxygen-glucose deprivation and reperfusion

Correspondence
Quanbin Zhang, MD and Zuoquan Chen, MM, Shanghai Tenth People's Hospital, No. 301 Yanchang Road, Shanghai 200072, China.
Email: zqblab@163.com and czqlab@163.com

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S100A12 is a member of S100 calcium-binding proteins with effect to promote inflammation in brain damage and stroke. However, the role of S100A12 in ischemia/reperfusion (I/R) remains to be clarified. This study aimed to explore the effect of S100A12 on I/R and discover the possible mechanism. Oxygen-glucose deprivation and reperfusion (OGD/R) was used to induce I/R injury model *in vitro*. Knockdown or overexpression of S100A12 was utilized to explore the role of S100A12 in I/R-induced inflammation and apoptosis. Results indicated that S100A12 expression was dramatically upregulated after OGD/R. Knockdown of S100A12 inhibited, while overexpression of S100A12 enhanced, the activation of ERK1/2 protein. OGD/R also triggered the occurrence of inflammation and oxidative stress, while these effects were blunted by S100A12 silencing and aggravated by S100A12 overexpression, and the presence of MAP kinase signaling system (ERK) inhibitor MK-8353 counteracted the effect of S100A12 overexpression. Besides, S100A12 silencing abolished, while its overexpression restored, the OGD/R-induced increased apoptosis rate and pro-apoptotic proteins expression. Similarly, ERK inhibitor MK-8353 reversed the effects of S100A12 overexpression. In conclusion, S100A12 promoted OGD/R-induced inflammation, oxidative stress and apoptosis via activation of ERK signaling *in vitro*.

KEYWORDS
apoptosis, calcium-binding protein, inflammation, MAP kinase signaling system, reperfusion injury

INTRODUCTION

Stroke, especially cerebral ischemia, is a destructive cerebrovascular accident that has become the major cause of mortality and morbidity around the world.[1] Strokes most commonly originate from ischemia, which is caused by vessel occlusion in the cerebral circulation. Ischemic stroke can be reversed by removing the obstruction and restoring blood flow if diagnosed in a timely manner.[2] However, reperfusion, but not ischemia itself, may result in additional, substantial brain damage called 'ischemia/reperfusion (I/R) injury'.[3] Until now, massive efforts have been devoted to find neuroprotective agents that target pathological mechanisms of I/R injury, including inflammation, apoptosis, and

blood-brain barrier (BBB) disruption, and considerable achievements have been reported in both *in vitro* and *in vivo* models of cerebral I/R injury.[4,5] Nevertheless, due to the limitations of these candidates, safe and effective therapeutic agents for cerebral I/R still need to be developed.[6]

It is well-established that multiple pathological processes are implicated in I/R injury.[3,7,8] Reactive oxygen species (ROS) are widely believed to be the major cause of reperfusion-induced damage to microvessels and parenchymal organs in ischemic tissue. After the restoration of blood supply in ischemic tissue, excessive free radicals will attack the cells of this tissue and cause damage, namely 'tissue ischemia/reperfusion injury'.[9] I/R is also associated with a pathological activation of the immunity and inflammation. Tissue ischemia can result in toll-like receptors (TLR)-dependent induction of proinflammatory cytokines including tumor necrosis factor α (TNFα), interleukin (IL)-1β and IL-18.[10] TLR expression can be increased by ROS, and production of the anti-inflammatory cytokine IL-10 will contribute to decreased levels of TNFα and ROS.[11] In addition, I/R leads to the activation of cell death programs including apoptosis, autophagy and necrosis. Increasing evidence has demonstrated the existence of excessive proinflammatory factors, proapoptotic proteins and oxidative stress products in cerebral I/R injury.[5,8,12] Therefore, targeting inflammation and apoptosis could be a prospective therapy for treating cerebral I/R injury.

The S100A12, also named calcium-binding protein in amniotic fluid-1 (CAAF1s), calgranulin C or extracellular newly identified receptor for advanced glycation end products (EN-RAGE), is a member of the S100 proteins, which are small, acidic, calcium-binding proteins with 'EF-hand type' conformation and comprise more than 20 low-molecular-weight protein members. It is well-known that RAGE can promote inflammation by activation of non-glycation products. As a ligand of RAGE, the S100 family was found to be involved in inflammatory responses, in which S100A8, S100A9 and S100A12 have been confirmed to play an important role in aggravating inflammation.[13] Recently, it has been reported that S100A12 treatment significantly increased the levels of inflammasome NOD-like receptor family, pyrin domain-containing 3 (NLRP3), apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), caspase-1, and proinflammatory cytokines IL-1β, IL-18.[14] Besides, serum S100A12 was indicated to be a prognostic biomarker of severe traumatic brain injury.[15] Also, in acute ischemic stroke, high plasma S100A12 levels are associated with a poor functional outcome.[16] The preceding data suggested the potential prognostic role of S100A2 in I/R. However, whether S100A2 could contribute to the development of cerebral I/R injury and the underlying mechanism remains to be elucidated.

Furthermore, the RAS–RAF–MEK–ERK pathway (ERK signaling) is an evolutionary conserved signaling cascade that transmits signals from cell surface receptors to promote cell proliferation and survival.[17] It has been confirmed that the ERK pathway is associated with inflammation, and blocking it could inhibit release of inflammatory cytokines.[17,18] Meanwhile, some studies have shown that S100A12-induced damage was mediated by the activation of ERK1/2 signaling, and blocking the ERK signaling could inhibit S100A12-induced apoptosis and inflammatory response.[19–21]

PC12 cells, which derived from rat adrenal pheochromocytoma, can differentiate into neuron phenotype cells under the treatment of nerve growth factor (NGF), thereby exerting characteristics of neuronal cells in morphology, physiology and biochemistry.[22] Therefore, PC12 has been a commonly used cell model for studying neurological diseases. Taken together, the purpose of this study was to investigate the role of S100A12 in I/R injury and determine whether the effect of S100A12 is dependent on ERK signaling in PC12 cells.

MATERIALS AND METHODS

Regents and antibodies

The ERK inhibitor MK-8353 (SCH900353) was purchased from Selleck (Houston, TX, USA), Dulbecco's modified eagle medium (DMEM) and fetal bovine serum (FBS) were obtained from Gibco (Grand Island, NY, USA). S100A12 knockdown and overexpression vectors were constructed by Genscript Biotechnology (Nanjing, China). Enzyme-linked immunosorbent assay kits were obtained from Abcam Biotechnology (Cambridge, UK). Reactive oxygen species (ROS), lipid peroxidation (MDA), lactate dehydrogenase (LDH) and superoxide dismutase (SOD) Activity Assay kit were purchased from Nanjing Jiancheng Bioengineering (Nanjing, China). The primary antibodies were as follows: anti-ERK1, anti-ERK2, anti-p-ERK1/2, anti-NLRP3, anti-ASC, anti-caspase1, anti-eNOS, anti-p-eNOS, anti-Bcl-2, anti-Bax, anti-cleaved caspase3, anti-cleaved caspase9 and anti-GAPDH were obtained from Santa Cruz Biotechnology (Dallas, TX, USA).

Cell culture

PC12 cells were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA) and maintained in DMEM medium supplemented with 10% FBS and 1% antibiotics, in a 5% CO_2 humidified atmosphere at 37°C. After treatment with 100 ng/mL nerve growth factor (NGF) for about 5 days, PC12 cells that had differentiated were utilized for the next experiments (Fig. S1).

Oxygen-glucose deprivation and reperfusion (OGD/R)

For simulation of an *in vitro* cerebral ischemia/reperfusion injury model, the OGD/R process was performed based on the method described previously.[23] Briefly, PC12 cells were cultured in a deoxygenated glucose-free Hanks' balanced salt solution inside an anaerobic chamber, which contains 5% CO_2 and 95% N_2, for 2 h. Then cells were removed from the anaerobic environment and transferred to normal conditions for 12 h. Cells that were not exposed to OGD and incubated under normal conditions were used as control groups.

Cell transfection

Recombinant S100A12 shRNA (target sequence: CCGGGTCGACTTTCAAGAATT-CATACTCGAGTATGAATTC TTGAAAGTCGACTTTTTG) or full-length S100A12 cDNA was cloned into the pcDNA3 vector, and the empty vector was used as a negative control. PC12 cells were seeded in 24-well plates and cultured to 70–80% density, then transfected with indicated expressing vectors using Lipofectamine 2000 (Invitrogen, Waltham, MA, USA) according to the manufacturer's instruction.

Western blot analysis

For total proteins extraction, PC12 were washed twice with PBS and lysed using RIPA buffer (Beyotime, Shanghai, China). After centrifugation and quantification, each sample was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred onto polyvinylidene fluoride (PVDF) membranes (Bio-Rad, Hercules, CA, USA). After being blocked using nonfat milk at room temperature for 1 h, the membranes were incubated with primary antibodies against ERK1, ERK2, p-ERK1, p-ERK2, NLRP3, ASC, caspase1, eNOS, p-eNOS, Bcl-2, Bax, cleaved-caspase3 and cleaved-caspase9 at 4ºC overnight. Anti-GAPDH antibody was selected as internal reference. Next, the membranes were washed with Tris-buffered saline and incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies at room temperature for 2 h. Finally, the membranes were visualized by the ECL (electrochemiluminescence) system (Amersham, Chicago, IL, USA).

Real-time quantitative polymerase chain reaction (RT-qPCR)

For detection of the messenger RNA (mRNA) level, total RNA of PC12 cells was extracted using TRIzol reagent (Invitrogen). Then agarose gel electrophoresis and ultraviolet spectrophotometry were applied to determine RNA concentration and purity. Total RNA was converted to cDNA using the PrimeScript™ RT reagent kit with gDNA Eraser (Takara Biotechnology, Beijing, China). RT-qPCR was performed with SYBR Green (Invitrogen) in a Bio-Rad real-time PCR detection system. Primers were as follows: S100A12, forward 5'-ATTCCTGTGCATTGAGGGGTTA-3', reverse 5'-TGTCAAAATGCCCCTTCCGA-3'.

Detection of inflammatory and oxidative markers

The levels of proinflammatory cytokines TNF-α, IL-1β, IL-18 and IL-10 in the culture medium of PC12 cells were detected using ELISA kit following the manufacturer's instructions. Cellular ROS assay kit, MDA Assay kit, LDH and SOD

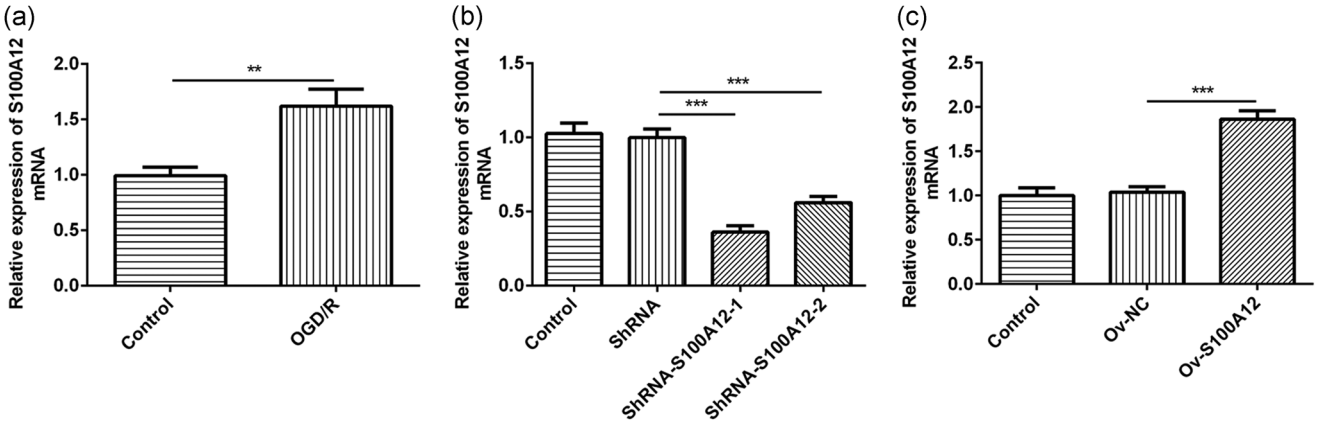

Figure 1 S100A12 messenger RNA (mRNA) was upregulated after oxygen-glucose deprivation and reperfusion (OGD/R). (**a**) Relative mRNA expression of S100A12 in control and OGD/R-treated PC12 cells (n = 3). $^{**}P < 0.01$ versus control. (**b**) Relative mRNA expression of S100A12 before and after S100A12 was knockdown (n = 3). short hairpin RNA (ShRNA), shRNA-S100A12-1 and shRNA-S100A12-2 indicate the cells were transfected with empty plasmids, shRNA-S100A12-1 and shRNA-S100A12-2, respectively. $^{***}P < 0.001$ versus shRNA group. (**c**) Relative mRNA expression of S100A12 before and after S100A12 was overexpressed (n = 3). Ov-NC means overexpression negative control. Ov-S100A12 means overexpression of S100A12. $^{***}P < 0.001$ versus Ov-NC group.

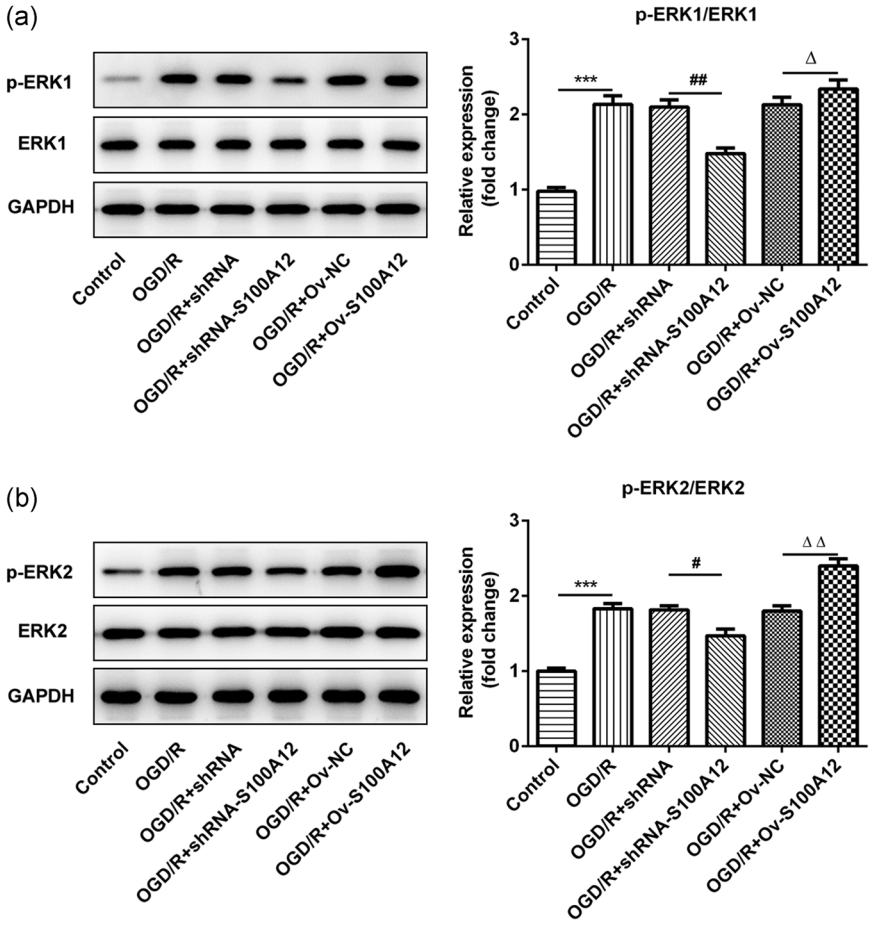

Figure 2 Effects of S100A12 on ERK1/2 protein expression. (**a, b**) Representative immunoblot analysis and quantification for p-ERK1/ERK1 and p-ERK2/ERK2 in different groups (n = 3). Oxygen-glucose deprivation and reperfusion (OGD/R) means oxygen-glucose deprivation and reperfusion. Ov means overexpression. $^{***}P < 0.001$ versus control. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ versus OGD/R + shRNA. $^{\Delta}P < 0.05$ and $^{\Delta\Delta}P < 0.01$ versus OGD/R + Ov-NC.

Activity Assay were used to determine the generation of ROS, MDA, LDH and SOD.

Flow cytometry

Cell apoptosis was assessed by flow cytometry using propidium iodide (PI) staining. Briefly, cells were gently washed twice with PBS, digested with 0.25% trypsin, and centrifuged at 200*g* for 5 min. After gently resuspension of the cell pellet with 1 mL NaCl/Pi, cells were incubated in PI for 15 min in a darkroom and immediately analyzed by flow cytometer (Becton Dickinson, USA). Data were analyzed by flow cytometry software.

Statistical analysis

All results were expressed as mean ± standard deviation, the statistical analyses were performed using the GraphPad Prism 6 (La Jolla, CA, USA). Differences between the data were tested using Student's *t*-test and one-way analysis of variance. $P < 0.05$ was considered statistically significant.

RESULTS

S100A12 was upregulated after cerebral I/R in PC12 cells

To determine whether S100A12 was involved in cerebral I/R, the mRNA level of S100A12 in PC12 cells was detected first. As shown in Fig. 1a, expression of S100A12 mRNA was significantly upregulated in the OGD/R group. The mRNA alteration of S100A12 after OGD/R treatment indicated a role of it in cerebral I/R injury. Next, to confirm the detailed functions of S100A12 in cerebral I/R injury, we constructed two short hairpin RNA (shRNA) expression vectors, shRNA-S100A12-1 and shRNA-S100A12-2, to knockdown the expression of S100A12. Results showed a better knockdown effect of shRNA-S100A12-1, so we chose shRNA-S100A12-1 for the following study (Fig. 1b). One

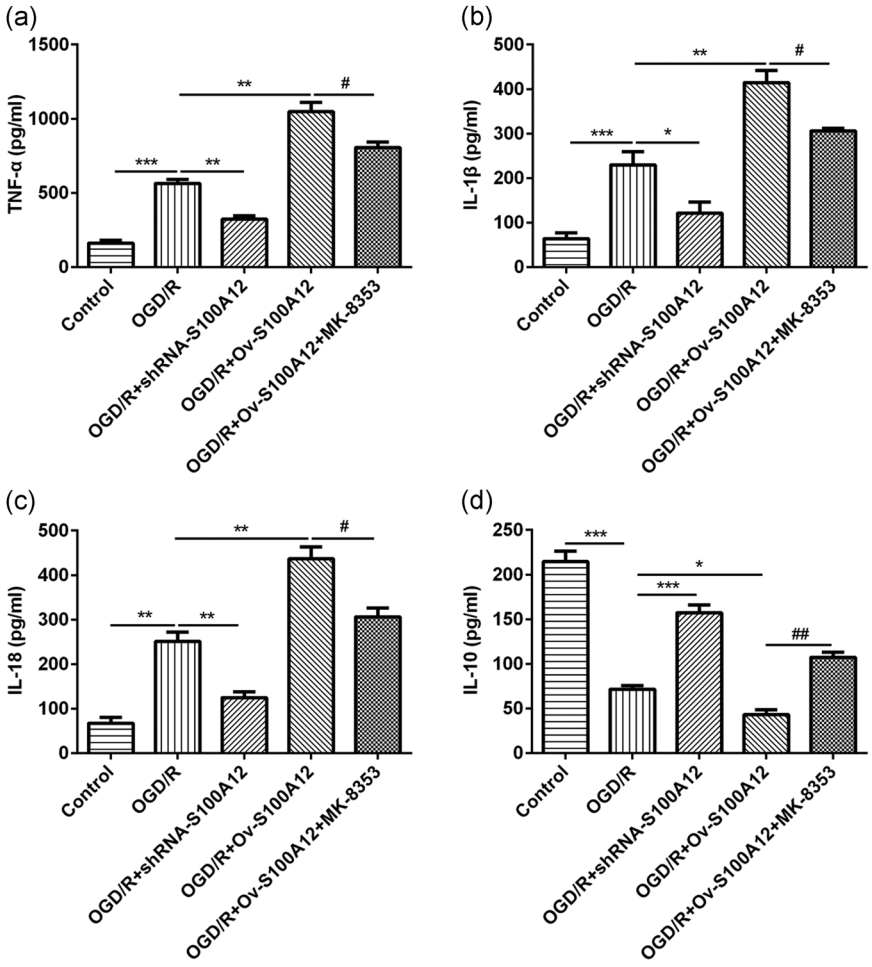

Figure 3 Effects of S100A12 on generation of inflammatory cytokines. The activities of tumor necrosis factor α (TNF-α) (**a**), interleukin (IL)-1β (**b**), IL-18 (**c**) and IL-10 (**d**) in different groups (n = 3). Oxygen-glucose deprivation and reperfusion (OGD/R) means oxygen-glucose deprivation and reperfusion. Ov means overexpression. OGD/R means oxygen-glucose deprivation and reperfusion. Ov means overexpression. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ versus OGD/R. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ versus OGD/R + Ov-S100A12.

full-length S100A12 vector was transfected into PC12 cells to overexpress S100A12, the efficiency of S100A12 overexpression was confirmed by RT-qPCR (Fig. 1c).

S100A12 regulated the expression of ERK1/2 proteins

Considering the previous evidence that the proinflammatory effect of S100A12 is mediated by ERK signaling, western blot analysis was used to detect ERK1 and ERK2 protein expression with or without S100A12 knockdown and overexpression. The empty expressing vectors were used as negative control. Results revealed that the relative expression of activated ERK1 and ERK2 was markedly enhanced by OGD/R treatment and recovered after S100A12 knockdown, but further increased by S100A12 overexpression (Fig. 2). These data suggested the stimulative effect of S100A12 on ERK signaling. At the same time, we can conclude that the negative controls of S100A12 knockdown and overexpression have no additional effect on ERK1/2 protein expression, so these two negative control groups will be excluded in the next study.

Effect of S100A12 on OGD/R-induced inflammation

To identify whether S100A12 could mediate inflammation in cerebral I/R injury, PC12 cells transfected with or without S100A12 knockdown and overexpression vectors were exposed to OGD/R, and the levels of inflammatory cytokines and the protein expression of inflammasome were assessed. Results showed that silencing of S100A12 was blunted, while overexpression of S100A12 aggravated the OGD/R-induced release of proinflammatory factors TNFα, IL-1β and IL-18 together with decrease of anti-inflammatory IL-10.

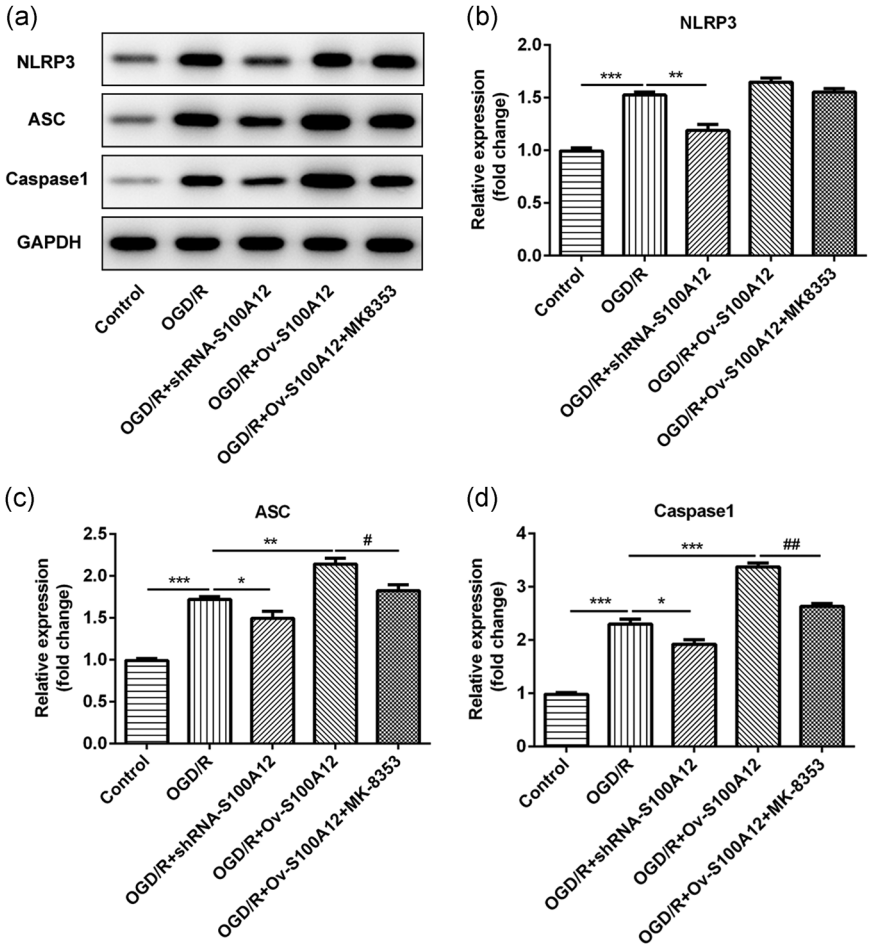

Figure 4 Effects of S100A12 on protein expression of inflammasome. Representative immunoblot analysis (**a**) and quantification for NOD-like receptor family, pyrin domain-containing 3 (NLRP3) (**b**), apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) (**c**) and Caspase1 (**d**) in different groups (n = 3). Oxygen-glucose deprivation and reperfusion (OGD/R) means oxygen-glucose deprivation and reperfusion. Ov means overexpression. OGD/R means oxygen-glucose deprivation and reperfusion. Ov means overexpression. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ versus OGD/R. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ versus OGD/R + Ov-S100A12.

In addition, the selective ERK1/2 inhibitor MK-8353 was applied to further determine the ERK-dependent effect of S100A12. As shown in Fig. 3, the presence of MK-8353 abolished the proinflammatory effect of S100A12 after OGD/R treatment. Similarly, silencing or overexpression of S100A12 and treatment with MK-8353 exerted the same effect on OGD/R-enhanced protein expression NLRP3, ASC and caspase1, respectively (Fig. 4). These results indicated the incentive effect of S100A12 on inflammation in cerebral I/R, which is mediated by ERK pathway.

Effect of S100A12 on oxidative stress

To observe the influence of S100A12 on oxidative stress, the activities of ROS, MDA, LDH and SOD were evaluated. Results revealed that OGD/R brought about the excitation of oxidative products ROS, MAD and LDH, while cut down the activity of antioxidase SOD. However, these effects were blunted by S100A12 silencing and restored by its' overexpression, and MK-8353 counteracted the effect of S100A12 overexpression (Fig. 5a–d).

Endothelial nitric oxide synthase (eNOS) is reported to be involved in vascular oxidative stress, and its product NO exerts neuroprotective effects.[24] We utilized western blot analysis to investigate the alteration of eNOS expression. Results suggested that the expression of eNOS was obviously reduced after OGD/R, S100A12 silencing rescued, while S100A12 overexpression weakened eNOS protein expression. At the same time, MK-8353 abrogated the effect of S100A12 overexpression, suggesting the accelerative effect of S100A12 on I/R-induced oxidative stress via ERK signaling (Fig. 5e).

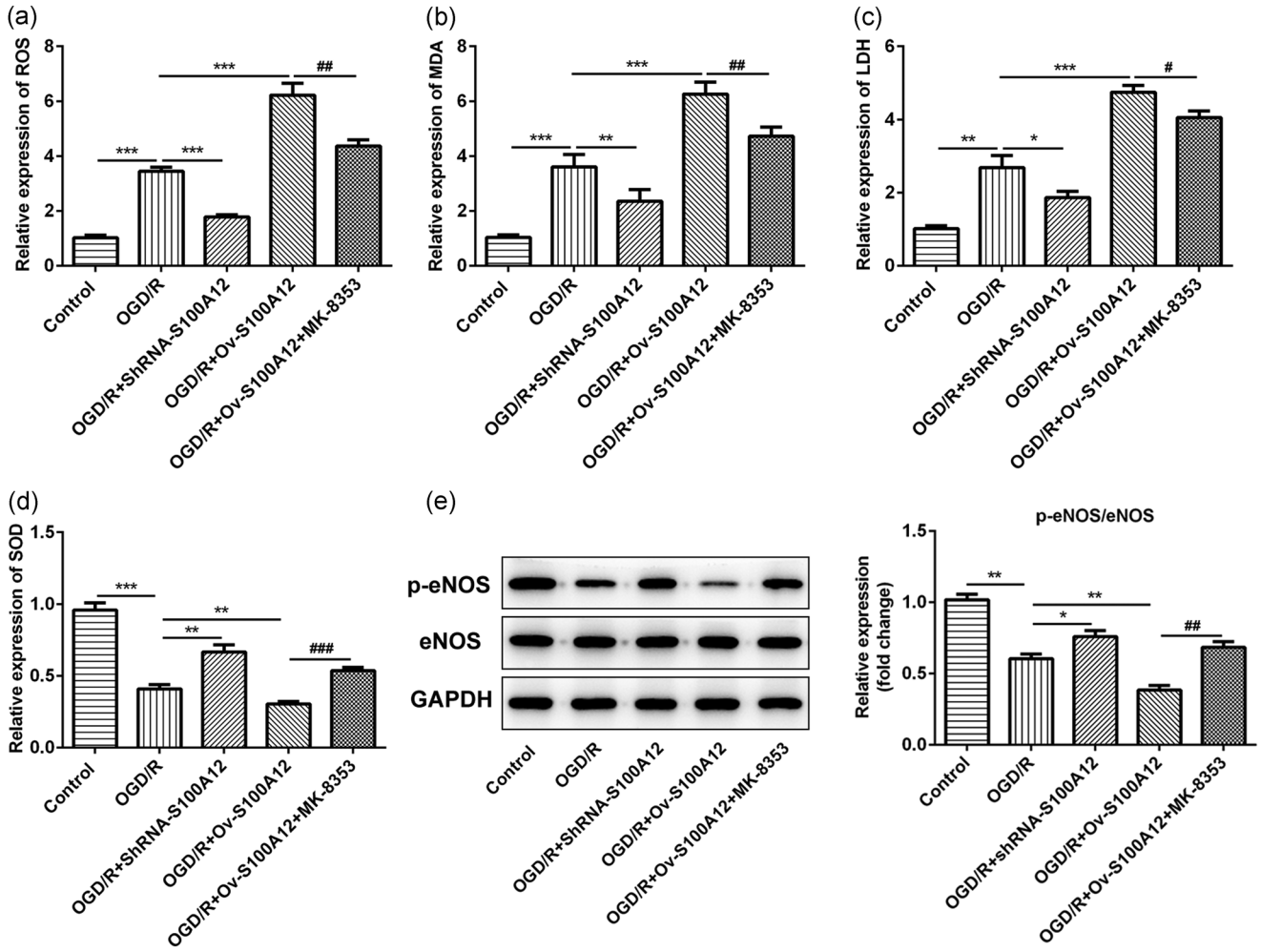

Figure 5 Effects of S100A12 on oxidative stress. (**a**–**d**) Relative expression of reactive oxygen species (ROS) (**a**), lipid peroxidation (MDA) (**b**), lactate dehydrogenase (LDH) (**c**) and superoxide dismutase (SOD) (**d**). (**e**) Representative immunoblot analysis and quantification for p-endothelial nitric oxide synthase (eNOS)/eNOS in different groups. Oxygen-glucose deprivation and reperfusion (OGD/R) means oxygen-glucose deprivation and reperfusion. Ov means overexpression. OGD/R means oxygen-glucose deprivation and reperfusion. Ov means overexpression. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ versus OGD/R. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ versus OGD/R + Ov-S100A12.

Effect of S100A12 on OGD/R-induced apoptosis

To examine the role of S100A12 in OGD/R-induced cell apoptosis, we detected the ratio of apoptotic cells and the expression of apoptosis-related proteins. As demonstrated in Fig. 6, compared with OGD/R groups, shRNA- S100A12 attenuated, while overexpression-S100A12 restored the OGD/R-induced increase of cell apoptosis rate.

As shown in Fig. 7, the expression of antiapoptotic protein Bcl-2 was decreased, but the expression of proapoptotic proteins Bax, cleved-caspase3 and -caspase9 was enhanced after OGD/R treatment. Meanwhile, all these effects were blunted by S100A12 knockdown, while rescued by S100A12 overexpression. The enhanced effect of S100A12 overexpression on cell apoptosis was also reversed by ERK inhibitor MK-8353. These data revealed that S100A12 play an aggravating function in OGD/R-produced apoptosis via ERK signaling.

DISCUSSION

The present study is the first to shed light on the effects of S100A12 on I/R injury. Here, we confirmed that S100A12 promotes OGD/R-induced inflammation, oxidative stress and cell apoptosis in PC12 cells via activating the ERK signaling pathway.

Cerebral I/R injury is the most common complication in stroke patients undergoing thrombolytic therapy. The specific mechanism of cerebral I/R injury is poorly understood,[25] therefore, uncovering the potential mechanism of cerebral I/R injury and developing effective agents for treating it are of great clinical importance.

S100A12 is a member of the S100 proteins, which have been reported to be involved in inflammatory response. Increasing evidence has suggested the proinflammatory effect of S100A12 in inflammation-related diseases.[14,26,27]

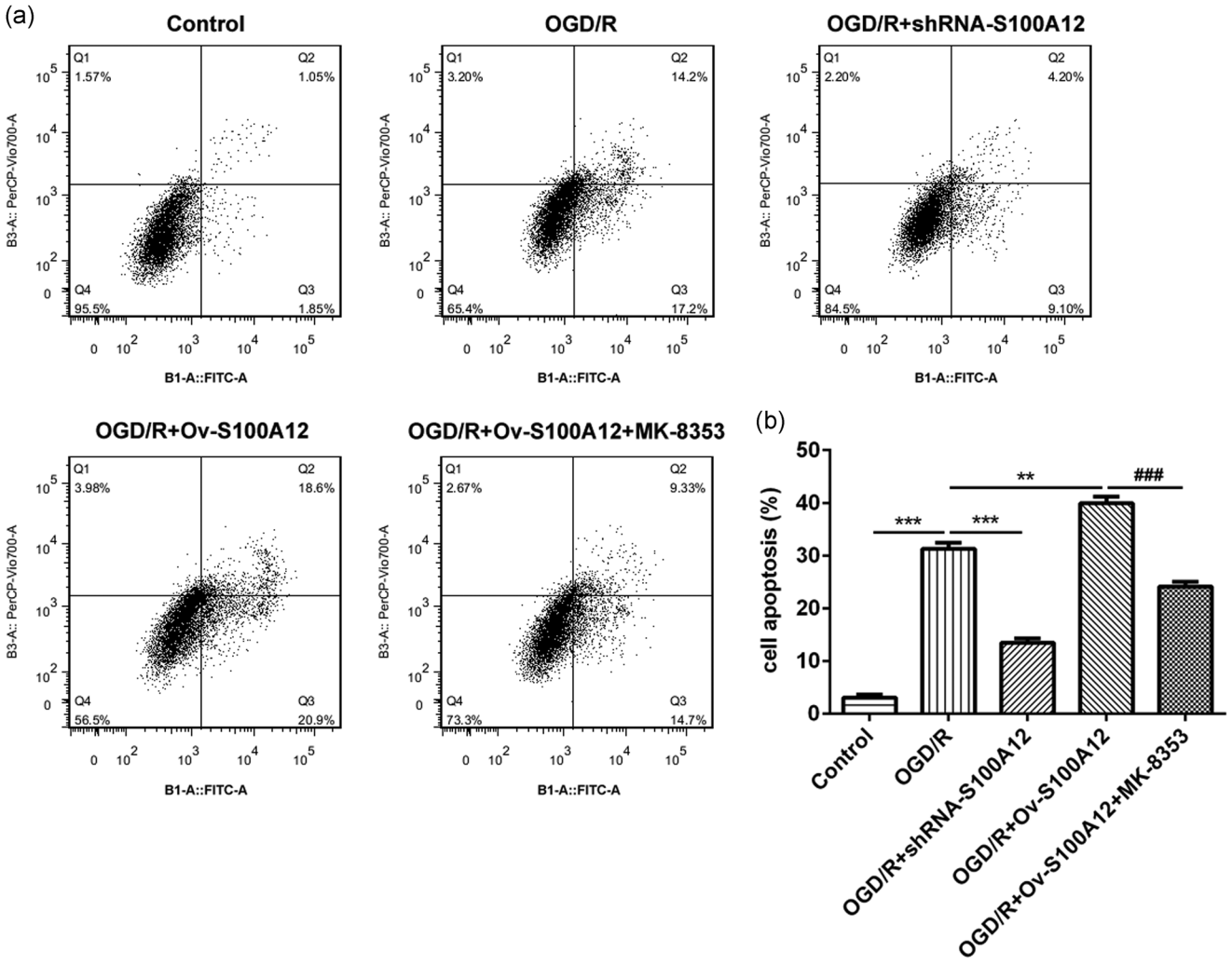

Figure 6 Effects of S100A12 on apoptosis rate. (**a**) Cell apoptosis was assessed by flow cytometry. (**b**) Quantification of apoptosis rate in different groups. Oxygen-glucose deprivation and reperfusion (OGD/R) means oxygen-glucose deprivation and reperfusion. Ov means overexpression. OGD/R means oxygen-glucose deprivation and reperfusion. Ov means overexpression. $^{**}P < 0.01$ and $^{***}P < 0.001$ versus OGD/R. $^{\#\#\#}P < 0.001$ versus OGD/R + Ov-S100A12.

Associated with previous studies,[15,16] we found that the level of S100A12 mRNA was significantly upregulated in the I/R injury *in vitro* model (Fig. 1a), suggesting the possible role of S100A12 in I/R injury. To further investigate the effects of S100A12 in I/R injury, we knocked down and overexpressed S100A12 in PC12 cells to observe the protein expression of ERK1 and ERK2. Results indicated that the expression of activated ERK1 and ERK2 proteins was obviously increased after OGD/R (Fig. 2). These data are consistent with others' findings that the ERK pathway would be activated in response to I/R injury.[28,29] Also, S100A12 silencing blunted, while its' overexpression enhanced the OGD/R-induced activation of ERK1/2 (Fig. 2). This result can be explained by previous studies which inferred that S100A12-induced damage would be dependent on activation of ERK1/2 signaling.[19–21] Based on these findings, we speculated that S100A12 could aggravate OGD/R-induced injury in PC12 cells through activating ERK signaling.

It is widely accepted that inflammation and oxidative stress play a pivotal role in the pathogenesis of I/R injury because of the overproduction of proinflammatory cytokines and free oxygen radicals, leading to extensive cell apoptosis and death.[30] We next examined the alteration of OGD/R-induced inflammation, oxidative stress and cell apoptosis. Our results showed that following OGD/R treatment, the generation of proinflammatory factors TNFα, IL-1β and IL-18; the level of inflammasome NLRP3, ASC and caspase1; the activities of oxidative products ROS, MDA and LDH; the ratio of cell apoptosis; the expression of proapoptotic proteins Bax, caspase3 and caspase9 were significantly increased. On the contrary, the generation of anti-inflammatory factors IL-10; the level of antioxidase SOD and eNOS; the expression of antiapoptotic protein

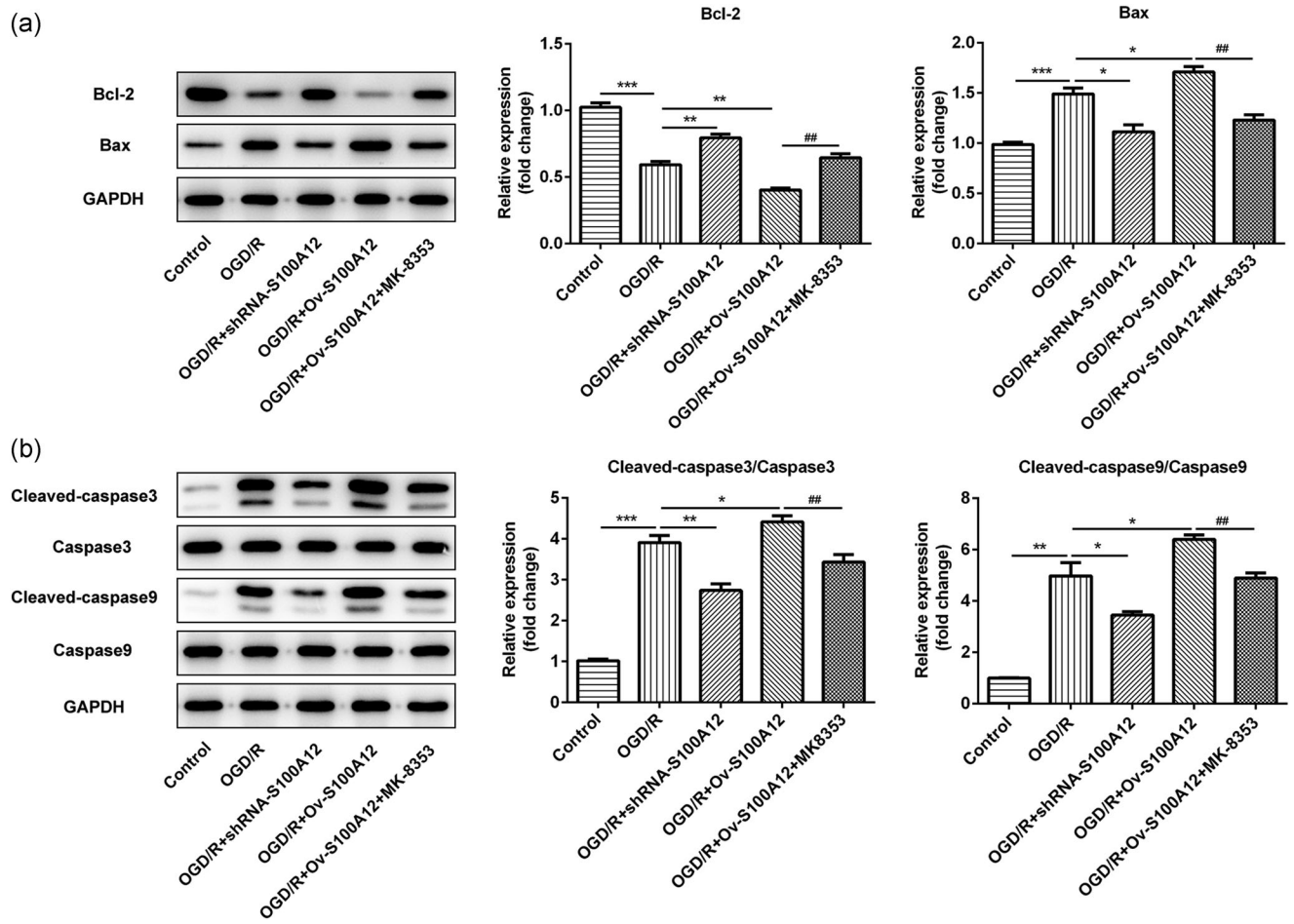

Figure 7 Effects of S100A12 on expression of apoptotic proteins. (**a**) Representative immunoblot analysis and quantification for Bcl-2 and Bax in different groups. (**b**) Representative immunoblot analysis and quantification for cleaved-caspase3 and cleaved-caspase9 in different groups. Oxygen-glucose deprivation and reperfusion (OGD/R) means oxygen-glucose deprivation and reperfusion. Ov means overexpression. OGD/R means oxygen-glucose deprivation and reperfusion. Ov means overexpression. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ versus OGD/R. $^{\#\#}P < 0.01$ versus OGD/R + Ov-S100A12.

Bcl-2 were obviously decreased. Briefly, OGD/R produced the occurrence of inflammation, oxidative stress and apoptosis in PC12 cells. However, all these effects were cut down by S100A12 silencing and exacerbated by its overexpression, confirming the promoting effect of S100A12 on cerebral I/R injury.

Furthermore, we incubated cells that were transfected into S100A12-overexpressed plasmids with ERK1/2 inhibitor MK-8353 to observe the effect of ERK signaling on S100A12-enhanced I/R damage in brain, simultaneously. The results that MK-8353 partially abolished the enhanced effects of S100A12 overexpression on the OGD/R-induced inflammation, oxidative stress and apoptosis further confirmed our hypothesis that S100A12 exerted its effects via activating the ERK pathway. However, the presence of MK-8353 didn't completely eliminate the functions of S100A12 overexpression when compared with OGD/R groups, suggesting the existence of other pathways or receptors which are in charge of the signal transmission of S100A12 besides ERK signaling. In addition, as a calcium-binding protein, S100A12 can activate and participate in a variety of signaling pathways including nuclear factor (NF)-κB, mitogen actived protein kinas (MAPK) and ERK by binding to Ca^{2+} such as calmodulin kinase II, protein kinase C (PKC) and phospholipase C (PLC).[31,32] Therefore, multiple signaling may involve in the effect of S100A12 on I/R injury, more *in vitro* and in *vivo* research is still needed to be launched to reveal the detailed mechanism of S100A12 actions.

In conclusion, our results display a new role of S100A12 in mediating OGD/R-induced inflammation, oxidative stress and apoptosis in PC12 cells. Considering the characteristics of PC12 is same with neuronal cells in morphology, physiology, and biochemistry, this study indicated that inhibiting S100A12 might be a prospective therapy for treating or preventing I/R in brain. Future investigation can be focused on developing S100A12 antagonist and investigating its curative effect in cerebral I/R as well as other inflammation-related diseases.

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DISCLOSURE STATEMENT

None declared.

AUTHOR CONTRIBUTIONS

QZ and ZC: conception and design of the study; XZ and RS: acquisition and analysis of data; ZS: drafting the manuscript or figures.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.