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Inhibition of perilipin 2 attenuates cerebral ischemia/reperfusion injury by blocking NLRP3 inflammasome activation both in vivo and in vitro

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Abstract

Cerebral ischemia/reperfusion (CI/R) usually causes neuroinflammation within the central nervous system, further prompting irreversible cerebral dysfunction. Perilipin 2 (Plin2), a lipid droplet protein, has been reported to exacerbate the pathological process in different diseases, including inflammatory responses. However, the role and mechanism of Plin2 in CI/R injury are unclear. In this study, the rat models of transient middle cerebral artery occlusion followed by reperfusion (tMCAO/R) were established to mimic I/R injury, and we found that Plin2 was highly expressed in the ischemic penumbra of tMCAO/R rats. The siRNA-mediated knockdown of Plin2 significantly decreased neurological deficit scores and reduced infarct areas in rats induced by I/R. Detailed investigation showed that Plin2 deficiency alleviated inflammation of tMCAO/R rats as evidenced by reduced secretion of proinflammatory factors and the blockade of NLR family pyrin domain containing 3 (NLRP3) inflammasome activation. In vitro experiments showed that Plin2 expression was upregulated in mouse microglia subjected to oxygen–glucose deprivation/reoxygenation (OGD/R). Plin2 knockdown inhibited OGD/R-induced microglia activation and the accumulation of inflammation-related factors. Taken together, this study demonstrates that lipid droplet protein Plin2 contributes to the pathologic process of CI/R damage by impacting inflammatory response and NLRP3 inflammasome activation. Thus, Plin2 may provide a new therapeutic direction for CI/R injury.

Keywords Cerebral ischemia/reperfusion injury · Perilipin 2 · Neuroinflammation · NLRP3 inflammasome

Introduction

Ischemic stroke (IS) is the driving cause of morbidity and mortality in the world with few treatment options available (Turner *et al*. 2013), and its ubiquity rises with age (Margaill *et al*. 2005). Currently, the most effective treatment for IS is recanalization therapy, which can restore nutrients and oxygen along with clear toxic metabolites (Kraft *et al*. 2012; Shafie and Yu 2021). However, blood reperfusion exacerbates cerebral damage and dysfunction after ischemia, which is called cerebral ischemia/reperfusion (CI/R) injury (Chomova and Zitnanova 2016). CI/R as an extremely complex cascade generally leads to a series of biological processes, such as inflammation, mitochondrial dysfunction, and oxidative stress (Sarkar *et al*. 2019). The mechanism of CI/R complexity is elusive; thus, it is imperative to investigate potential molecular targets to recover the function after CI/R.

Neuroinflammation is a well-orchestrated process in the central nervous system (CNS) and it exerts a vital role in various neurological disorders (Yang and Zhou 2019). Neuroinflammation refers to the inflammatory response that occurs in the cerebral tissues in many acute pathologies of the brain, including IS and CI/R injury (Jurcau and Simion 2021), and it is reported to act as a promising target for IS (Candelario-Jalil *et al*. 2022). During the presence of the blood–brain barrier, the immune cells from the periphery are difficult to get into the brain, and this blockade may trigger inflammation due to stagnant

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blood flow and the further release of pro-inflammatory cytokines (Jurcau and Simion 2021). It is widely established that cytokines play a significant role in inflammatory processes, which can lead to the pathologic process of IS (Dhanesha *et al*. 2022). Anti-inflammation appears to be an underlying therapeutic strategy for neuroinflammatory damage after IS since it plays a significant role in the CI/R pathogenesis (Peng *et al*. 2019). The NLR family pyrin domain containing 3 (NLRP3) inflammasome is a multimeric cytosolic protein complex that regulates caspase-1 activation and the release of proinflammatory cytokines (Sharma and Kanneganti 2021). The important role of the NLRP3 inflammasome is to respond to microbial infection and cellular damage, and aberrant NLRP3 activation can trigger a chronic inflammatory state in the body (Kelley *et al*. 2019). In CI/R injury, the activation of the NLRP3 inflammasome triggers neuroinflammation, and initial NLRP3 inhibition prevents CI/R injury by attenuating inflammation (Franke *et al*. 2021). Thus, investigating new immunomodulation strategies that balance neuroinflammatory injury may provide a viable way to reduce CI/R injury.

The protein family of perilipin (Plin) as the protein of lipid droplets (LDs) is found in almost every type of mammalian cell and regulates intracellular lipid metabolism (Sztalryd and Brasaemle 2017). Plin2, also called adipophilin, is the first identified protein in the Plin family, and it is reported to involve in LD formation, stability, and binding of lipids with high affinity (Frolov *et al*. 2000; Fukushima *et al*. 2005). A recent study suggests that Plin2-mediated moderate LD mobilization is essential for maintaining the pluripotency of embryonic stem cells (Wu *et al*. 2022). Plin2 has been deemed as the symbol of LDs, but its role is not limited to maintaining the homeostasis of lipid metabolism. Plin2 is revealed to augment inflammatory response in macrophages by upregulating the expression and secretion of many inflammatory cytokines (Chen *et al*. 2010). The impeditive effects of Plin2 on insulin-induced glucose uptake in myoblasts were illustrated by activating the NLRP3 inflammasome (Cho and Kang 2015). These findings indicate that Plin2 exerts an important role in the inflammatory response. However, whether Plin2 plays a pro-inflammatory or anti-inflammatory role in the process of CI/R has not been described.

In this study, we aimed to examine the role and underlying mechanism of Plin2 in cerebral dysfunction induced by I/R. We found that Plin2 was widely expressed in the ischemia of tMCAO/R rats. The possible role of Plin2 in inflammatory response was explored by performing neuropathological analyses in CI/R rats. In addition, oxygen–glucose deprivation/reoxygenation (OGD/R)–induced microglia were utilized to explore the effects of Plin2 on inflammation.

Materials and methods

Animals and transient middle cerebral artery occlusion followed by reperfusion (tMCAO/R) models This study was performed according to guidelines provided by the Animal Care Use Committee and was approved by the committee of the First Affiliated Hospital of Jinzhou Medical University (number: 2020413). Male Sprague–Dawley rats aged 8–12 wk were fed adaptively for 1 wk. The rats were randomly divided into three groups: the sham group, the 24-h reperfusion group (tMCAO/R-24 h), and the 72-h reperfusion group (tMCAO/R-72 h). In anesthetized rats, a nylon monofilament was inserted through the external carotid artery into the internal carotid artery. When there is a slight resistance, the middle cerebral artery was occluded by a monofilament that blocked blood flow. After that, the monofilament was left in place for 2 h and then was removed slowly to facilitate reperfusion. The established tMCAO/R rats showed palsy or looping of the left foreleg, and no cerebral hemorrhage in the infarcted tissues after removing the brain. The control group underwent the same procedure except for the carotid artery occlusion and reperfusion.

Preparation and injection of siRNA In this study, the rats were divided into four groups: the sham group, the tMCAO/R group, the tMCAO/R–si-NC (siRNA-negative control) group, and the tMCAO/R–si-Plin2 group (6 rats were used per experimental group). si-NC or si-Plin2 (15 μL) was injected into the left ventricle at 1.0 mm posterior to the bregma, 2.0 mm from the midline, and 3.5 mm below the skull. After the injection, the needle was kept in place for 10 min and then withdrawn slowly. siRNA liquids were injected at a rate of 2 μL/min to prevent siRNA reflux. After 24 h-injection, the rats were given the MCAO administration and followed by the 24-h reperfusion.

Evaluation of neurological deficit score The rats underwent a blind evaluation using the Zea-Longa Neurological Deficit Score after 24-h or 72-h reperfusion. The Zea-Longa scores were as follows: 0, with no symptoms of neurological damage; 1, unable to fully extend the contralateral forepaw; 2, turning to the hemiplegic side when walking; 3, falling to the hemiplegic side while walking; 4, unable to walk voluntarily and consciousness disorder; 5, death.

2,3,5-Triphenyltetrazolium chloride (TTC) staining The degree of cerebral infarction was evaluated using TTC staining as described before (Sakamula and Thong-Asa 2018). After 24-h reperfusion, the rat brains were removed, frozen for 20 min at − 20℃, and then sectioned into five coronal slices (2 mm thick). Next, the brain slices were incubated with 1% TTC (5 mL, Solarbio, Beijing, China) at 37℃ for

15 min in the dark, and the reverse side of the slices was stained for 15 min under the same condition. After TTC staining, normal brain tissues appeared red, while infarcted tissues appeared pale. ImageJ software (version IPP6.0) was used to capture the pictures and calculated the infarct area.

Cell culture and transfection Mouse BV2 microglia were purchased from iCell Bioscience Inc (Shanghai, China), and they were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum and 1% GlutaMAX (Gibco, Grand Island, NY). The BV2 cells were seeded in a 6-well plate at 37 °C in a humidified atmosphere with 5% CO_2. According to the manufacturer's instructions, the cells were transfected with si-NC or si-Plin2 using Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA) until they were at 70% confluency. After 48-h incubation, the transfected cells were used for western blot assay.

Oxygen–glucose deprivation/reoxygenation (OGD/R) models For OGD/R models, BV2 cells were incubated in glucose-free DMEM (Procell, Wuhan, China), and incubated in a hypoxia chamber (95% N_2, 5% CO_2, and 0.1% O_2) for 4 h. Subsequently, the cells were cultured in DMEM with high sugar and placed in an incubator with 74% N_2, 21% O_2, and 5% CO_2 for 24 h. Next, cells were cultured in a normal medium and incubated in normal cell culture incubators containing 95% air and 5% CO_2. Then the OGD/R models were established, and cells in the control group were treated identically without OGD/R. In addition, the BV2 cells were subjected to OGD/R treatment after 48-h infection of si-NC or si-Plin2.

Immunofluorescence The brains were removed, fixed, and embedded in paraffin. Coronal sections at 5 μm were used for immunofluorescence. After that, the brain tissues or cells were placed on coverslips and fixed with 4% paraformaldehyde for 15 min. For immunofluorescence, the sections were treated with 0.1% tritonX-100 (Beyotime, Shanghai, China) for 30 min. For double immunofluorescence, the embedded samples were deparaffinized, rehydrated, and then blocked by 1% bovine serum albumin (BSA) for 15 min. Then the samples were incubated with primary antibodies against Plin2 (ABclonal, Wuhan, China, Cat.No.A6276), NLRP3 (ABclonal, Cat.No.A5652), and ionized calcium binding-adaptor molecule 1 (Iba1, Abcam, Cambridge, UK, Cat. No.Ab283319) overnight at 4 °C. Subsequently, the samples were visualized by incubation with secondary antibody conjugated to cyanine 3 (Cy3, Invitrogen, Cat.No.A-21424) or fluorescein isothiocyanate (FITC, Abcam, Cat.No.ab6717) at room temperature for 1 h, along with 4′,6-diamidino-2-phenylindole (DAPI, Aladdin, Shanghai, China, Cat. No.D106471-5 mg). Then the pictures were obtained using a microscope (Olympus, Tokyo, Japan).

Real-time quantitative PCR Total RNA was extracted from the brain tissues of ischemia and BV2 cells by TRIpure reagent (BioTeke, Beijing, China), and RNA concentration was assessed using a NanoDrop spectrophotometer (Thermo, Pittsburgh, PA). BeyoRT II M-MLV reverse transcriptase (Beyotime) was used to synthesize cDNA. The expression of Plin2 was measured utilizing the SYBR Green (Solarbio), and the expression levels were normalized against that of GAPDH. The $2^{-\Delta\Delta CT}$ method was used to calculate the fold change in gene expression. The primers used were as follows (5′-3′): Rat Plin2, forward: CCA GCACAGTCTCAGGG, reverse: CAGCCGTTCATAGTA TCTTT; Mus Plin2, forward: GCTCTACCTACGACCTTG; reverse: GTCTTTCCTCCATCCTGT.

Western blot The samples were lysed in RIPA buffer (Solarbio), and protein concentration was determined with the BCA Protein Assay Kit (Solarbio). Equal amounts of samples were subjected to SDS–polyacrylamide gel electrophoresis (PAGE) and then transferred onto polyvinylidene difluoride membranes (Millipore, Billerica, MA). After blocking, the membranes were incubated with specific primary antibodies (Plin2: ABclonal, Cat.No.A6276; NLRP3: ABclonal, Cat.No.A5652; apoptosis-associated speck-like protein containing a CARD (ASC): ABclonal, Cat.No.A16672; cleaved caspase-1: affinity, China, Cat. No.AF4005; GAPDH: ABclonal, Cat.No.A19056) overnight at 4℃. Subsequently, the membranes were incubated with the horseradish peroxidase (HRP)–conjugated secondary antibody (Solarbio, Cat.No.SE134) for 1 h. The optical density of the protein bands was analyzed by using Image quant software (Tanon, Shanghai, China).

Enzyme-linked immunosorbent assay (ELISA) The tissues of the ischemic penumbra were mechanically homogenized under ice bath conditions and then centrifuged at 430 g for 10 min. BV2 cells were centrifuged at 300 g for 10 min to obtain the cellular supernatants. In the brain homogenates and cellular supernatants, the following cytokines were measured using the ELISA kits according to the supplier's instructions. The contents of tumor necrosis factor-α (TNF-α), interleukin (IL)-6, and IL-1β were measured using rat ELISA kits (MultiSciences Biotech, Hangzhou, China). IL-18 level was measured using a rat IL-18 ELISA Kit (Wuhan Fine Biotech, Wuhan, China). Mouse High Sensitivity ELISA Kits (MultiSciences Biotech) were used to detect the levels of TNF-α, IL-6, IL-1β, and IL-18 in the cellular supernatant. The corresponding absorbance read by the ELISA plate reader (Biotek, Winooski, VT) was calculated according to the standard curve.

Statistical analysis Statistical analyses were performed using GraphPad Prism 8.0, and all data were presented

as the mean ± SD. Unpaired *t*-test and ordinary one-way ANOVA were performed to determine *P* values. All data were represented after at least three repeated experiments with a similar pattern.

Results

Plin2 is significantly expressed in tMCAO/R rats The tMCAO/R rat models were established to mimic I/R injury in vivo, and the neurologic behavior of rats was assessed using the neurological deficit scores. We found that tMCAO/R rats displayed high neurological deficit scores, among which the ischemia rats subjected to 24-h reperfusion had the higher scores ($P < 0.001$, Fig. 1*a*). The mRNA and protein levels of Plin2 were upregulated after ischemia (Fig. 1*b*). According to the results of double immunofluorescence, we observed that the expression of Plin2 and Iba1 was significantly increased in the ischemic penumbra of tMCAO/R rats. Plin2 expression was significantly greater after 24-h reperfusion compared to 72-h reperfusion in ischemia rats (Fig. 1*c*).

Plin2 knockdown attenuated CI/R injury in tMCAO/R rats After 24-h reperfusion, the ischemia rats were injected with si-Plin2 liquid, and we found that the rats with tMCAO/R treatment had higher neurologic scores compared with the normal rats, while the scores were significantly decreased after the knockdown of Plin2 (Fig. 2*a*). As shown by TTC staining, the rats with the sham operation induced no infarction, but tMCAO/R treatment increased infarct areas. After Plin2 knockdown, the infarct volumes of

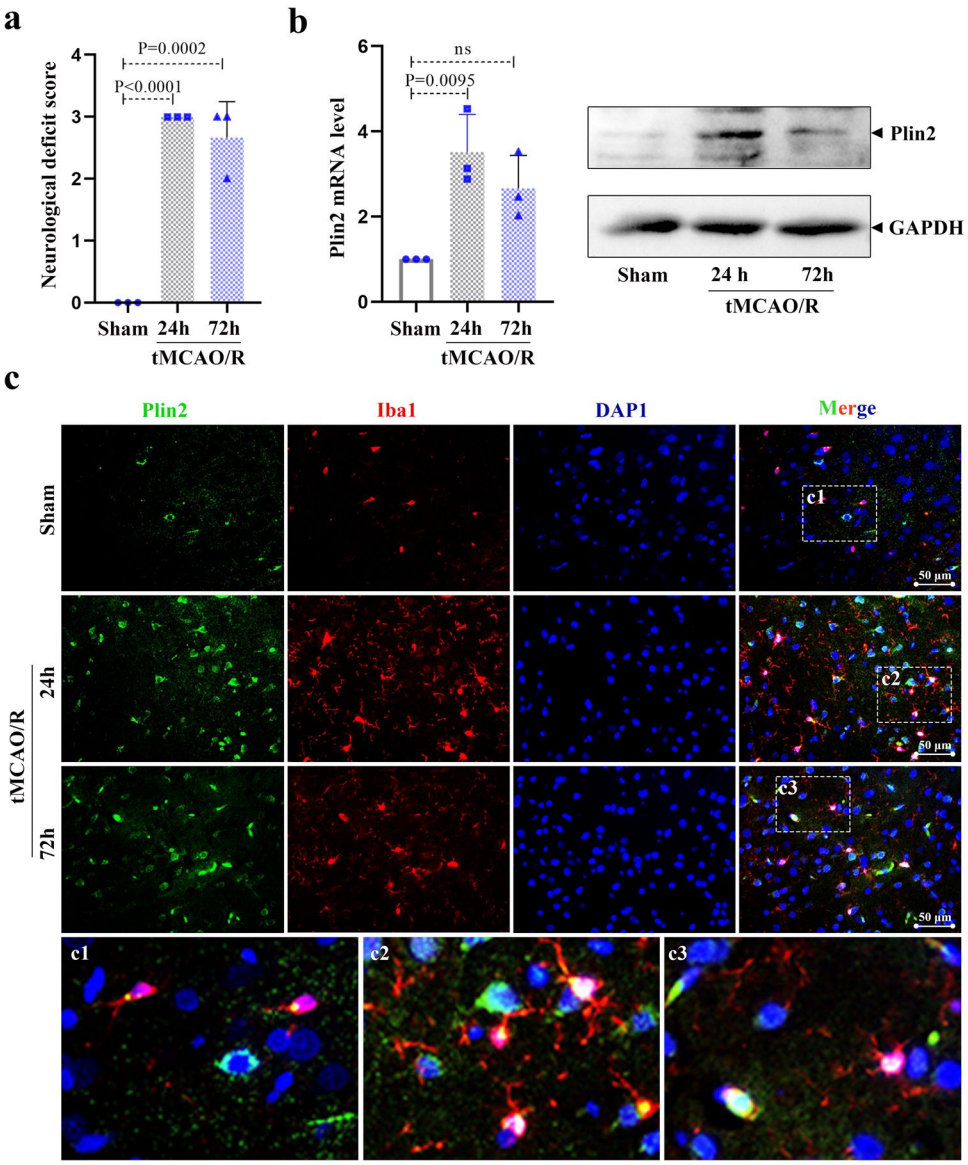

Figure 1. The expression of Plin2 in tMCAO/R rats. The tMCAO/R rat models were established to mimic ischemia/reperfusion injury. (*a*) After 24-h or 72-h reperfusion, the neurological deficit scores were assessed. (*b*) Quantitative RT-PCR and western blot assays were used to detect the transcriptional and protein levels of Plin2 in the ischemic penumbra of rats. (*c*) Double immunofluorescence staining for Plin2 with Iba1 in tMCAO/R rats. Panels *c1–c3* were the partially enlarged *picture* of fluorescence staining. Plin2, perilipin 2; tMCAO/R, transient middle cerebral artery occlusion followed by reperfusion; Iba1, ionized calcium-binding adapter molecule 1.

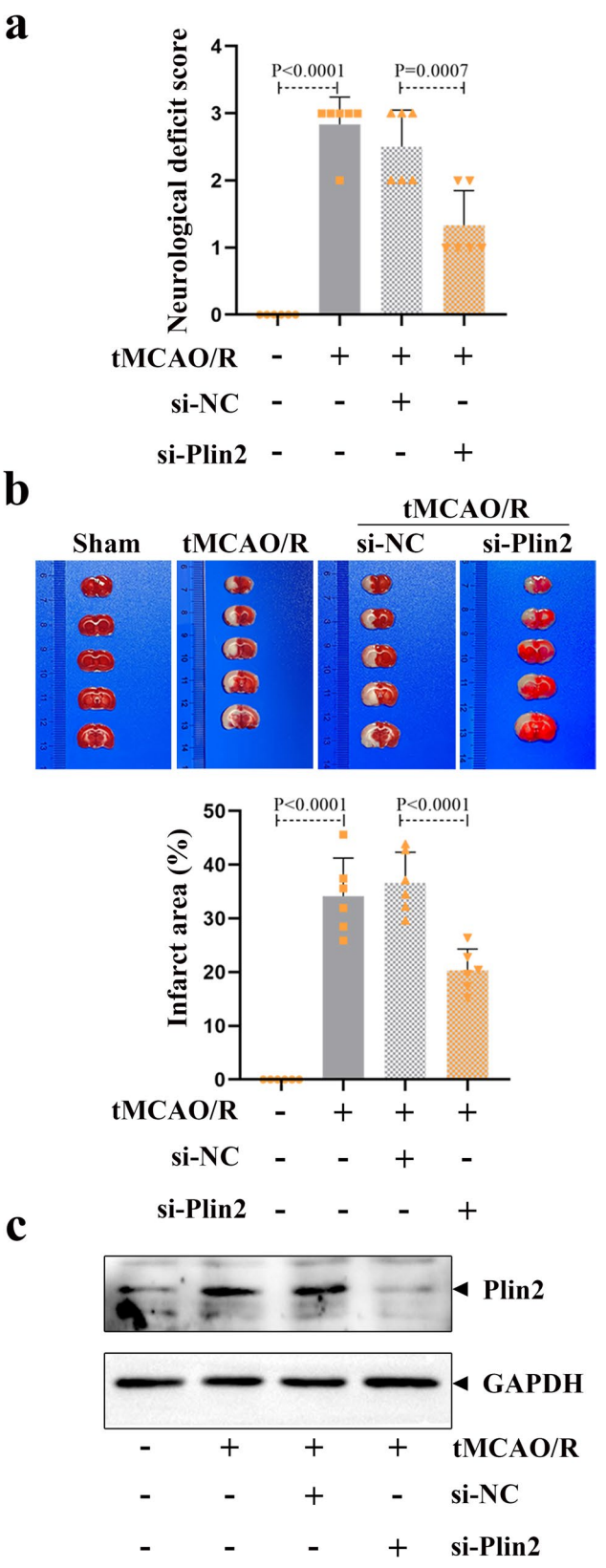

Figure 2. Effects of Plin2 deficiency on cerebral ischemia/reperfusion injury in tMCAO/R rats. After 24-h reperfusion, the tMCAO rats were injected with si-NC or si-Plin2 liquid at a rate of 2 μL/min. (*a*) Neurological deficit scores were assessed in rats after Plin2 knockdown. (*b*) Evaluation of infarct area with TTC staining in the brain. (*c*) Western blot assays were used to detect the protein level of Plin2 in the ischemic penumbra. si, siRNA; NC, negative control.

the brain were significantly reduced (Fig. 2*b*). In addition, the protein level of Plin2 detected by western blot verified the efficiency of Plin2 knockdown (Fig. 2*c*).

Plin2 knockdown inhibits inflammatory response and NLRP3 inflammasome activation in tMCAO/R rats TNF-α and IL-6, the important pro-inflammatory cytokines in the CNS, were used to further explore Plin2 function in CI/R. ELISA data showed that Plin2 knockdown decreased the levels of TNF-α and IL-6 in the ischemic penumbra of I/R rats (Fig. 3*a*). To investigate the effects of Plin2 on NLRP3 inflammasome, immunofluorescence staining for NLRP3 and Iba1 after tMCAO/R was performed in the ischemic penumbra. The increased NLRP3-positive and Iba1-positive cells were shown in tMCAO/R rats. In comparison, Plin2 silencing reduced the cell number of both. Moreover, double staining indicated that there was co-location between NLRP3 and Iba1 in the ischemic penumbra of the brain (Fig. 3*b*). Then the protein amounts of NLRP3 inflammasome–related factors were detected, and the results indicated that the loss of Plin2 led to the downregulated expression of NLRP3, ASC, and cleaved caspase-1 in tMCAO/R-induced rats (Fig. 3*c*). The levels of NLRP3 inflammasome–induced cytokines (IL-1β and IL-18) were also declined in tMCAO/R rats with Plin2 deficiency (Fig. 3*d*).

Plin2 is significantly expressed in OGD/R-induced microglia To systemically assess the role of Plin2 in cerebral damage induced by I/R, the OGD/R cell models were established by OGD/R administration. We found that Plin2 was highly expressed in OGD/R-induced microglia both in transcriptional and protein levels (Fig. 4*a*). Immunofluorescent staining showed that the number of Plin2-labelled cells was increased after OGD/R treatment as compared to the control group (Fig. 4*b*).

Plin2 knockdown inhibits inflammatory response and NLRP3 inflammasome activation in OGD/R-induced microglia The efficiency of Plin2 knockdown was validated by western blot analysis (Fig. 5*a*). Immunofluorescence analysis showed that Iba1 immunoreactivity in OGD/R-incurred cells was increased, while Plin2 knockdown presented the opposite effects (Fig. 5*b*). The levels of TNF-α and IL-6 were markedly declined in Plin2-silenced cells subjected to OGD/R (Fig. 5*c*). The upregulated protein levels of NLRP3, ASC, and cleaved caspase-1 caused by OGD/R activation were

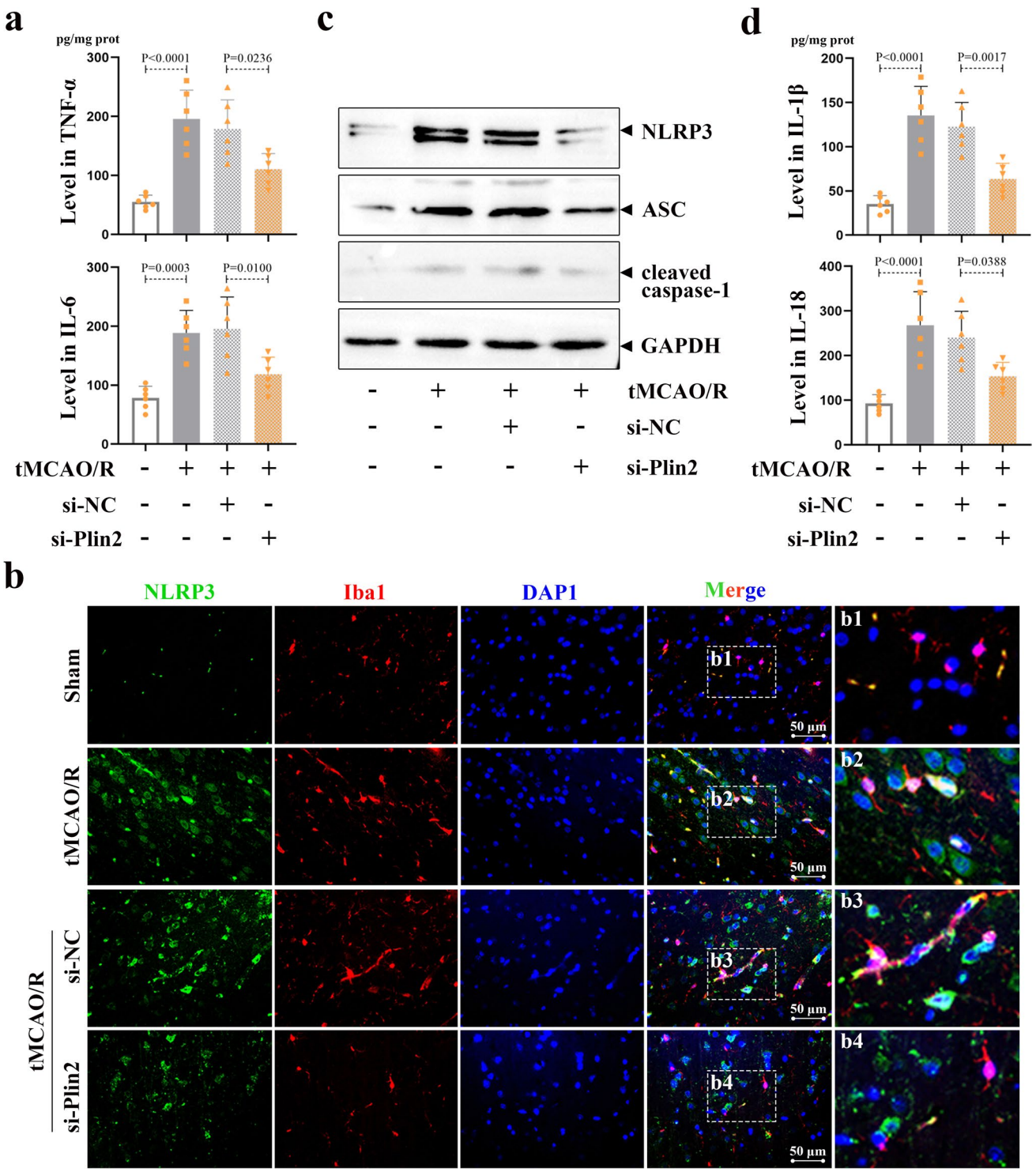

Figure 3. Effects of Plin2 deficiency on inflammatory response and NLRP3 inflammasome activation in tMCAO/R rats. (*a*) The contents of TNF-α and IL-6 after ischemia/reperfusion were quantified by ELISA. (*b*) Double immunofluorescence staining for NLRP3 with Iba1 in the ischemic penumbra of rats. Panels *b1–b4* were the partially enlarged *picture* of fluorescence staining. (*c*) Western blot analysis of NLRP3, ASC, and cleaved caspase-1 in rats. (*d*) The contents of IL-1β and IL-18 after ischemia/reperfusion were quantified by ELISA.

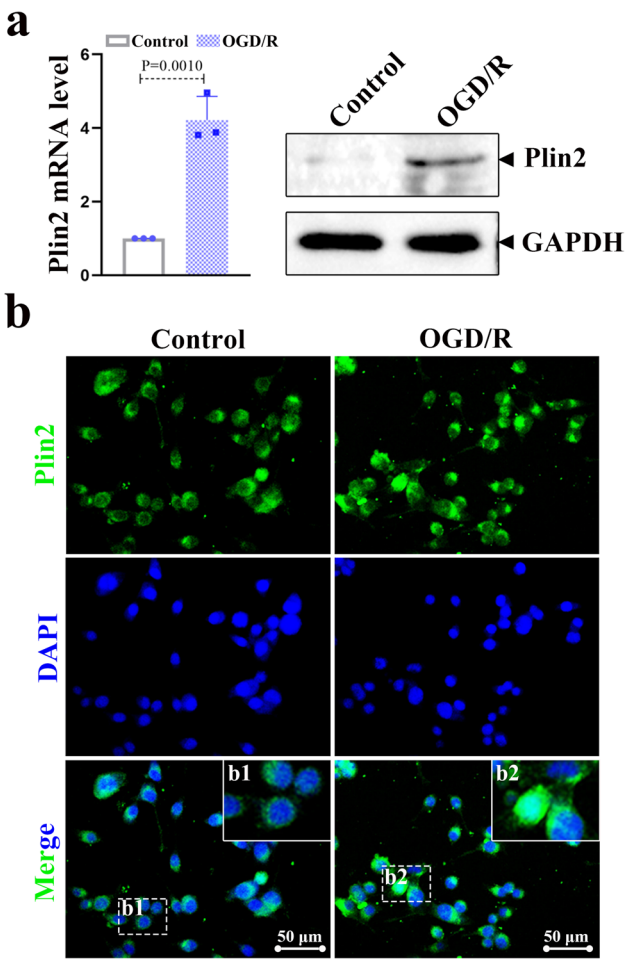

Figure 4. The expression of Plin2 in OGD/R-induced BV2 cells. (*a*) Quantitative RT-PCR and western blot assays were used to detect the transcriptional and protein levels of Plin2. (*b*) The expression of Plin2 was assessed by immunofluorescence staining. Panels *b1–b2* were the partially enlarged *picture* of fluorescence staining. OGD/R, oxygen–glucose deprivation/reoxygenation.

inhibited after Plin2 deficiency (Fig. 5*d*). In addition, we found that OGD/R treatment caused the enhancement of IL-1β and IL-18 levels, which were reduced after Plin2 knockdown (Fig. 5*e*).

Discussion

Cerebral ischemia is the primary cause of disability and mortality worldwide (Turner *et al*. 2013; Lee *et al*. 2018). However, the underlying mechanisms referred to I/R injury are currently unknown; thus, it is limited to develop effective therapy (Kawabori and Yenari 2015). In this study, we showed that Plin2 was widely expressed in I/R rats and OGD/R-induced microglia. The knockdown of Plin2 decreased the neurological deficit scores and infarct areas in tMCAO/R rats and alleviated inflammatory responses after I/R. Moreover, the significance of Plin2 knockdown in the maintenance and protection of I/R injury was further confirmed by in vitro experiments in microglia. Therefore, Plin2 may serve as a target for the therapy for I/R-induced cerebral damage.

Neuroinflammation and systemic inflammation are thought to be crucial components in the pathogenesis of neurodegenerative and psychiatric dysregulation (Medina-Rodriguez *et al*. 2018; Teleanu *et al*. 2022). It is widely known that the pathogenesis and etiology of cerebral ischemia are strongly influenced by inflammation (Jurcau and Simion 2021). After CI/R, ischemic brain tissues showed a dramatical increase in the expression of inflammatory markers, and this remarkable manifestation triggers various mechanisms that cause brain tissue damage (Talma *et al*. 2016). Here, this study found that Plin2 expression was upregulated in I/R rats, and Plin2 contributed to pathological changes and inflammatory responses, demonstrating the effects of Plin2 on cerebral inflammation after ischemia. The consistent results revealed in macrophages that Plin2 played a significant role in proinflammatory cytokine secretion (Chen *et al*. 2010).

NLRP3 inflammasome is a multi-protein complex of the innate immune system that detects cellular homeostasis deviation as a dangerous signal and triggers inflammation (Hoffman and Broderick 2016). When cells are harmed or subjected to microbial infection, the NLRP3 inflammasome can mediate caspase-1 activation and secrete the proinflammatory cytokines IL-1 and IL-18 (Huang *et al*. 2021). Plin2 is also demonstrated to trigger NLRP3 inflammasome in insulin-induced glucose uptake in myoblasts (Cho and Kang 2015). Although it is widely acknowledged that a neuroinflammatory response is induced during cerebral ischemia, it is unknown if Plin2 contributes to the inflammatory response induced by CI/R injury. In the present study, the pro-inflammatory function of Plin2 was demonstrated in tMCAO/R rats, which is consistent with the previous report conducted by Chen *et al*. (Chen *et al*. 2010). Moreover, it has been demonstrated that the loss of Plin2 relieves diet-induced hepatic steatosis and inflammation incurred by mediating caspase-1 activation to secrete and release IL-1β (Najt *et al*. 2016). In addition to the inflammatory response, a series of biological processes have also been activated after ischemia. For example, it has been noted that ischemia-induced neuronal autophagy promotes microglial inflammatory injury after IS (He *et al*. 2019). Whether Plin2 is involved in neuronal autophagy in I/R injury remains unclear, but the regulatory role of Plin2 in autophagy has been demonstrated in the liver (Tsai *et al*. 2017). Plin2 is widely expressed in renal I/R injury mice, and Plin2 overexpression markedly enhanced cell apoptosis after hydrogen peroxide treatment (Xu *et al*. 2021). In addition, the important roles of Plin2 in oxidative stress are reported in human dermal fibroblasts

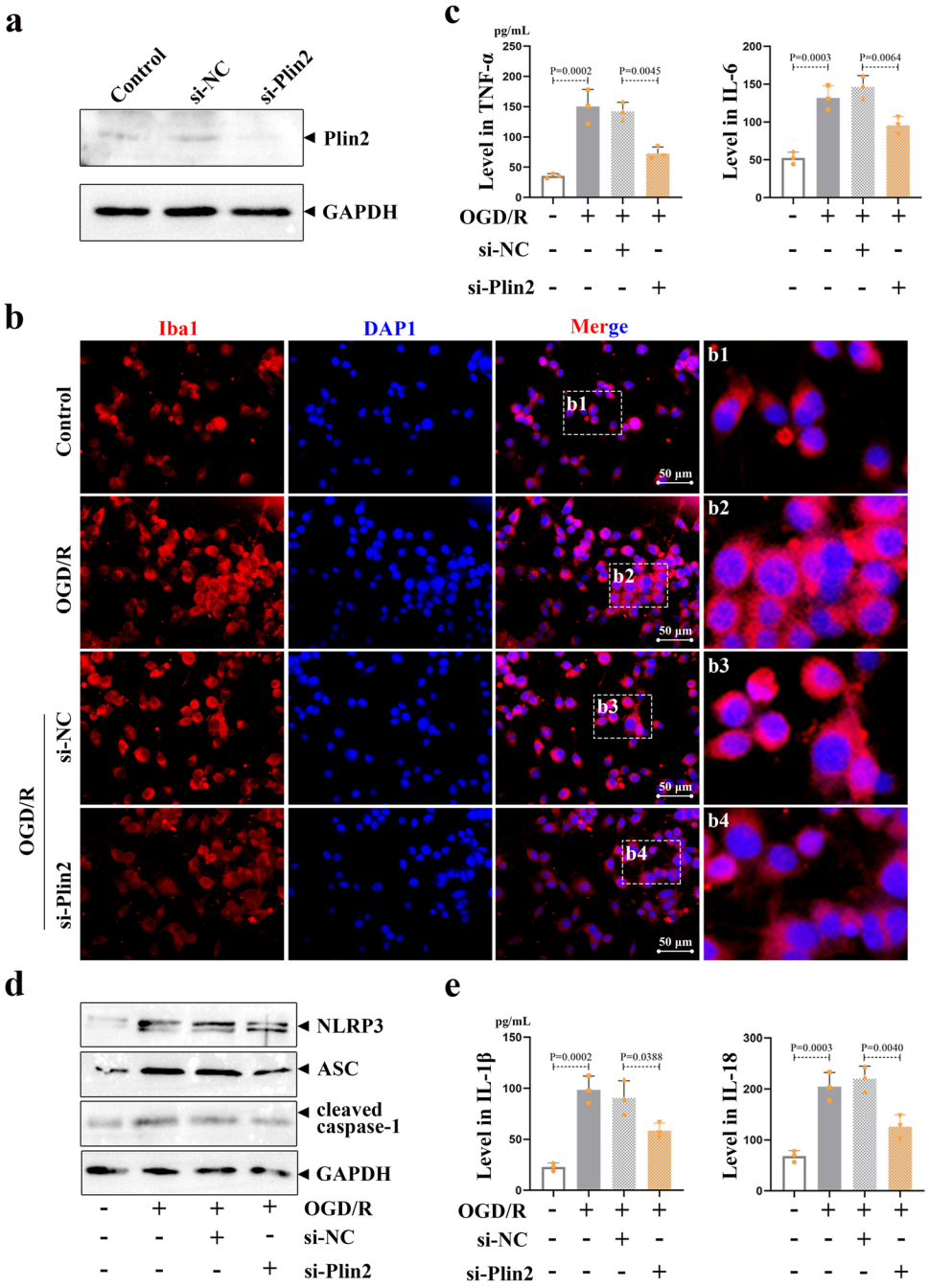

Figure 5. Effects of Plin2 deficiency on inflammatory response and NLRP3 inflammasome activation in OGD/R-induced BV2 cells. (*a*) The efficiency of Plin2 knockdown was verified by western blot. BV2 cells were treated with OGD/R after 48-h transfection of siRNA. (*b*) The expression of Iba1 after OGD/R administration was assessed by immunofluorescence staining, and panels *b1–b4* showed partially enlarged *pictures*. (*c*) The levels of TNF-α and IL-6 after OGD/R treatment were measured using ELISA. (*d*) Western blotting analysis of NLRP3, ASC, and cleaved caspase-1 in OGD/R-induced cells. (*e*) The levels of IL-1β and IL-18 after OGD/R treatment were measured using ELISA.

and stress-induced hypertension (Lu *et al*. 2020; Zhang *et al*. 2021). These findings confirm the function of lipid droplet protein Plin2 is not limited to lipid formation but performs multiple functions.

Microglia, the resident immune cells of CNS, are reported to represent 5–20% of the glial population and serve as the most mobile cells (Guruswamy and ElAli 2017; Yang and Zhou 2019). When the CNS is exposed to risky stimulations or pathological conditions, microglia are frequently activated earlier than other glial cells (Davalos *et al*. 2005). In response to internal or external stimuli, microglia are activated and secrete cytokines and other inflammatory mediators (Bachiller *et al*. 2018). Abundant evidence indicates that endogenous immune and inflammatory responses, particularly brain-resident microglia, and infiltrating macrophages, cause I/R injury in the brain (Kanazawa *et al*. 2017; Choi and Pile-Spellman 2018). After IS, the activated microglia easily lead to alterations of morphology and phenotype (Guruswamy and ElAli 2017). An analysis of postmortem human stroke injury reveals that inflammation initiates shortly after ischemia and the neighboring penumbral tissue is swiftly encircled by the activated microglia (Spiteri

et al. 2022). Given the important role of microglia in the CNS response to I/R (Xia *et al*. 2022), in this study, we established OGD/R-incurred microglia to verify the function of Plin2 on inflammatory response in the CNS. The data suggested that Plin2 was widely expressed in the microglia undergoing OGD/R, and its knockdown alleviated cerebral damage by preventing the expression of inflammatory factors. Consistently, studies have shown that Plin2 mediates microglial polarization/proliferation in stress-induced hypertension (Zhang *et al*. 2021).

Conclusion

In summary, this study revealed that Plin2 was highly expressed in the ischemic penumbra of I/R rats, and siRNA-mediated knockdown of Plin2 presented a decreased neurological deficit score and infarct area after ischemia–reperfusion in I/R rats. Additionally, our data demonstrated that Plin2 contributed to CI/R injury by impacting proinflammatory cytokines and the NLRP3 inflammasome, which provides a theoretical basis for the prevention of cerebral injury induced by I/R.

Author contribution XYL and RBS conceived the study. XYL, QSL, and RBS participated in its design and coordination. XYL performed most of the experiments and analyzed the data. XYL and WHY contributed to the establishment of tMCAO/R rat models. XYL, YQ, and FFZ performed behavioral testing experiments and analyzed the data. XYL, QSL, XHM, and QWY drafted the manuscript. RBS supervised the project and edited the paper. All authors have read and approved the final manuscript.

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Data availability All data generated or analyzed during this study are included in the article.

Declarations

Conflict of interest The authors declare no competing interests.

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