doi: 10.4149/gpb_20240381

Senkyunolide A attenuates cerebral ischemia-reperfusion injury by inhibiting NLRP3-mediated ferroptosis in PC12 cells

Qian Zhang[1,*], Yale Wang[1,2,*], Yihong Xiu[3], Zhiqiang Zhang[1], Tianyu Zou[1], Hongyan Wu[1,2] and Yaping Quan[1]

[1] *Shanghai University of Traditional Chinese Medicine, Shenzhen Hospital, Shenzhen, Guangdong, China*
[2] *Shanghai University of Traditional Chinese Medicine, Pudong New District, Shanghai, China*
[3] *Shenzhen Kangning Hospital, Shenzhen Mental Health Center, Shenzhen, Guangdong, China*

Abstract. Cerebral ischemia-reperfusion (I/R) is a serious complication in patients with ischemic stroke. Senkyunolide A (SenA) can alleviate neuronal cell damage induced by cerebral I/R; however, the exact action mechanism remains unclear. An *in vitro* cellular injury model was established by inducing PC-12 cells with OGD/R. The viability of SenA-treated PC-12 cells with or without OGD/R induction was detected with CCK-8 assay while the cell apoptosis was detected using TUNEL. The secretion of inflammatory cytokines, the activity of ROS, mitochondrial membrane potential and mtROS level were measured with ELISA, ROS assay kits, JC-1 staining and MitoSOX Red assay, respectively. The level of Fe^{2+} was detected with Fe^{2+} assay kits and lipid peroxidation was detected with TBARS assay. The expressions of lipid peroxides were measured using corresponding assay kits. Western blot was used to measure the expressions of NLRP3, apoptosis-, and ferroptosis-related proteins. The transfection efficiency of OV-NLRP3 was also detected using Western blot. The present study showed that SenA could attenuate viability damage, inflammatory response, oxidative stress, apoptosis and ferroptosis in OGD/R-induced PC-12 cells and it was identified that the cytoprotective effects of SenA on PC-12 cells stimulated by OGD/R may be associated with the inhibition of NLPR3. Collectively, SenA protects neuronal cells against cerebral I/R injury through the inhibition of NLRP3-mediated ferroptosis.

Key words: Cerebral ischemia-reperfusion — Senkyunolide A — NLPR3 — Ferroptosis

Introduction

Ischemia/reperfusion (I/R) injury is characterized by the limited blood supply to organs, followed by the restoration of blood flow and reoxygenation (Lv et al. 2021). As is known to all, I/R includes two events, which are ischemia and reperfusion (Soares et al. 2019). It is reported that various organs such as kidney, liver, heart and brain are vulnerable to I/R injury (He et al. 2022). Cerebral I/R is defined by the impairment of brain function that occurs during the reconstruction of blood supply to brain tissue after ischemia (Zhang et al. 2019). Previous studies have revealed that multiple pathophysiological processes are involved in cerebral I/R, such as inflammatory response, oxidative stress and apoptosis (Liu et al. 2021). At the present, the treatment of cerebral I/R still remains to be a challenge for clinicians.

Herbal medicines have been extensively applied to treat cerebral I/R injury because of their low toxicity and neuroprotective effects. For instance, Longxuetongluo capsule protects cerebral

* These authors contributed equally to this work.
Correspondence to: Yaping Quan, Shanghai University of Traditional Chinese Medicine, Shenzhen Hospital, 16 Xiantong Road, Luohu District, Shenzhen, Guangdong 518004, China
E-mail: Quanyapyes@163.com
Hongyan Wu, Shanghai University of Traditional Chinese Medicine, Shenzhen Hospital, 16 Xiantong Road, Luohu District, Shenzhen, Guangdong 518004, China
E-mail: Wuhongy1988@163.com

neuronal cells against I/R injury through endoplasmic reticulum stress (Pan et al. 2021). Xie et al. have elucidated that scutellarin can ameliorate oxidative stress and neuroinflammation in cerebral I/R injury (Xie et al. 2023). Senkyunolide A (SenA), a prominent natural product, is one of the principal bioactive constituents derived from Ligusticum chuanxiong (Chan et al. 2007). In recent years, the anti-inflammatory, neuroprotective, and antioxidant properties of SenA have recently attracted considerable attention (Zheng et al. 2018; Shao et al. 2022). It is worthwhile to mention that SenA has been demonstrated to protect against cerebral I/R injury in rats (Lin 2016). However, the regulatory mechanism of SenA and the key signaling pathways involved in cerebral I/R injury haven't been explored yet.

As is known to all, NLRP3 inflammasome, a member of the inflammatory vesicle, can regulate stroke-induced inflammatory response, thereby exacerbating cerebral I/R injury (Franke et al. 2021). Ferroptosis has been increasingly recognized as a mechanism of cell death after ischemia in various organs (Tuo et al. 2022). It has been evidenced that the inhibition of NLRP3 can suppress ferroptosis in cerebral I/R (Wu et al. 2023). Moreover, Shao et al. have shown that SenA can block NLRP3 signaling pathway (Shao et al. 2022). Considering this, it is reasonable to speculate that SenA protects against cerebral I/R injury *via* inhibiting NLRP3-mediated ferroptosis.

In summary, this study explored the role of SenA in the inflammation, oxidative stress and ferroptosis in OGD/R-induced PC-12 cells and revealed the relevant reaction mechanism associated with NLRP3, which might provide insights into the development of novel therapeutic agents in treating cerebral I/R injury.

Materials and Methods

Cell culture and treatment

The rat pheochromocytoma cell line PC-12 was obtained from BeNa Culture Collection (Henan, China) and maintained in Dulbecco's modified Eagle's medium (Gibco; Thermo Fisher Scientific, Inc.) containing 10% fetal bovine serum (GE Healthcare Life Sciences) and 1% penicillin-streptomycin (Sigma-Aldrich) at 37°C in a humidified atmosphere with 5% CO_2. SenA was dissolved in dimethyl sulfoxide (DMSO) and configured as the stock solution, which was further diluted to 20, 40 and 80 μg/ml as a working solution and then used to pre-treat PC-12 cell for 48 h (Shao et al. 2022). Cells were treated with DMSO of the same volume in the drug group as control group.

Oxygen-glucose deprivation/reperfusion (OGD/R) model

To simulate cerebral I/R injury *in vitro*, OGD/R model was established. PC-12 cells were incubated in glucose-free DMEM under hypoxic conditions (1% O_2, 94% N_2, and 5% CO_2) for 4 h. After that, PC-12 cells were incubated in normal medium under normoxic conditions (5% CO_2 and 95% air) at 37°C for 24 h.

Cell transfection

For transfection, PC-12 cells were cultured to 80–90% confluence. Overexpressing plasmids carrying NLRP3 (OV-NLRP3) and the empty vector (OV-NC) were synthesized by GenePharma (Shanghai, China). The cell transfection was conducted using Lipofectamine® 2000 (cat. no. 12566014; Thermo Fisher Scientific, Inc.; Shanghai, China) for 48 h at 37°C. After transfection for 48 h, the transfection efficiency was examined with Western blot.

Cell counting kit-8 (CCK-8) assay

To explore the effects of SenA on cell viability, CCK-8 assay kits (cat. no. #ZP328-3, Jiancheng Institute of Biotechnology, Nanjing, China) were performed. In brief, PC-12 cells were seeded in 96-well plates at a density of 4×10^3 cells/well and cultured for 24 h. Following OGD/R treatment as above mentioned, each well was added with 10 μl CCK-8 solution and PC-12 cells were incubated for additional 2 h. Finally, the absorbance at 450 nm was determined using a spectrophotometer (cat. no. 840-298000; Thermo Fisher Scientific, Inc.; Shanghai, China).

TUNEL staining

Following the incubation to 70–80% confluence, PC-12 cells were fixed with 4% formaldehyde at 37°C for 15 min and permeated with 0.25% Triton-X 100 for 20 min at room temperature. After that, PC-12 cells were incubated with TUNEL solution for 1 h at 37°C, followed by the staining with 0.1 μg/ml DAPI for 5 min at room temperature. Finally, the apoptotic cells in five randomly selected fields of view were observed with a fluorescent microscope (cat. no. F36913; Thermo Fisher Scientific, Inc.; Shanghai, China).

Detection of mitochondrial membrane potential

The mitochondrial membrane potential (MMP) was detected with JC-1 staining. Briefly, PC-12 cells with indicated treatment were seeded into 6-well plates and then incubated with 10 μM JC-1 staining for 30 min at 37°C in the dark, making JC-1 enter the cells and form aggregates or monomers according to the MMP. Finally, the stained PC-12 cells were observed using fluorescence microscopy.

Measurement of mitochondrial reactive oxygen species (mtROS)

The production of mtROS was detected using MitoSOX Red probe according to the manufacturer's instructions. In brief,

PC-12 cells were exposed to Hank's balanced salt solution supplemented with 5 μmol/l MitoSOX at 37°C for 15 min in the dark. Finally, a fluorescence microscopy (Olympus, Japan) was used for observation.

Enzyme-linked immunosorbent assay (ELISA)

The levels of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and interleukin 1beta (IL-1β) in the supernatant of PC-12 cells were detected using ELISA kits related to IL-6 assay kits (cat. no. EK0412; Wuhan Boster Biological Technology, Ltd.), TNF-α assay kits (cat. no. EK0393; Wuhan Boster Biological Technology, Ltd.) and IL-1β assay kits (cat. no. EK0526; Wuhan Boster Biological Technology, Ltd.) according to the manufacturer's instructions. The optical density of each well was measured at 450 nm using a microplate reader (Thermo Fisher Scientific, Inc.)

Determination of iron levels

Following centrifugation at 3,000×*g* at 4°C for 10 min, the concentrations of Fe^{2+} and total iron in the cell supernatants were detected using the Cell Ferrous Iron Colorimetric Assay Kit (cat. no. TC1011; Leagene, China). Subsequently, the optical density at 562 nm was measured with a spectrophotometer (Thermo Fisher Scientific) to quantify the iron level.

Thiobarbituric acid-reactive substances (TBARS) assay

For the detection of lipid peroxidation, TBARS approach was used. The supernatants of the cell lysates were mixed with 0.1% thiobarbituric acid solution and vortexed for 30 min at 95°C. Following the collection of supernatants, the absorbance at 532 nm was determined with a spectrophotometer.

Detection of lipid peroxide

The activities of malondialdehyde (MDA), superoxide dismutase (SOD), ROS, glutathione peroxidase (GSH-Px) and 4-hydroxynonenal (4-HNE) in PC-12 cells were detected using MDA assay kits (cat. no. A003-1-2, Nanjing Jiancheng Bioengineering Institute), SOD assay kits (cat. no. A001-3-2, Nanjing Jiancheng Bioengineering Institute), ROS assay kits (cat. no. S0033S, Beyotime), GSH-px assay kits (cat. no. A005-1-2, Nanjing Jiancheng Bioengineering Institute) and 4-HNE assay kits (cat. no. ab238538, Abcam) according to the manufacturer's instructions.

Western blot analysis

The total proteins were extracted from PC-12 cells using RIPA buffer (Solarbio, Beijing, China) and the concentration of proteins was quantified with bicinchoninic acid (BCA) protein assay kits (Thermo Fisher Scientific Inc.) according to the manufacturer's instructions. Separated by 10% SDS-PAGE (Bio-Rad, Hercules, California, USA), equal amount of proteins (20 μg *per* lane) were then transferred onto PVDF membranes (Millipore, USA). Following the inhibition with 5% BSA for 2 h at room temperature, the membranes were immunoblotted with primary antibodies targeting Cleaved caspase3 (cat. no. #9664; 1:1000; Cell Signaling Technology), Bax (cat. no. ab32503; 1: 1000; Abcam), BCL2 (cat. no. ab194583; 1: 2000; Abcam), caspase3 (cat. no. ab184787; 1:2000; Abcam), SLC7A11 (cat. no. ab307601; 1:1000; Abcam), GPX4 (cat. no. ab125066; 1:1000; Abcam), ACSL4 (cat. no. ab155282; 1:10000; Abcam), TFR1 (cat. no. ab269513; 1:5000; Abcam), NLRP3 (cat. no. ab263899; 1:1000; Abcam), and GAPDH (cat. no. ab181602; 1:10000; Abcam) at 4°C overnight. Subsequently, the membranes were incubated with horseradish peroxidase-labeled goat anti-rabbit secondary antibodies (cat. no. ab6721; 1:2000; Abcam) or goat anti-mouse secondary antibodies (cat. no. ab205719; 1:2000; Abcam) at room temperature for 1 h. Protein bands were visualized using enhanced ECL detection reagent (Yeasen Biotech), and the protein density was analyzed with ImageJ software (Version 1.49; National Institutes of Health).

Statistical analyses

All experiments were repeated for three times. The collected data were analyzed with GraphPad Prism 8 software (GraphPad Software, Inc.) and presented as mean ± standard deviation. Comparisons among multiple groups were shown using one-way ANOVA followed by Tukey's *post hoc* test. p-value < 0.05 indicated statistical significance.

Results

SenA mitigates PC-12 cell viability damage induced by OGD/R

To explore the effects of SenA on PC-12 cell viability, SenA with varying concentrations was initially used to treat PC-12 cells and CCK-8 assay was performed. Notably, SenA had no significant effects on the viability of PC-12 cells compared with the 0 μg/ml group (Fig. 1A). Compared with the Control group, the viability of PC-12 cells was significantly reduced following OGD/R induction, which was then revived by SenA treatment in a concentration-dependent manner (Fig. 1B). Besides, TUNEL results showed that OGD/R stimulation significantly promoted the apoptosis of PC-12 cells compared with the Control group. However, the increased cell apoptosis in OGD/R group was then sig-

nificantly decreased by SenA treatment, accompanied with decreased expressions of Cleaved caspase3 and Bax as well as increased BCL2 expression (Fig. 1C,D). Evidently, SenA treatment could alleviate the viability damage in PC-12 cells stimulated by OGD/R.

SenA alleviates OGD/R-induced inflammation and oxidative stress in PC-12 cells

To investigate the effects of SenA on inflammation and oxidative stress stimulated by OGD/R, the levels of inflam-

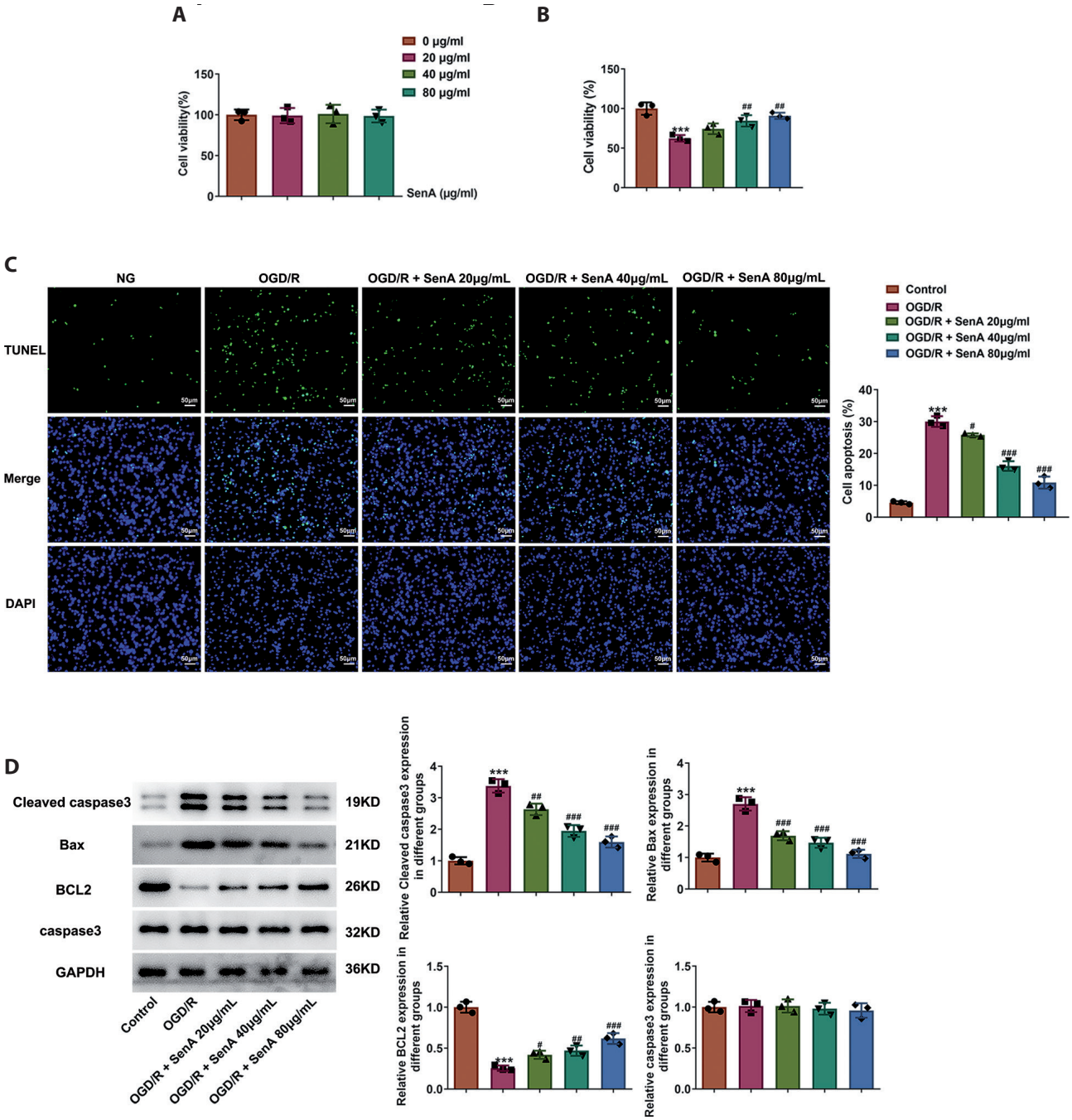

Figure 1. SenA mitigates PC-12 cell viability damage induced by OGD/R. **A.** The viability of SenA-treated PC-12 cells was detected using CCK-8 assay. **B.** The viability of OGD/R-induced PC-12 cells with SenA treatment was detected using CCK-8 assay. **C.** The cell apoptosis was detected using TUNEL. Scale bar = 50 μm. **D.** The expressions of apoptosis-related proteins were detected using Western blot. *** $p < 0.001$ *vs.* Control; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ *vs.* OGD/R group. (For color figure see online version.)

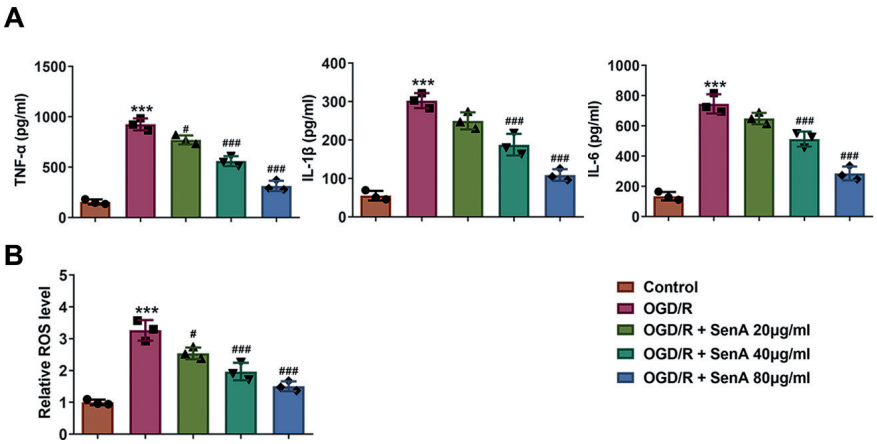

Figure 2. SenA alleviates OGD/R-induced inflammation and oxidative stress in PC-12 cells. **A.** The levels of inflammatory cytokines were detected using ELISA-related assay kits. **B.** The activity of ROS was detected using ROS assay kits. *** $p < 0.001$ *vs.* Control; # $p < 0.05$, ### $p < 0.001$ *vs.* OGD/R group. (For color figure see online version.)

matory cytokines and oxidative stress marker were detected. As shown in Figure 2A, OGD/R stimulation significantly increased the levels of TNF-α, IL-6 and IL-1β compared with the Control group, which were all decreased following the treatment of SenA. In addition, it was also found that OGD/R induction greatly increased ROS activity compared with the Control group, which was then concentration-dependently decreased by SenA treatment (Fig. 2B). The empirical evidence presented in this section suggests that SenA could reduce inflammation and oxidative stress in OGD/R-induced PC-12 cells.

SenA suppresses ferroptosis in OGD/R-induced PC-12 cells

The effects of SenA on ferroptosis in OGD/R-induced PC-12 cells were initially investigated through JC-1 staining. It was noted that OGD/R induction increased JC-1 monomers whereas it decreased JC-1 aggregates compared with the Control group, which were subsequently reversed by SenA treatment (Fig. 3A). Meanwhile, results obtained from MitoSOX Red assay demonstrated the inhibitory effects of SenA on mitochondrial damage (Fig. 3B). In addition, we quantified Fe^{2+} and total iron levels and found that Fe^{2+} and total iron levels in OGD/R group were significantly increased compared with the Control group, which were then decreased by SenA in a concentration-dependent manner (Fig. 3C). Subsequently, TBARS approach was used to detect lipid peroxidation and the results revealed that the TBARS production of PC-12 cells was significantly increased after OGD/R induction. However, the increased TBARS production in OGD/R group was then concentration-dependently reduced by SenA treatment (Fig. 3D). Compared with the Control group, OGD/R stimulation increased the expressions of MDA and 4-HNE whereas it decreased the expressions of SOD and GSH-Px, which were all reversed by SenA treatment (Fig. 3E). The expressions of proteins associated with ferroptosis were detected with Western blot and the results illustrated that OGD/R induction decreased the contents of SLC7A11 and GPX4 while increasing the contents of ACSL4 and TFR1 compared with the Control group, which were all reversed following the treatment of SenA (Fig. 3F).

SenA inhibits NLRP3 expression in OGD/R-induced PC-12 cells

The effects of SenA on NLRP3 expression were explored through Western blot. Compared with the Control group, NLRP3 expression was significantly increased in OGD/R group, which was then decreased following the treatment of SenA in a concentration-dependent manner (Fig. 4A). To upregulate NLRP3 expression, OV-NLRP3 was transfected into PC-12 cells and the transfection efficacy was examined with Western blot. Compared with the OV-NC group, the protein expression of NLRP3 in PC-12 cells was greatly increased by OV-NLRP3 (Fig. 4B).

SenA alleviates the viability damage of OGD/R-induced PC-12 cells via inhibiting NLRP3

To explore the mechanism of SenA associated with NLRP3 in cerebral I/R injury, OV-NLRP3 was transfected into cells and above functional experiments were conducted again. As Figure 5A displayed, the decreased cell viability in OGD/R group was partially revived by SenA treatment, which was then decreased again after overexpressing NLRP3 expression. Besides, the decreased cell apoptosis in OGD/R+SenA group was partially increased by NLRP3 overexpression, accompanied with increased expressions of Cleaved caspase3 and Bax and decreased BCL2 expression (Fig. 5B,C). These findings imply that NLRP3 overexpression counteracted the inhibitory effects of SenA on viability damage and apoptosis of OGD/R-induced PC-12 cells.

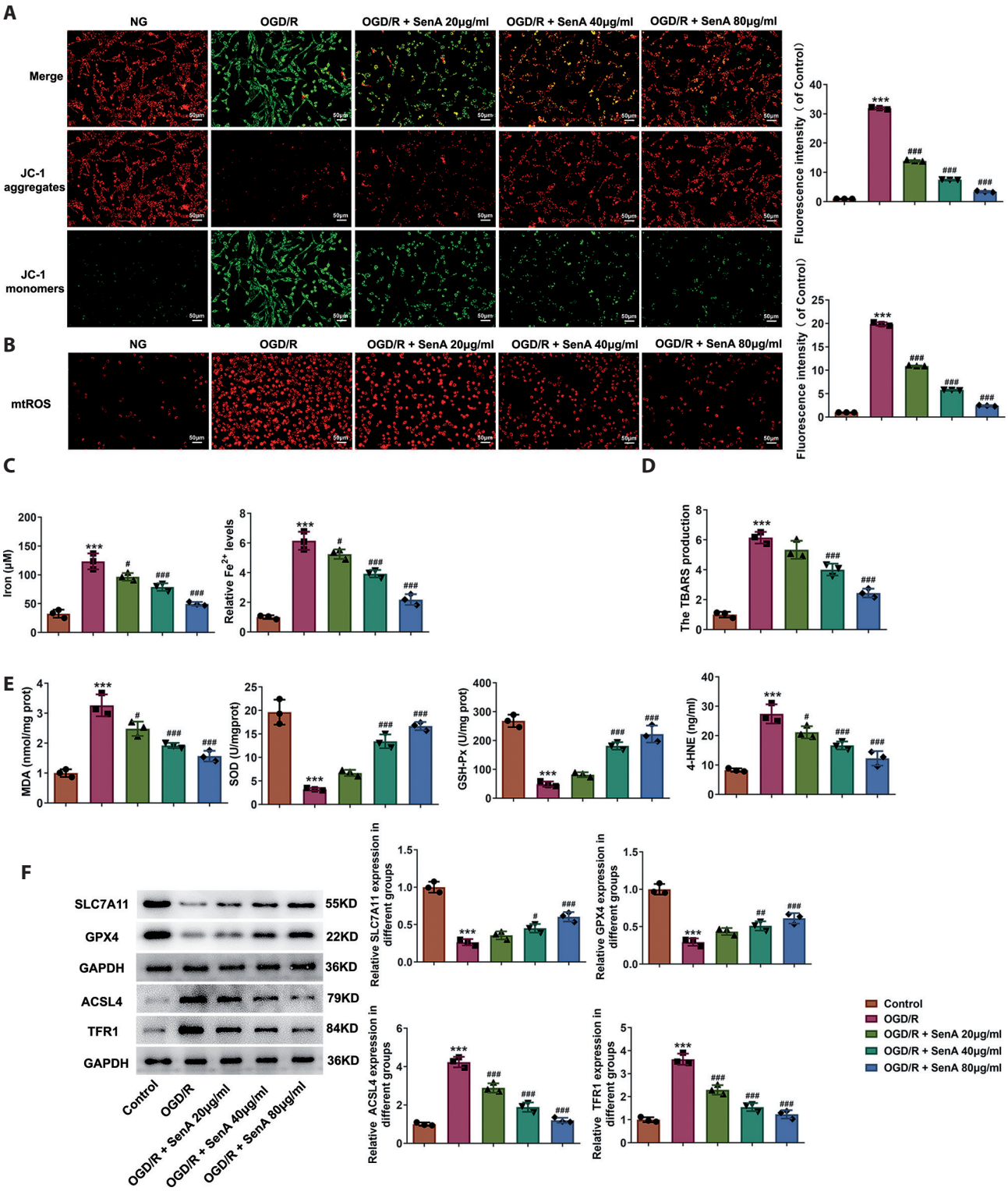

Figure 3. SenA suppresses ferroptosis in OGD/R-induced PC-12 cells. **A.** The mitochondrial membrane potential was detected using JC-1 staining. **B.** The level of mtROS was detected using MitoSOX Red probe. Scale bar = 50 μm. **C.** Fe^{2+} and total iron levels were detected using corresponding assay kits. **D.** The production of TBARS was detected using TBARS assay. **E.** The expression levels of MDA, SOD, GSH-Px and 4-HNE were detected using corresponding assay kits. **F.** The expressions of ferroptosis-related proteins were detected using Western blot. *** $p < 0.001$ *vs.* Control; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ *vs.* OGD/R group. (For color figure see online version.)

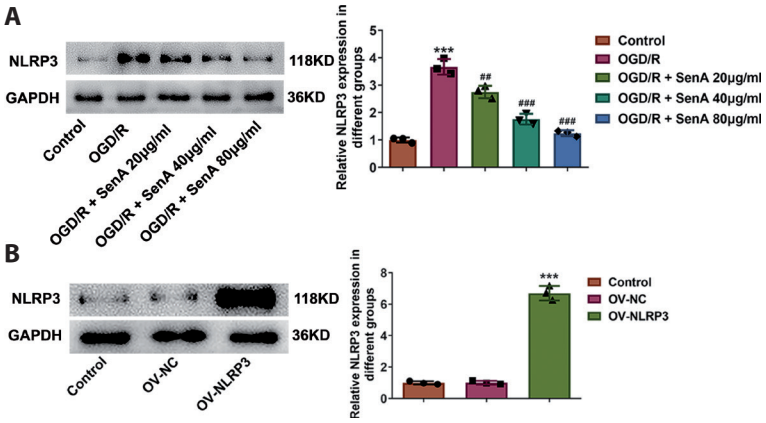

Figure 4. SenA inhibits NLRP3 expression in OGD/R-induced PC-12 cells. **A.** The expression of NLRP3 was detected using Western blot. *** $p < 0.001$ *vs.* Control; ## $p < 0.01$, ### $p < 0.001$ *vs.* OGD/R group. **B.** The transfection efficacy of OV-NLRP3 was examined with Western blot. *** $p < 0.001$ *vs.* OV-NC. (For color figure see online version.)

SenA alleviates OGD/R-induced inflammatory and oxidative stress damage in PC-12 cells via inhibiting NLRP3

Compared with OGD/R group, SenA treatment significantly decreased the levels of TNF-α, IL-6 and IL-1β in OGD/R+SenA group, which were subsequently increased after overexpressing NLRP3 expression (Fig. 6A). Similarly, the increased ROS activity in OGD/R-induced PC-12 cells was greatly decreased by SenA treatment. However, NLRP3 overexpression exhibited opposite effects on it, evidenced by increased ROS activity in OGD/R+SenA+OV-NLRP3 group (Fig. 6B). The above

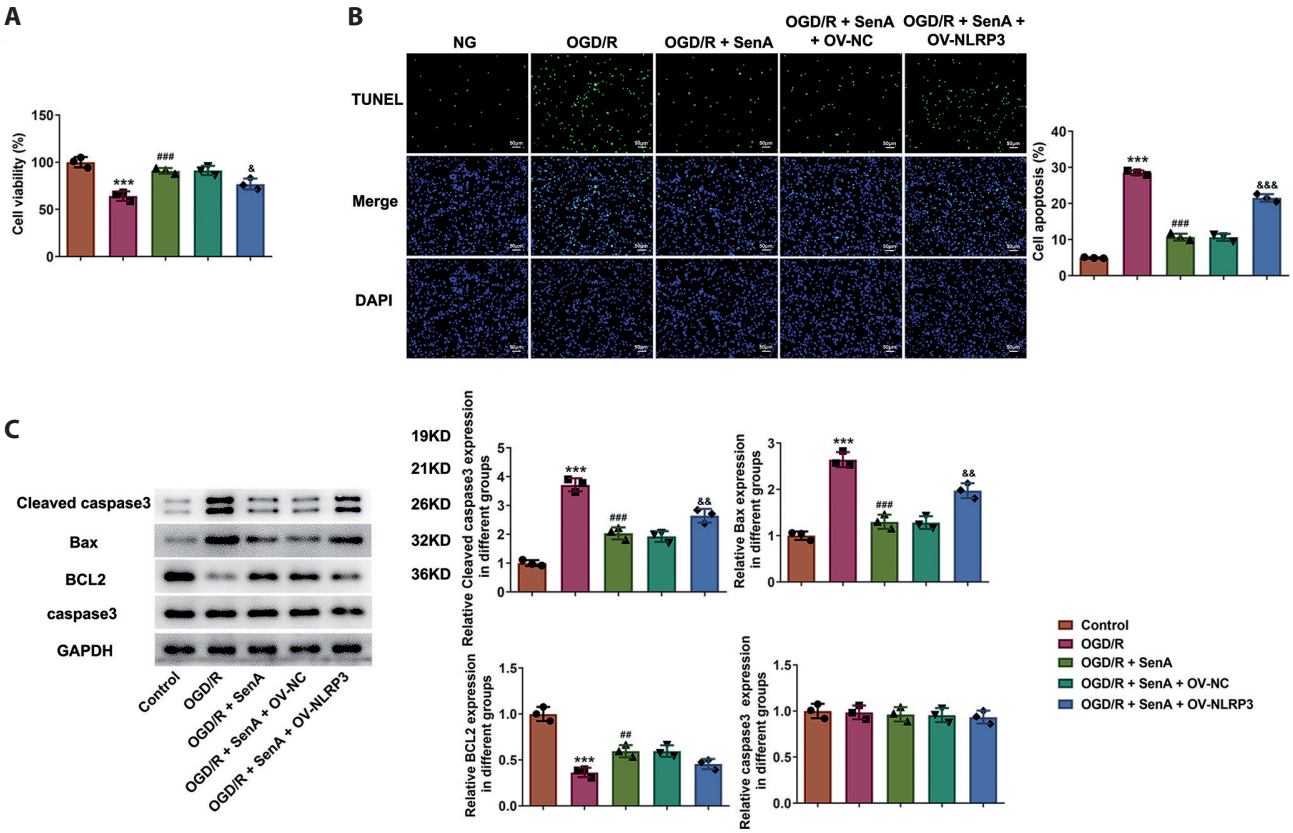

Figure 5. SenA alleviates the viability damage of OGD/R-induced PC-12 cells *via* inhibiting NLRP3. **A.** The cell viability was detected using CCK-8 assay. **B.** The cell apoptosis was detected using TUNEL. Scale bar = 50 μm. **C.** The expressions of apoptosis-related proteins were detected using Western blot. *** $p < 0.001$ *vs.* Control; ## $p < 0.01$, ### $p < 0.001$ *vs.* OGD/R; & $p < 0.05$, && $p < 0.01$, &&& $p < 0.001$ *vs.* OGD/R+SenA+OV-NC. (For color figure see online version.)

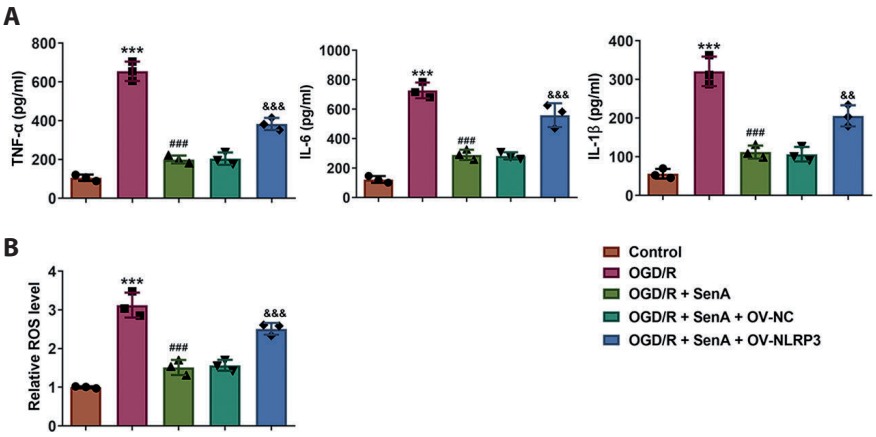

Figure 6. SenA alleviates OGD/R-induced inflammatory and oxidative stress damage in PC-12 cells *via* inhibiting NLRP3. **A.** The levels of inflammatory cytokines were detected using ELISA-related assay kits. **B.** The activity of ROS was detected using ROS assay kits. *** $p < 0.001$ *vs.* Control; ### $p < 0.001$ *vs.* OGD/R; && $p < 0.01$, &&& $p < 0.001$ *vs.* OGD/R+SenA+OV-NC. (For color figure see online version.)

results imply that SenA inhibited inflammatory response and oxidative stress in OGD/R-induced PC-12 cells *via* the inhibition of NLRP3.

SenA alleviates ferroptosis of OGD/R-induced PC-12 cells through inhibiting NLRP3

Compared with the OGD/R group, SenA treatment decreased JC-1 monomers while increasing JC-1 aggregates, which were subsequently reversed after overexpressing NLRP3 (Fig. 7A). Compared with OGD/R+SenA+OV-NC group, the decreased mtROS level in OGD/R-induced PC-12 cells with SenA treatment was partially increased in OGD/R+SenA+OV-NLRP3 group (Fig. 7B). Besides, the decreased Fe^{2+} and total iron levels in OGD/R+SenA+OV-NC were significantly increased following the transfection with OV-NLRP3 (Fig. 7C). Compared with the OGD/R+SenA+OV-NC group, NLRP3 overexpression increased the production of TBARS and the levels of MDA and 4-HNE whereas it decreased the levels of SOD and GSH-Px (Fig. 7D,E). Additionally, results obtained from Western blot revealed that SenA treatment increased the contents of SLC7A11 and GPX4 while decreasing the contents of ACSL4 and TFR1 compared with the OGD/R group, which were all reversed following the overexpression of NLRP3 (Fig. 7F).

Discussion

Brain I/R injury and its cellular mechanisms have been the focus of extensive research (Zhao et al. 2019). In the present study, brain I/R injury and its cellular mechanisms were thoroughly investigated, and complicated changes in oxidative stress, inflammatory response, and apoptosis were found. It was found that SenA has the potential to attenuate brain I/R injury and inhibit oxidative stress and inflammatory response. Meanwhile, SenA has regulatory effects on NLRP3 expression in OGD/R-induced PC-12 cells and can be used to target NLRP3-mediated ferroptosis.

SenA has attracted wide attention due to its anti-inflammatory, anti-endoplasmic reticulum stress, antioxidant, and anti-apoptotic properties (Shao et al. 2022). It has been evidenced that SenA can be used for the treatment of cerebrovascular diseases. For instance, SenA demonstrates immunomodulatory effects in the amelioration of atherosclerosis *via* downregulating AP-1 and NF-κB expression (Lei et al. 2019). Hu et al. have proved that SenA has certain protective effects on blood-brain barrier (Hu et al. 2016). Intriguingly, SenA has been reported to inhibit the excessive releases of pro-inflammatory cytokines and alleviate oxidative stress in rats with cerebral I/R injury (Lin 2016). However, the detailed reaction mechanism of SenA in cerebral I/R injury hasn't been discussed yet. Previous studies have shown that inflammation, apoptosis and oxidative stress are pivotal factors in brain damage during cerebral I/R injury (Gong et al. 2017; Zhang et al. 2017) and the intervention of these pathological processes is a viable therapy for cerebral I/R injury. The present study evidenced that SenA could alleviate PC-12 cell viability damage, oxidative stress and inflammation induced by OGD/R.

Ferroptosis is characterized by the over-production of lipid, which can cause oxidative stress and increase iron content. It has been implicated in various brain diseases, including cerebral ischemic disease (She et al. 2020). Accumulated studies have illuminated that targeting ferroptosis might be an effective method to ameliorate cerebral I/R injury (Guo et al. 2021; Li et al. 2021). In the present study, it was discovered that SenA could reduce mtROS level, decrease Fe^{2+} level, reduce the production of TBARS as well as decrease the levels of MDA, 4-HNE, ACSL4 and TFR1 in OGD/R-stimulated PC-12 cells, indicating the inhibitory effects of SenA on ferroptosis in cerebral I/R injury.

Previous study has suggested the potential of the NLRP3 inflammasome as a reliable pharmacological target for

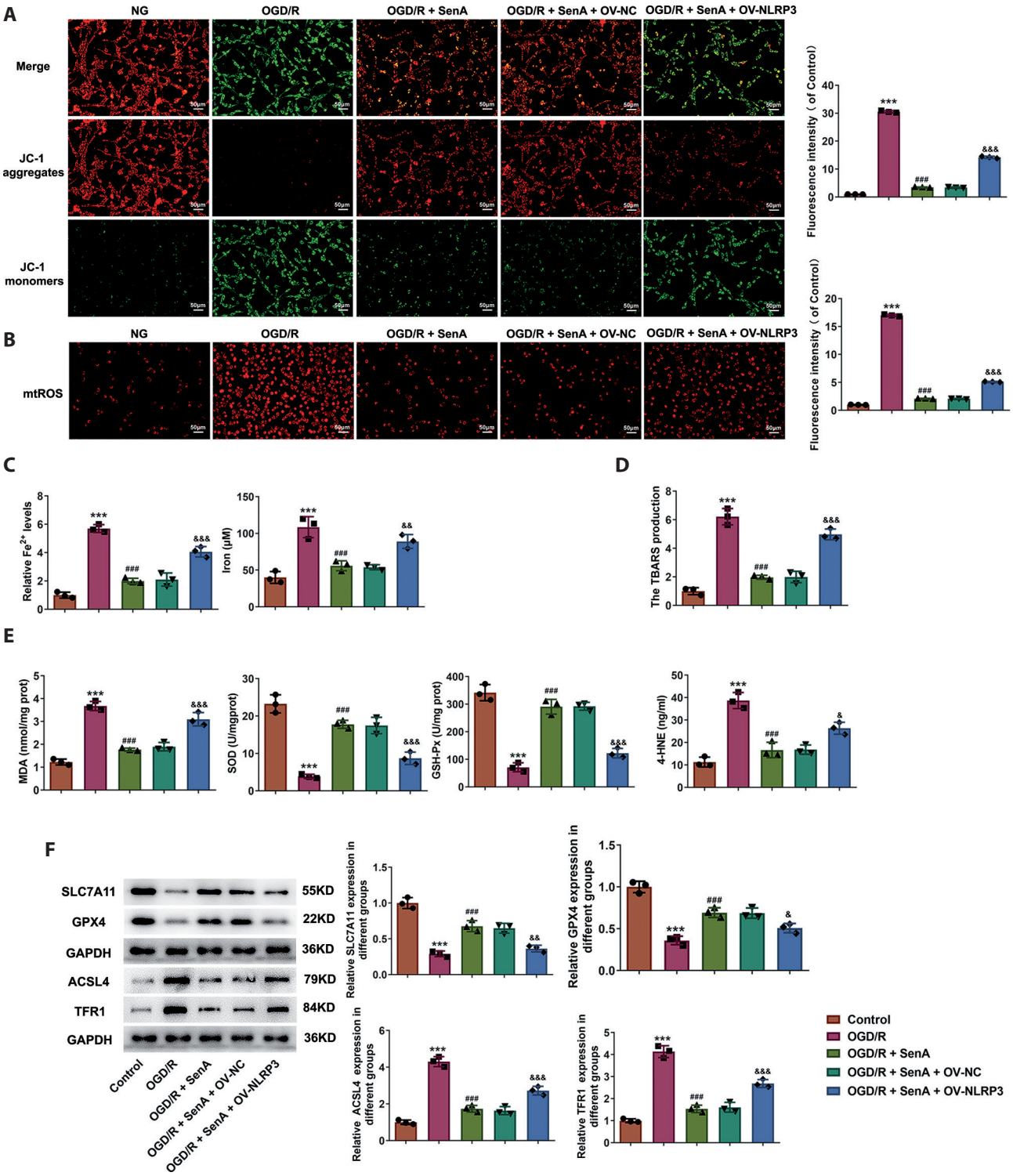

Figure 7. SenA alleviates ferroptosis of OGD/R-induced PC-12 cells through inhibiting NLRP3. **A.** The mitochondrial membrane potential was detected using JC-1 staining. **B.** The level of mtROS was detected using MitoSOX Red probe. Scale bar = 50 μm. **C.** Fe^{2+} and total iron levels were detected using corresponding assay kits. **D.** The production of TBARS was detected using TBARS assay. **E.** The expression levels of MDA, SOD, 4-HNE and GSH-Px were detected using corresponding assay kits. **F.** The expressions of ferroptosis-related proteins were detected using Western blot. *** $p < 0.001$ *vs.* Control; ### $p < 0.001$ *vs.* OGD/R; & $p < 0.05$, && $p < 0.01$, &&& $p < 0.001$ *vs.* OGD/R+SenA+OV-NC. (For color figure see online version.)

ameliorating cerebral I/R injury (Xu et al. 2022). Increasing studies have illustrated the relationship between the mechanisms of plant natural products alleviating cerebral I/R injury and the NLRP3 signaling pathway. For instance, Meisoindigo has been attested to protect against cerebral I/R injury *via* inactivating NLRP3 inflammasome both *in vivo* and *in vitro* (Ye et al. 2019). Peng et al. have shown that idebenone ameliorates brain damage of rats with I/R injury through inhibiting NLRP3 inflammasome activity (Peng et al. 2020). In this study, it was also revealed that SenA could decrease the expression of NLRP3 in PC-12 cells stimulated by OGD/R, which was consistent with the findings of previous study (Shao et al. 2022), indicating that SenA could block NLRP3 inflammasome activation.

Additionally, recent study has implicated that the activation of NLRP3 inflammasome can induce various types of cell death, including apoptosis and ferroptosis (Huang et al. 2021). The rescue experiments revealed that NLRP3 overexpression partially offset the inhibitory effects of SenA on viability damage, apoptosis, inflammation, oxidative stress and ferroptosis induced by OGD/R in PC-12 cells, implying that SenA protected against brain damage in I/R *via* inhibiting NLRP3 expression.

Conclusion

In summary, this study demonstrates the protective effects of SenA on OGD/R-induced cell viability damage, apoptosis, inflammation and oxidative stress in PC-12 cells as well as discloses that SenA can inhibit NLRP3 expression, which for the first time reveals the mechanism of by which SenA protects against cerebral I/R injury. However, it is still imperative to further investigate the mechanisms of SenA in cerebral I/R injury, especially its interactions with other signaling pathways.

Availability of data and materials. The analyzed data sets generated during the present study are available from the corresponding author on reasonable request.

Conflict of interest. The authors declare no competing financial interests.

Acknowledgements. This work was funded by Guangdong Basic and Applied Basic Research Foundation (2021A1515110473); Shenzhen Science and Technology Program (JCYJ20220530151401003); Luohu District soft science research project (LX202202045, LX202302094, LX202202048, LX20210101, LX202202127, LX202302101, LX202302102); Sanming Project of Medicine in Shenzhen (SZZYSM202201007), National Natural Science Foundation of China (82405329) and Guangdong Basic and Applied Basic Research Foundation (2019A1515011789).

Authors' contributions. QZ, HYW and YPQ conceived the experiments. QZ and YLW performed the experiments. YHX, ZQZ and TYZ analyzed the data. QZ and YLW confirmed the authenticity of all the raw data. All authors have read and approved the final manuscript.

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https://doi.org/10.1002/ptr.5985

Received: July 19, 2024
Final version accepted: September 2, 2024