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Aerobic exercise regulates the cGAS/STING signaling pathway and inhibits ferroptosis to alleviate ischemic stroke

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Abstract. Aerobic exercise at the appropriate intensity alleviates motor dysfunction after ischemic stroke (IS); however, its regulatory mechanisms remain to be elucidated. In this study, the neuroprotective effects of exercise and the related mechanisms were investigated using a rat middle cerebral artery occlusion (MCAO) model and an HT22/PC12 cell oxygen–glucose deprivation/reperfusion (OGD/R) model. The rats were subjected to daily exercise training for durations of 3, 7 or 14 days post-surgery. The results revealed that exercise, particularly the 14-day regimen, significantly reduced the mNSS and infarct area, restored the number of Nissl bodies, and ameliorated cortical pathology. Exercise also decreased Fe^{2+}, MDA, and IREB2 levels and increased SOD, GPX4, and SLC7A11 levels, indicating the inhibition of ferroptosis. In addition, the ferroptosis inhibitor Fer-1 attenuated OGD/R-induced damage in HT22 and PC12 cells. In terms of molecular mechanisms, we revealed that the cGAS/STING signaling pathway was activated and that knockdown of cGAS or STING restored cell viability and mitochondrial structure by inhibiting ferroptosis and reducing cell damage. Importantly, exercise also alleviated IS progression in rats by downregulating the expression of cGAS and STING. In conclusion, aerobic exercise inhibits ferroptosis *via* suppression of the cGAS/STING pathway, thereby alleviating IS progression.

Key words: Ischemic stroke — Exercise — cGAS/STING signaling pathway — Ferroptosis

Introduction

Stroke is the second leading cause of death and the third leading cause of disability worldwide, and its prevalence is increasing rapidly in low- and middle-income countries (Owolabi et al. 2022). Ischemic stroke (IS) accounts for approximately 80–85% of all strokes (Cui et al. 2021), can cause permanent damage to patients that affects their movement and cognitive communication ability, and may cause learning and memory impairment (Knecht 2011; Zhang et al. 2013). Recent clinical interventions have focused on blood flow restoration to salvage the ischemic penumbra, but once blood flow is restored, a condition known as ischemia-reperfusion (I/R) triggers a series of rapid neuropathic events, including but not limited to disorders of cell bioenergetic and redox balance; these disorders play multifaceted roles in further aggravating brain injury because the overproduction of reactive oxygen species (ROS) can induce cell death (Jin et al. 2022). Recent studies have shown that the use of iron chelators, such as deferoxamine mesylate (DFO), can prevent the production of free radicals and reduce the level of superoxide in the serum of stroke patients (Selim 2010). Interestingly, selenium supplementation after stroke effectively suppresses ferroptosis by increasing the expression of GPX4 (Alim et

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al. 2019). Therefore, inhibition of ferroptosis may be critical for the treatment of IS.

As a nondrug treatment, exercise can effectively reduce the incidence, severity and mortality of IS (Di Raimondo et al. 2020). Exercise has a variety of potential mechanisms through which it exerts therapeutic effects on stroke, including increasing the levels of neurotrophic factors (especially brain-derived neurotrophic factor (BDNF), reducing oxidative stress, inhibiting inflammation, and improving cerebral blood perfusion (Alkadhi 2018). Recent studies have shown that regular aerobic exercise attenuates IS-induced cognitive decline, making it a promising intervention for enhancing cognitive recovery and functional independence in patients (Choi et al. 2024). Furthermore, previous animal studies utilizing a middle cerebral artery occlusion (MCAO) model have demonstrated that exercise training improves motor function; alters the expression of plasticity-related proteins (BDNF and TrkB); and promotes cerebral angiogenesis, neurogenesis, functional changes in neuronal structure, and the resistance of hippocampal neurons to injury (Cui et al. 2020). Although the positive effect of exercise on functional prognosis after stroke has been widely recognized, whether exercise affects the progression of IS through the regulation of ferroptosis remains unclear.

In recent years, increasing research has focused on the emerging role of the cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) pathway. The cGAS-STING pathway is a crucial signaling cascade of the innate immune system that was initially studied extensively in the context of pathogen infection (Collins et al. 2015; Thomsen et al. 2016; Gallego-Marin et al. 2018; Domizio et al. 2022) and is now recognized as a significant factor in various ischemic diseases (King et al. 2017; Cao et al. 2018). By approximately 2020, its importance in IS began to gain wider recognition in the scientific literature (Li et al. 2020). Moreover, activation of the cGAS-STING pathway is related to excessive iron deposition in cells. Studies have confirmed that excessive activation of the cGAS-STING pathway during cerebral ischemia leads to NCOA4 overexpression, resulting in iron accumulation in neurons (Ma et al. 2023). The increase in the number of intracellular free iron ions induced by the cGAS-STING pathway is a key factor in ferroptosis. Notably, existing research suggests that exercise has the potential to modulate the cGAS-STING pathway. For instance, studies have shown that aerobic exercise can alleviate depression-like behaviors in female mice through the inhibition of the cGAS-STING signaling pathway (Chunchun et al. 2025). More importantly, the exercise-induced myokine irisin has been demonstrated to protect against contrast-induced acute kidney injury by inhibiting the cGAS-STING pathway (Long et al. 2025). This collective evidence indicates that exercise, as a physiological intervention, can regulate the cGAS-STING pathway. However, whether exercise can ameliorate ferroptosis after IS by regulating the cGAS-STING pathway remains to be elucidated and warrants further investigation.

In summary, this study aimed to investigate whether aerobic exercise slows the progression of IS by inhibiting ferroptosis through regulation of the cGAS-STING pathway, thereby providing a new theoretical basis and potential intervention target for the prevention and treatment of this disease.

Materials and Methods

Establishment and treatment of the middle cerebral artery occlusion (MCAO) rat model

Eight-week-old male Sprague-Dawley rats (220–240 g) were purchased from Beijing Charles River Laboratory Animal Technology Co., Ltd. The rats were housed in a specific pathogen-free facility. After one week of acclimatization, the rats were randomly divided into the following groups: Sham group, MCAO-3d group, MCAO-7d group, MCAO-14d group, MCAO+EXE-3d group, MCAO+EXE-7d group, MCAO+EXE-14d group, MCAO+sh-cGAS group, and MCAO+EXE+OE-cGAS group, with 5 rats in each group. After the rats were anesthetized with 4% isoflurane, a midline incision was made in the neck region to expose the right common carotid artery and external carotid artery. The distal end of the external carotid artery was ligated with a 6.0 mm nylon monofilament. Another suture was passed through the external carotid artery and tied near the bifurcation of the common carotid artery. The common carotid artery was clamped with an arterial clip, and the nylon monofilament was inserted into the external carotid artery and internal carotid artery and further advanced into the middle cerebral artery. After 1.5 h of ischemia, the monofilament was removed to restore blood flow, and the neck incision was sutured. In the Sham group rats, the structure of the common carotid artery was exposed up to the bifurcation, after which the incision was sutured. No other procedures were performed. At the end of the experiment, the rats were euthanized by cervical dislocation, and their brains were collected for the measurement of relevant indicators.

For the rats in the exercise (EXE) groups, acclimatization to aerobic treadmill training began 3 days prior to MCAO modeling. To avoid any impact of exercise preconditioning on the nervous system, acclimatization training was conducted at a speed of 1–2 m/min for 30 min to familiarize each rat with the treadmill environment. Formal aerobic training commenced on the 2nd day post-modeling (24 h later). The initial formal aerobic training was conducted at a speed of 6 m/min for 30 min starting from the 2nd day of training, the speed was adjusted to 12 m/min (Zhang et al.

2013), while the duration remained 30 min. Depending on the time point of euthanasia, the rats in the exercise groups were trained continuously for 3, 7, or 14 days. The training speed of 12 m/min adopted in this study is based on literature evidence for rats of the same strain (Fei et al. 2020), which has confirmed that this speed constitutes an effective "aerobic" exercise stimulus.

For rats in the cGAS knockdown or overexpression groups, an adenovirus for cGAS knockdown or overexpression was injected into the left lateral ventricle *via* stereotaxic injection 14 days before MCAO. Before virus injection, the rats were anesthetized with 4% isoflurane and fixed on a stereotaxic frame (RWD, China). The liquid (5 μl) was slowly injected into the lateral ventricle at a rate of 0.5 μl/min using a microinfusion pump (RWD, China), with a virus titer of 1×10^{13} vg/ml. The stereotaxic coordinates of the injection site from the bregma were as follows: posterior: 0.9 mm; medial and lateral: 1.4 mm; and dorsoventral: 3.8 mm.

Modified neurological severity score (mNSS)

The mNSS was determined according to the methods of Zhao et al. (2025). The mNSS is a comprehensive index of motor, sensory, reflex, and balance tests. Neurological function was graded according to 0–18 points, where 0 points indicated a normal level and a total score of 18 points indicated severe deficit.

2,3,5-Triphenyltetrazolium chloride (TTC) staining

After the animals were sacrificed, the brains were quickly removed and cut into 2 mm thick sections. The sections were then placed in 2% TTC solution (Beyotime, China) and stained at 37°C for 30 min, after which the container was gently shaken every 5 min. to allow sufficient staining. After staining, the sections were photographed to determine the ischemic infarct volume.

Nissl staining

The obtained paraffin-embedded brain tissue sections were dewaxed with xylene, hydrated through a graded alcohol series, and rinsed with distilled water. The samples were subsequently stained with Nissl staining solution (Beyotime, China) for 5 min, followed by two additional rinses with distilled water. The stained brain tissue was then observed under a microscope (Nikon, Japan).

Hematoxylin and eosin (H&E) staining

Brain tissue sections embedded in paraffin were stained with the reagents provided in an HE staining kit (Solarbio, China). Briefly, the sections were dewaxed in xylene, hydrated in a gradient series of alcohol solutions, stained with hematoxylin staining solution for 5 min, and then washed with distilled water to remove floating stains. Afterward, differentiation solution was added dropwise for 30 s, and the cells were subsequently washed twice with tap water. Next, they were stained with eosin solution for 1 min and then dehydrated and cleared by immersion in a gradient series of alcohol solutions and xylene. Finally, after the samples were sealed with neutral resin, they were observed and photographed using a light microscope (Olympus, Japan).

Cell culture and transduction

Mouse hippocampal neuronal HT22 cells and rat adrenal pheochromocytoma PC12 cells (differentiation) were purchased from Wuhan Procell Life Technology Co., Ltd. HT22 cells were cultured in DMEM (Gibco, USA), and PC12 cells were cultured in RPMI-1640 medium (Gibco, USA). Both media were supplemented with 10% fetal bovine serum (Gibco, USA) and 1% penicillin/streptomycin (Gibco, USA). The cells were routinely maintained in an incubator at 37°C with 5% CO_2. To establish an oxygen–glucose deprivation/reperfusion (OGD/R) model, HT22 and PC12 cells were cultured in 6-well plates until they reached 80% confluence. OGD was then performed using glucose-free and serum-free medium in a hypoxic chamber (1% O_2, 5% CO_2, 94% N_2). After 3 h of OGD, the cells were returned to normal culture conditions and incubated for an additional 24 h. To investigate the role of ferroptosis, varying concentrations (1.25 μM, 2.5 μM, 5 μM, 10 μM, and 20 μM) of the ferroptosis inhibitor ferrostatin-1 (Fer-1, HY-100579, MCE, USA) were added to the culture medium of the HT22 and PC12 cells at the onset of reperfusion. To explore the role of the cGAS-STING signaling pathway in OGD/R-induced cell injury, HT22 and PC12 cells were seeded in 6-well plates. When the cells reached approximately 50% confluence, they were infected with lentiviral vectors (GenePharma, Shanghai, China) carrying sh-STING, sh-cGAS, OE-STING, or the corresponding negative control at a titer of 1×10^{8} TU/ml (MOI = 100) for 14 h. Subsequently, the medium was replaced with complete culture medium. At 72 h post infection, cells were selected with 4 μg/ml puromycin (HY-K1057; MCE, USA) to establish stably infected cell lines, and the infection efficiency was validated by Western blotting.

CCK-8 assay

HT22 and PC12 cells (5×10^{3} cells/well) were seeded in 96-well plates and cultured at 37°C in a 5% CO_2 incubator for 24 h. The cells in each group were treated according to their groupings and then added to each well after treatment.

A total of 10 μl of CCK-8 reagent (Beyotime, China) was then added for another 2 h. The absorbance value of each well was measured at 450 nm using a microplate reader (Thermo Fisher Scientific, MA, USA).

Determination of MDA, SOD, and Fe^{2+} levels

The levels of malondialdehyde (MDA), superoxide dismutase (SOD), and ferrous iron (Fe^{2+}) in rat brain tissue or in HT22 and PC12 cells were evaluated according to the instructions of an MDA assay kit (A003-4-1; Nanjing Jiancheng Bioengineering Institute, China), a SOD assay kit (A001-3; Nanjing Jiancheng Bioengineering Institute, China), and an iron ion assay kit (BC5415; Solarbio, China), respectively.

ROS detection

HT22 and PC12 cells in each group were seeded into 6-well plates at 2×10^5 cells *per* well, cultured in a cell culture incubator with 5% CO_2 for 24 h, and then centrifuged. The medium was discarded. The cells were subsequently resuspended in D-Hanks solution. For each sample, 500 μl of D-Hanks solution containing 20 μl of DCFH-DA (Beyotime, China) was added, and the mixture was incubated in the incubator in the dark for 30 min. The mixture was fully combined and shaken once every 5 min. After centrifugation at 300×*g* for 5 min, the samples were washed twice with D-Hanks solution. The cells were subsequently resuspended in D-Hanks solution again, and the ROS level in the cells was detected *via* fluorescence microscopy (NiKon, Japan).

Western blot analysis

Total protein was isolated from rat brain tissue or from HT22 and PC12 cells using RIPA lysis buffer (Beyotime, China) supplemented with protease phosphatase inhibitor cocktail (Beyotime, China), and the protein concentration was determined using a BCA protein concentration assay kit (Beyotime, China). Equal amounts of protein were then separated by SDS-PAGE and transferred to PVDF membranes. After the membrane was blocked with PBS containing 5% skim milk for 1 h at room temperature, the membrane was incubated with primary antibodies against STING (1:2000; ab288157; Abcam, UK), cGAS (1:1000; PA5-141097; Thermo Fisher Scientific, USA), IREB2 (1:1000; PA5-19158; Thermo Fisher Scientific, USA), GPX4 (1:1000; ab125066; Abcam, UK), SLC7A11 (1:2000; ab307601; Abcam, UK), and β-actin (1:1000; ab8227; Abcam, UK) at 4°C overnight and then incubated with the corresponding secondary antibodies for 2 h at room temperature. Detection was performed using a Super Enhanced Chemiluminescence (ECL) detection kit (Thermo Fisher Scientific, USA), and analysis was conducted using ImageJ software.

Statistical analysis

All experimental data in this study are presented as the mean ± standard deviation (SD). For the animal experiments, each group contained 5 biological replicates (n = 5). For the cell experiments, each assay was independently performed three times (n = 3). Data analysis and graphing were performed using GraphPad Prism 8 software. Prior to parametric testing, all the data were assessed for normality using the Shapiro-Wilk test. For normally distributed data, comparisons between two groups were conducted using a two-tailed Student's t test, while comparisons among multiple groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's test for post hoc pairwise comparisons. A p value < 0.05 was considered to indicate statistical significance.

Results

Exercise alleviates ischemic stroke through the inhibition of ferroptosis

We first investigated the effects of different durations of exercise training on IS in rats. The mNSS results revealed that compared with the model group at each corresponding time point, rats that underwent 3, 7, or 14 days of exercise training after MCAO exhibited significantly lower neurological deficit scores (Fig. 1A). TTC staining revealed that exercise training markedly reduced the cerebral infarct volume (Fig. 1B). Nissl staining revealed that exercise alleviated neuronal edema, increased the number of neurons with normal morphology, and increased the quantity of Nissl bodies in the ischemic cortex (Fig. 1C). H&E staining further confirmed that exercise training ameliorated cellular structural disarray and neuronal damage in the ischemic cortex (Fig. 1D). In addition, studies have shown that ferroptosis plays important roles in the occurrence and development of stroke (Alim et al. 2019). Therefore, to explore whether exercise regulates ferroptosis, we examined relevant indicators. The results revealed that exercise significantly reduced the Fe^{2+} content and MDA levels but increased the activity of SOD in the brain tissue (Fig. 1E). Western blot analysis indicated that exercise downregulated the expression of IREB2 and upregulated the expression of GPX4 and SLC7A11 (Fig. 1F). These results suggest that exercise training alleviates neurological injury after IS by inhibiting ferroptosis.

Inhibition of ferroptosis can relieve OGD/R-induced neuronal injury

To investigate the role of ferroptosis in neuronal cell injury in vitro, HT22 and PC12 cells were treated with various concen-

trations (1.25, 2.5, 5, 10, and 20 μM) of the ferroptosis inhibitor Fer-1. The results revealed that 10 μM Fer-1 most strongly restored cell viability in OGD/R-treated HT22 cells, whereas 2.5 μM Fer-1 exerted the strongest restorative effect on cell viability in PC12 cells (Fig. 2A). Subsequent experiments were conducted using the respective optimal Fer-1 concentrations for each cell line. Biochemical assay results revealed that after OGD/R induction, the Fe^{2+} content and MDA content in

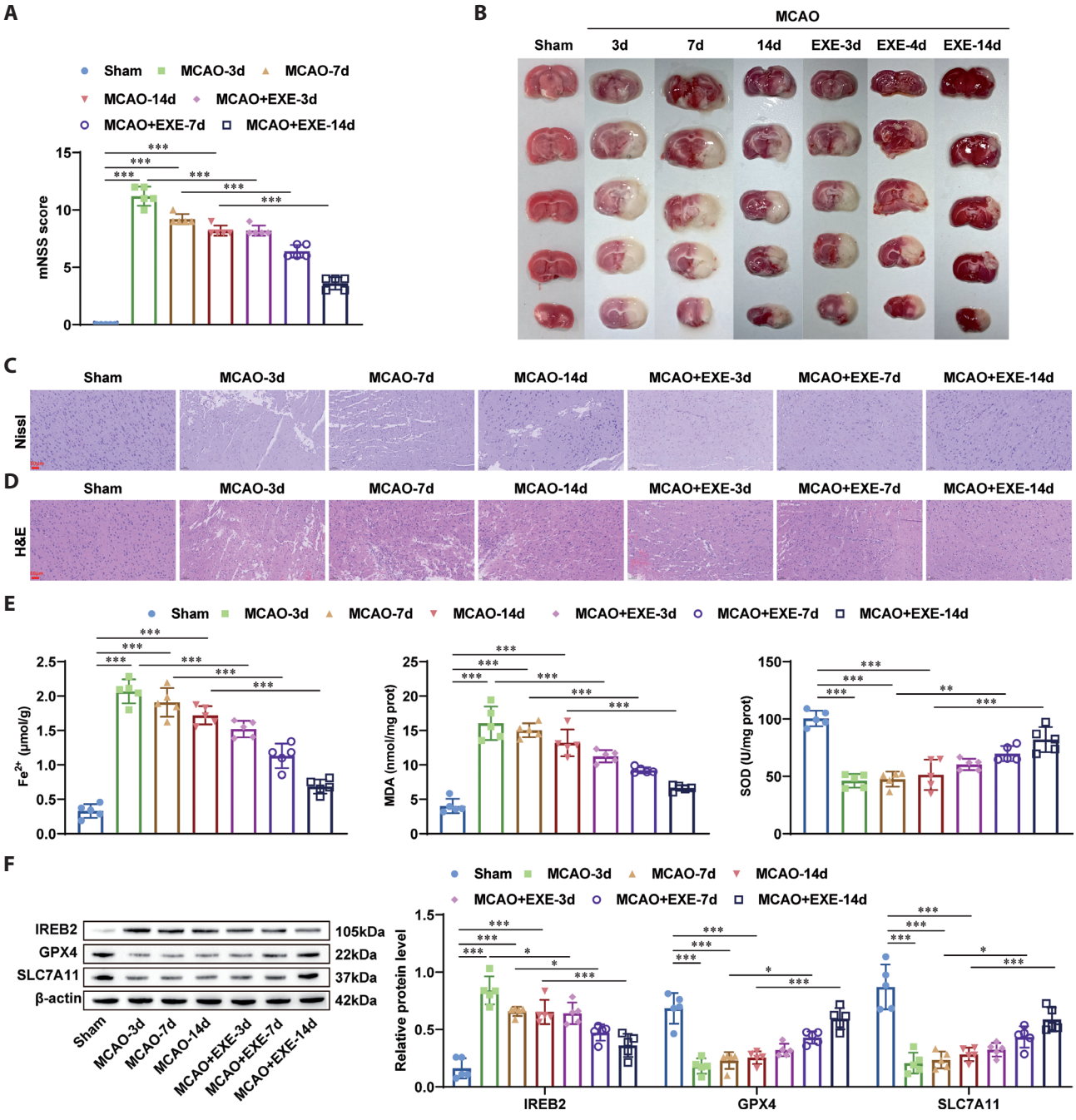


Figure 1. Exercise alleviates ischemic stroke through the inhibition of ferroptosis. **A.** Rat mNSS score. **B.** Cerebral infarct area in rats detected by TTC staining. **C.** Neuronal injury in the ischemic cortices of rats detected by Nissl staining. **D.** Pathological changes in the ischemic cortices of rats detected by H&E staining. **E.** The levels of Fe^{2+}, MDA, and SOD in rat brain tissue were measured using commercial kits. **F.** Western blot detection of the expression of the ferroptosis-related proteins IREB2, GPX4, and SLC7A11 in rat brain tissue. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

the HT22 and PC12 cells significantly increased, the SOD level significantly decreased, and the addition of Fer-1 partly attenuated the induction effect of OGD/R (Fig. 2B–D). The results of the immunofluorescence analysis revealed that OGD/R significantly increased the levels of ROS, whereas the addition of Fer-1 inhibited ROS production (Fig. 2E). Detection of the expression of ferroptosis-related proteins *via* Western blotting revealed that OGD/R increased the expression of IREB2 and decreased the expression of GPX4 and SLC7A11; the expression of these related proteins was

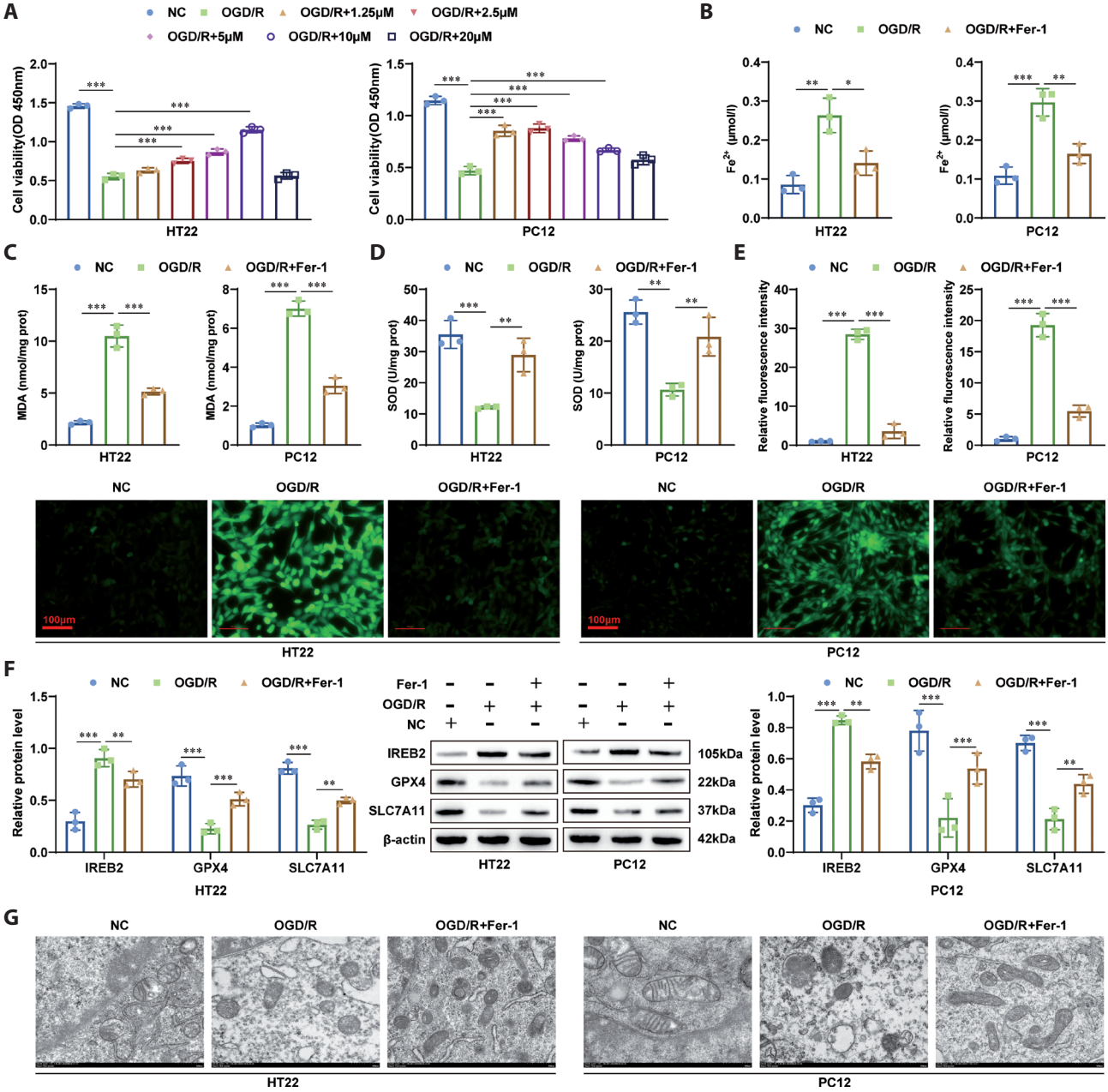


Figure 2. Inhibition of ferroptosis attenuated OGD/R-induced neuronal injury. **A.** CCK-8 assay for determining the viability of the HT22 and PC12 cells. **B.** Fe^{2+} content of the HT22 and PC12 cells measured using commercial kits. **C.** MDA content of the HT22 and PC12 cells measured using commercial kits. **D.** SOD content of the HT22 and PC12 cells measured using commercial kits. **E.** ROS content in the HT22 and PC12 cells detected by immunofluorescence. **F.** Western blot detection of the expression of the ferroptosis-related proteins IREB2, GPX4, and SLC7A11 in the HT22 and PC12 cells. **G.** The mitochondrial ultrastructures of the HT22 and PC12 cells were examined by TEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

partially attenuated after the addition of Fer-1 (Fig. 2F). Finally, transmission electron microscopy (TEM) analysis of mitochondrial ultrastructure revealed that OGD/R induced mitochondrial shrinkage, mitochondrial rounding, cristae loss, and occasional vacuolization in HT22 and PC12 cells. In contrast, Fer-1 treatment partially reversed these alterations, as evidenced by increased mitochondrial size, clearer cristae structures, reduced shrinkage, and restored morphological integrity (Fig. 2G). These results indicate that OGD/R induces neural injury by promoting ferroptosis.

Knocking down STING expression can alleviate nerve cell damage by inhibiting ferroptosis

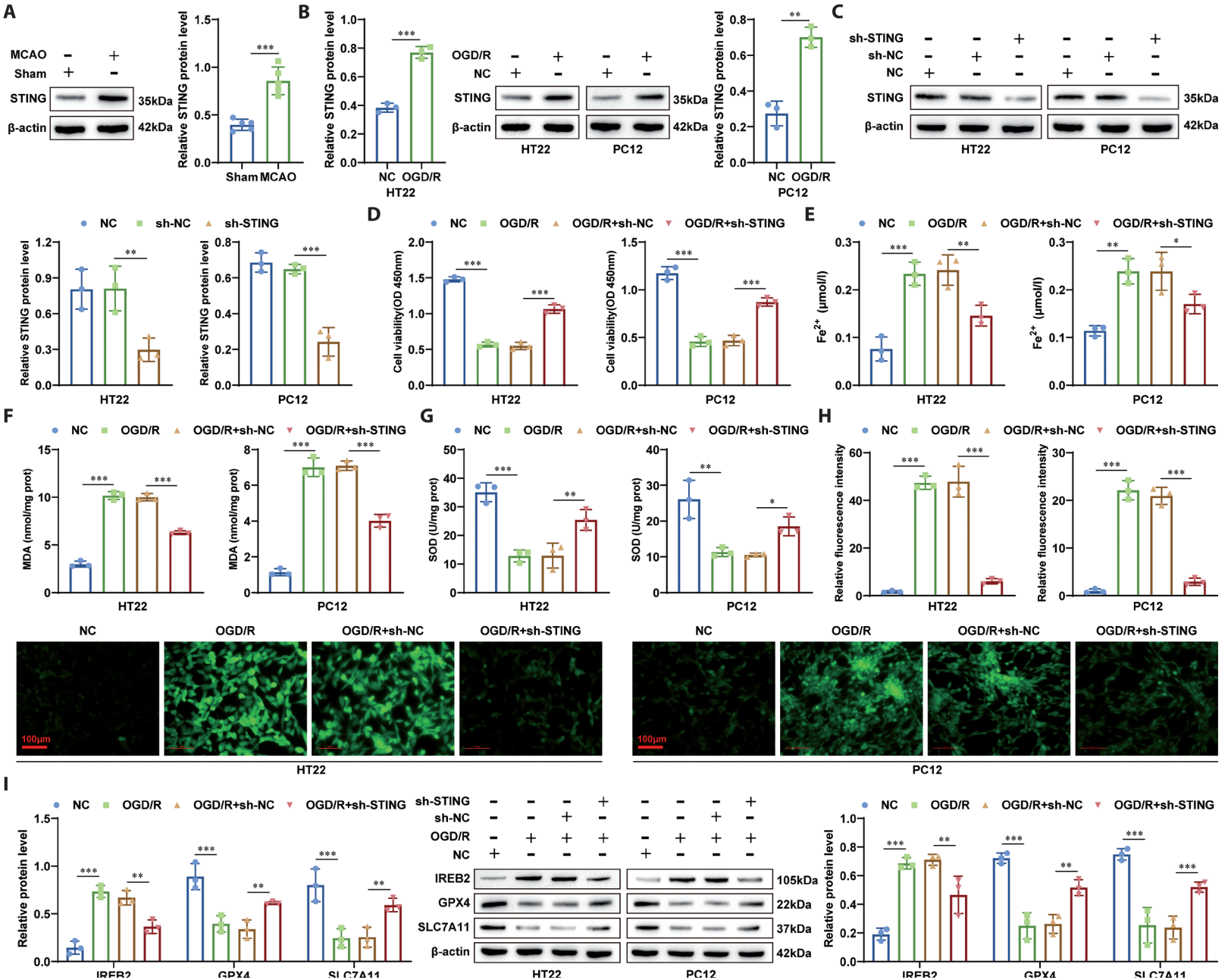
STING not only is associated with IS but also regulates the activation of ferroptosis (Ma et al. 2023). Therefore, we examined the expression of STING in IS and found that STING expression was upregulated in MCAO model rats and in HT22 and PC12 cells subjected to OGD/R (Fig. 3A,B). To verify the effect of STING, we knocked down STING in HT22 and PC12 cells, and the expression level of STING was significantly reduced in the sh-STING group (Fig. 3C). The results of the CCK-8 assay revealed that knocking down STING expression could partially restore the viability of the HT22 and PC12 cells (Fig. 3D). Biochemical assay and immunofluorescence assays revealed that compared with the OGD/R+sh-NC group, the groups in which STING was knocked down had significantly lower Fe^{2+} levels, MDA levels, and ROS levels but higher SOD levels in HT22 and PC12 cells (Fig. 3E–H). Moreover, knocking down STING decreased the expression of IREB2 and increased the expression of GPX4 and SLC7A11 (Fig. 3I). Finally, TEM revealed that compared with those in the OGD/R+sh-NC group, some mitochondria in the STING knockdown group exhibited normal morphology, clearly visible cristae (Fig. 3J). These results indicate that STING expression is upregulated in IS and that knocking down STING expression can relieve neuronal injury through the inhibition of ferroptosis.

OGD/R induction promotes ferroptosis in neuronal cells by activating the cGAS/STING signaling pathway

Furthermore, as an upstream regulator of STING, cGAS effectively activates STING expression, and the cGAS/STING signaling pathway has been shown to promote ischemic brain injury (Ma et al. 2023). Therefore, we further investigated the role of this signaling pathway in neuronal ferroptosis. First, Western blot analysis revealed that cGAS expression was also upregulated in MCAO model rats and in OGD/R-treated HT22 and PC12 cells (Fig. 4A,B). To investigate the role of the cGAS/STING signaling pathway in neuronal ferroptosis, we knocked down cGAS or overexpressed STING in HT22 and PC12 cells. The knockdown and overexpression efficiencies were validated by Western blot analysis (Fig. 4C,D). The results of the CCK-8 assay revealed that compared with OGD/R, knocking down cGAS expression significantly increased the viability of HT22 and PC12 cells, and further overexpression of STING reduced cell viability (Fig. 4E). Biochemical assay and immunofluorescence assays revealed that compared with OGD/R, cGAS knockdown significantly reduced the Fe^{2+} content, MDA level, and ROS level but increased the SOD level in HT22 and PC12 cells. Moreover, further overexpression of STING partially abolished the effects of cGAS knockdown (Fig. 4F–I). Moreover, knocking down cGAS decreased the expression of IREB2 and increased the expression of GPX4 and SLC7A11. STING overexpression partially attenuated the effect of cGAS knockdown (Fig. 4J). Finally, TEM observations of mitochondrial ultrastructure revealed that the beneficial effect of cGAS knockdown on mitochondrial structure could be weakened by STING overexpression (Fig. 4K). These results suggest that OGD/R-induced ferroptosis in neuronal cells is promoted through the activation of the cGAS/STING signaling pathway.

Exercise alleviates ischemic stroke by inhibiting ferroptosis through regulation of the cGAS/STING signaling pathway

To improve the scientific rigor and accuracy of the experiments, we validated whether exercise alleviates IS by regulating the cGAS/STING signaling pathway in a rat model. The mNSS results revealed that compared with the MCAO (3 d) group, both the exercise (14 d) group and the cGAS knockdown group had significantly lower neurological deficit scores. However, overexpression of cGAS in exercised rats partially reversed the protective effects of exercise, leading to increased mNSS score (Fig. 5A). The tissue staining results demonstrated that compared with the MCAO group, both the exercise group and the cGAS knockdown group had significantly reduced cerebral infarct volume, increased numbers of Nissl bodies, and ameliorated pathological damage in the ischemic cortex. However, overexpression of cGAS in exercised rats partially abrogated these exercise-induced protective effects on brain tissue (Fig. 5B–D). Furthermore, Biochemical assay results revealed that exercise or cGAS knockdown significantly reduced the Fe^{2+} content and MDA levels but increased the SOD level in brain tissue. However, overexpression of cGAS in exercised rats increased Fe^{2+} content and MDA levels and decreased SOD levels (Fig. 5E). Western blot analysis revealed that, compared with the MCAO group, exercise or cGAS knockdown significantly upregulated the expression of GPX4 and SLC7A11 and downregulated IREB2, cGAS, and STING expression. Notably, cGAS overexpression in exercised rats partially attenuated the regulatory effects of exercise on these proteins (Fig. 5F,G). Taken together, these findings indicate that exercise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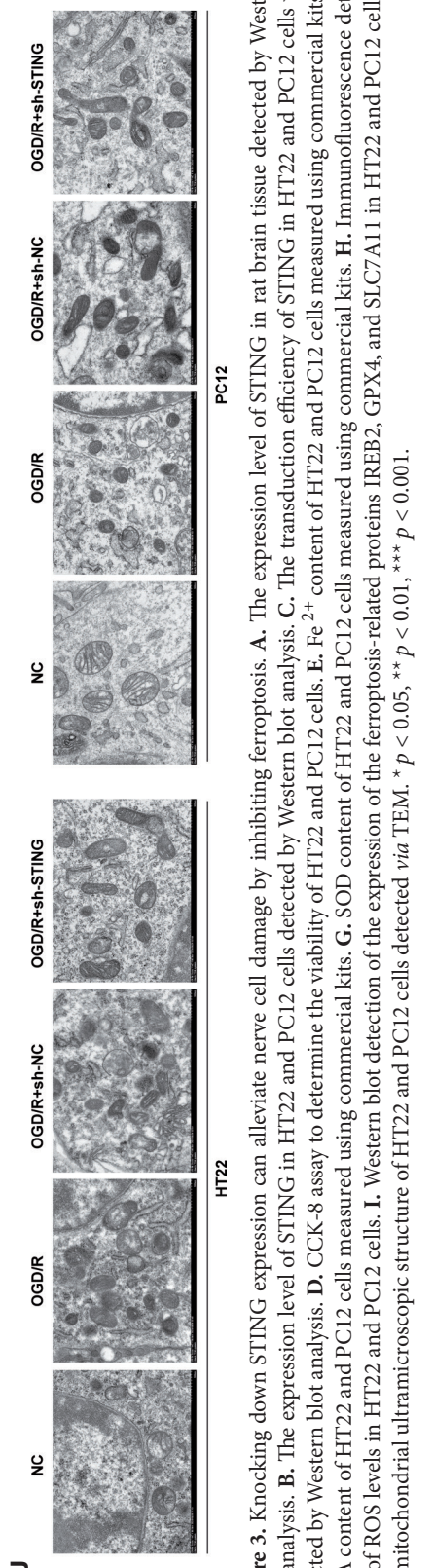


Figure 3. Knocking down STING expression can alleviate nerve cell damage by inhibiting ferroptosis. **A.** The expression level of STING in rat brain tissue detected by Western blot analysis. **B.** The expression level of STING in HT22 and PC12 cells detected by Western blot analysis. **C.** The transduction efficiency of STING in HT22 and PC12 cells was detected by Western blot analysis. **D.** CCK-8 assay to determine the viability of HT22 and PC12 cells. **E.** Fe^{2+} content of HT22 and PC12 cells measured using commercial kits. **F.** MDA content of HT22 and PC12 cells measured using commercial kits. **G.** SOD content of HT22 and PC12 cells measured using commercial kits. **H.** Immunofluorescence detection of ROS levels in HT22 and PC12 cells. **I.** Western blot detection of the expression of the ferroptosis-related proteins IREB2, GPX4, and SLC7A11 in HT22 and PC12 cells. **J.** The mitochondrial ultramicroscopic structure of HT22 and PC12 cells detected *via* TEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

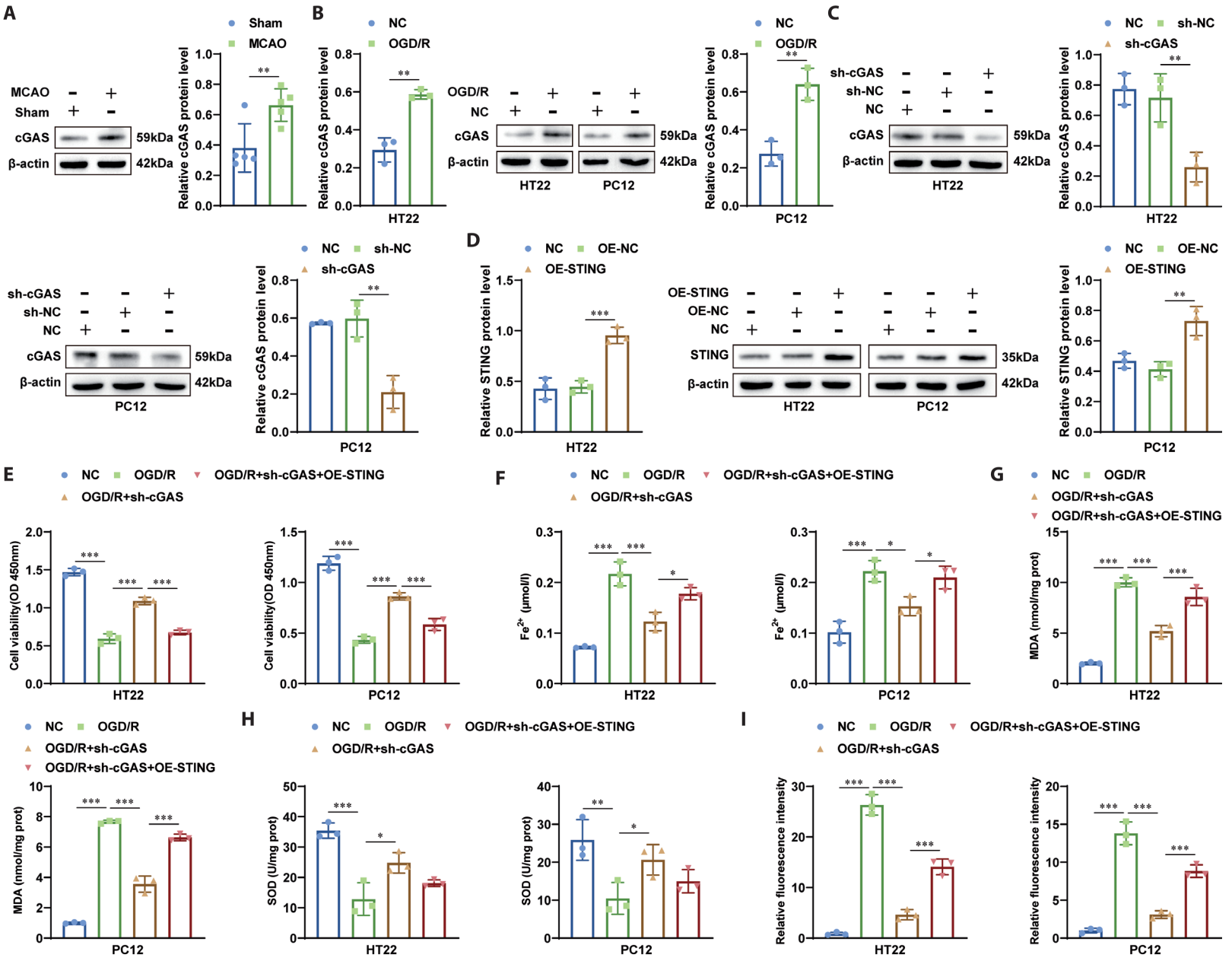
alleviates IS by inhibiting ferroptosis through modulation of the cGAS/STING signaling pathway.

Discussion

IS a major global health problem that is characterized by interruption of the blood supply to the brain, resulting in various neurological dysfunctions (Zhu et al. 2025). In this study, we revealed that exercise effectively reduced the mNSS, decreased the cerebral infarct area, restored the number of Nissl bodies, and ameliorated pathological damage to the ischemic cortices of MCAO model rats through the inhibition of ferroptosis. With respect to the underlying molecular mechanism, the expression of cGAS and STING was upregulated in MCAO-induced rats and OGD/R-induced HT22 and PC12 cells. Exercise protected against the development of IS by inhibiting the activation of the cGAS/STING signaling pathway, thereby suppressing ferroptosis.

In the treatment of stroke, exercise is a simple and widely used method with neuroprotective effects in humans and animals. A study comparing three groups of patients (exercise, social enrichment and control groups) revealed that exercise can improve cognitive function in elderly patients with chronic stroke in a cost-effective manner (Adjetey et al. 2023). Similarly, increasing and continuing light-intensity physical activity in the subacute stage after stroke can improve functional recovery at 6 months (Buvarp et al. 2023). Furthermore, in animal studies, exercise has been shown to enhance neuroprotection and neurogenesis, stimulate nerve repair, and promote functional recovery after IS (Cheng et al. 2020). Moreover, exercise can have therapeutic effects both before and after stroke. Wang et al. (2014) reported that treadmill exercise before ischemia could reduce cerebral infarct volume, cerebral edema, and neurological deficits in rats and alleviate brain injury after IS. Zhao et al. (2017) reported that in adult rats after IS, treadmill exercise can improve neurological recovery in ischemic rats by increasing the proliferation, migration and differentiation of infraventricular zone-derived neural stem cells (NPCs) in the ischemic penumbra. In this study, we explored mainly the effects of exercise training after IS on the progression of IS in rats. We found that continuous exercise for 3, 7, and 14 days reduced the mNSS and decreased the infarct area, with the 14-day exercise group showing the greatest improvement. Previous studies and our study both proved that exercise can protect against IS. However, some studies have reported that neuronal death is the leading cause of the progression of IS (Tani et al. 2023). Therefore, our research focused on how exercise regulates neuronal death.

A previous study revealed that neuronal necrosis and apoptosis play important roles in acute cerebral ischemia (Gilgun-Sherki et al. 2002). However, clinical experiments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Relative STING protein level
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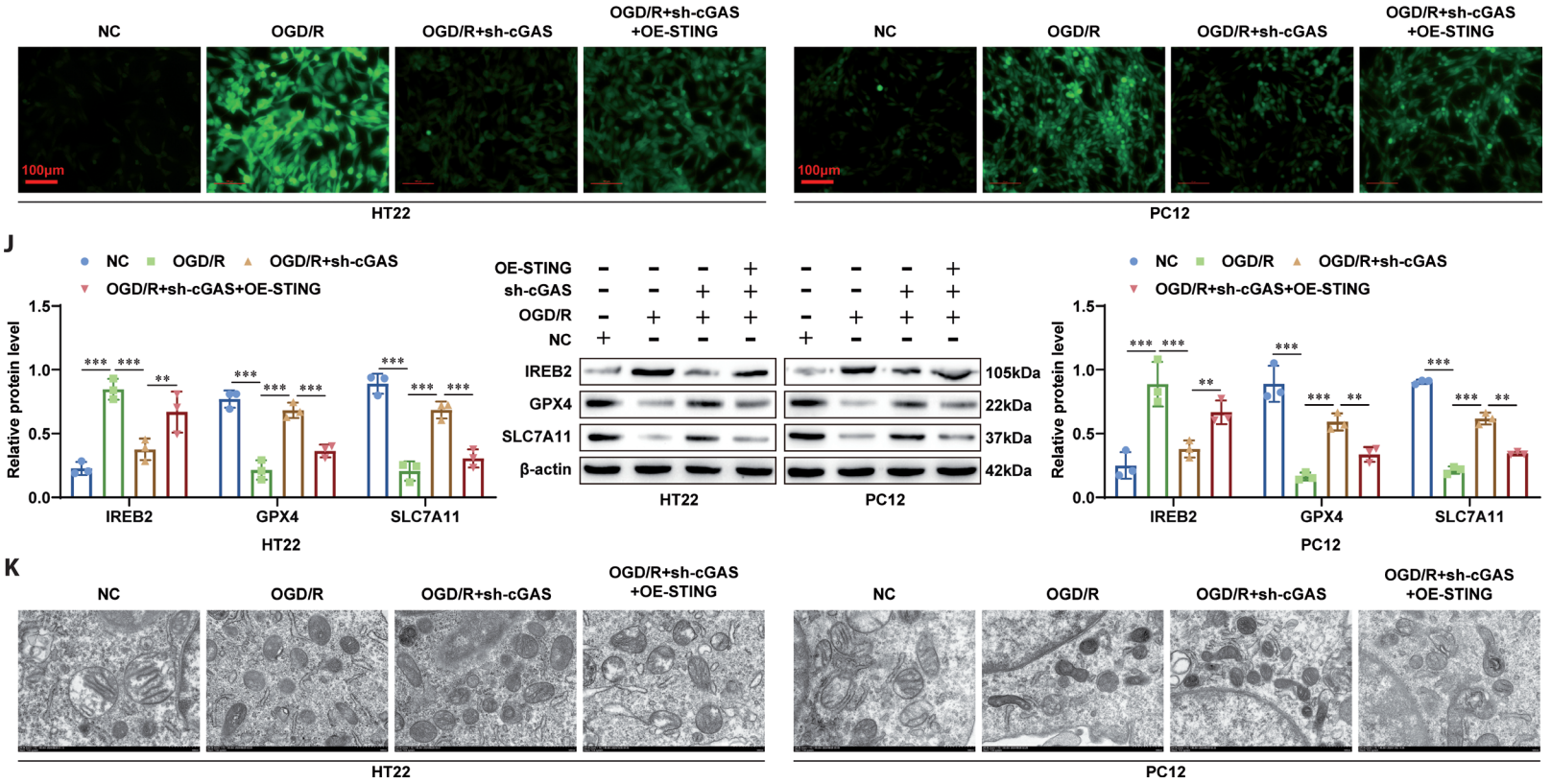

Figure 4. OGD/R induction promotes ferroptosis in neuronal cells by activating the cGAS/STING signaling pathway. **A.** Western blot detection of cGAS expression levels in rat brain tissue. **B.** Western blot detection of cGAS expression levels in HT22 and PC12 cells. **C.** Western blot detection of cGAS transduction efficiency in HT22 and PC12 cells. **D.** Western blot analysis was used to detect the transduction efficiency of STING in HT22 and PC12 cells. **E.** Cell viability of HT22 and PC12 cells assessed using a CCK-8 assay. **F.** Fe^{2+} contents in HT22 and PC12 cells measured using commercial kits. **G.** The MDA content in HT22 and PC12 cells was measured using commercial kits. **H.** SOD content in HT22 and PC12 cells measured using commercial kits. **I.** ROS content in HT22 and PC12 cells detected by immunofluorescence. **J.** Western blot detection of the expression of the ferroptosis-related proteins IREB2, GPX4 and SLC7A11 in HT22 and PC12 cells. **K.** The mitochondrial ultramicroscopic structure of HT22 and PC12 cells was examined *via* TEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

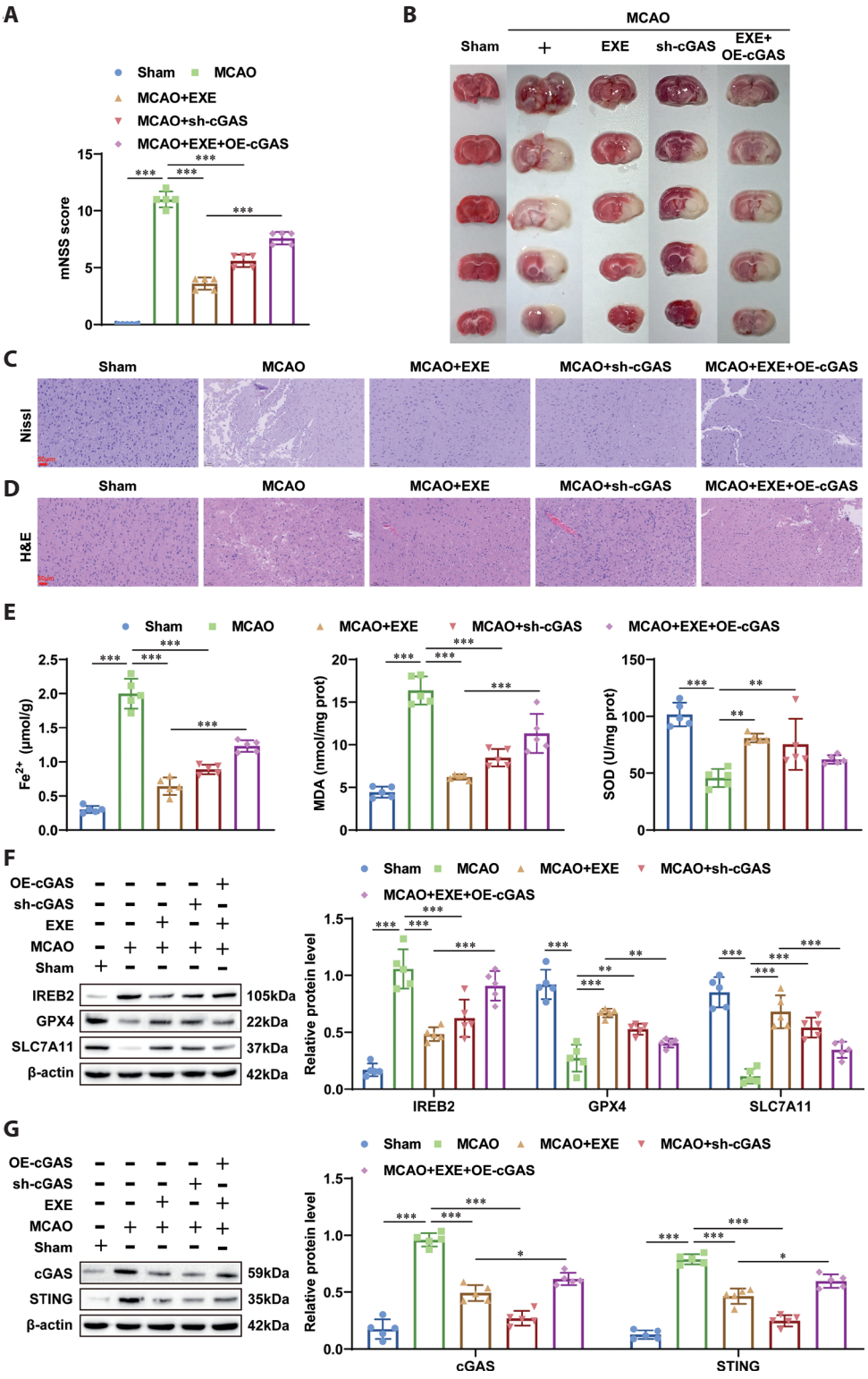

Figure 5. Exercise alleviates ischemic stroke by inhibiting ferroptosis through regulation of the cGAS/STING signaling pathway. **A.** Rat mNSS score. **B.** Cerebral infarct area in rats detected by TTC staining. **C.** Neuronal injury in the ischemic cortices of rats detected by Nissl staining. **D.** Pathological changes in the ischemic cortices of rats detected by H&E staining. **E.** The levels of Fe^{2+}, MDA, and SOD in rat brain tissue were measured using commercial kits. **F.** The expression of the ferroptosis-related proteins IREB2, GPX4, and SLC7A11 in rat brain tissue was detected by Western blot analysis. **G.** Western blot detection of the expression of cGAS and STING in rat brain tissue. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

targeting neuronal necrosis and apoptosis caused by cerebral ischemia have failed, indicating that other forms of neuronal death may also play key roles (Fricker et al. 2018; Hribljan et al. 2019). Ferroptosis, a newly discovered type of regulated cell death, is defined as an iron dependent, lipid peroxide-driven form of cell death that differs from other types of cell death processes (Tal and Brent 2018; Matthew et al. 2019). In IS, iron accumulation and redistribution, glutamate accumulation, oxidative stress, lipid peroxidation, and epigenetic regulation are the key driving factors of ferroptosis, making it a core mechanism in stroke pathology and neuronal death (Bu et al. 2021). Studies have shown that ferroptosis inhibitors can protect neurons from ischemia-reperfusion injury, strongly indicating that the inhibition of neuronal ferroptosis is a key therapeutic target for the treatment of IS and reperfusion injury (Liu et al. 2024). Huang et al. (2025) reported that Buyang Huanwu decoction inhibited ferroptosis through the Nrf2/GPX4 pathway, thus exerting its neuroprotective effect on IS. Similarly, Li et al. (2024) reported that caffeic acid inhibited ferroptosis through the Nrf2 signaling pathway, thereby attenuating IS injury in rats. Here, we also examined the changes in ferroptosis-related indicators in IS rats and reported that after MCAO modelling, the levels of Fe^{2+} and MDA as well as the expression of IREB2 significantly increased, whereas the levels of SOD and the expression of GPX4 and SLC7A11 significantly decreased. Exercise partially counteracted the effects of MCAO and inhibited the occurrence of ferroptosis. Furthermore, treatment of HT22 and PC12 cells with the ferroptosis inhibitor Fer-1 similarly attenuated the effects of OGD/R, resulting in increased cell viability, restored mitochondrial structure, and inhibition of ferroptosis. Our study is the first to indicate that poststroke exercise can alleviate the progression of ischemic stroke by inhibiting ferroptosis.

In the process of exploring the downstream mechanisms of exercise-regulated IS ferroptosis, some studies have shown that the STING pathway can promote ferroptosis in septic heart dysfunction, pancreatic cancer and acute kidney injury (Li et al. 2021; Kong et al. 2022; Jin et al. 2023). STING is a transmembrane homodimer located in the endoplasmic reticulum (Li and Chen 2018). A recent study revealed that the absence of TAK1 in liver cells caused oxidative stress-driven ferroptosis and accelerated the activation of STING macrophages, resulting in liver injury and liver fibrosis (Su et al. 2023). Other studies have shown that STING induces the release of free ferrous ions by interacting with NCOA4 in the cytoplasm and that the massive accumulation of ferrous ions triggers ferroptosis in macrophages, thereby promoting multiorgan damage in sepsis (Wu et al. 2022). Importantly, STING may also be involved in the development of IS (Ma et al. 2023). In this study, we revealed that STING expression was upregulated in IS and that STING knockdown reduced Fe^{2+} and MDA levels as well as IREB2 expression while increasing SOD levels and the expression of GPX4 and SLC7A11, thereby inhibiting ferroptosis and alleviating OGD/R-induced injury in HT22 and PC12 cells. In addition, as an upstream regulatory factor of STING, cGAS functions as a sensor of double-stranded DNA that triggers the activation of the STING pathway and downstream signaling pathways through the recognition of unbound DNA fragments in the cytoplasm (Zhang et al. 2024). Previous studies have shown that iron treatment increases the expression of cGAS, STING, and their downstream targets (Li et al. 2022). They reported that the cGAS-STING pathway regulates iron metabolism and exacerbates cerebral ischemic injury in mice by activating NCOA4-mediated siderophilin activity. Interestingly, inhibition of the cGAS-STING pathway also attenuated the brain injury caused by IS (Liu et al. 2023; Zhu et al. 2023). Here, we also detected the expression of cGAS and reported that cGAS expression was upregulated in IS and that the inhibitory effect of cGAS knockdown on ferroptosis in HT22 and PC12 cells was attenuated by the overexpression of STING. Additionally, we found through animal experiments that knocking down cGAS could relieve IS in rats through the inhibition of ferroptosis, whereas the overexpression of cGAS in exercising rats promoted the development of IS in rats. Our study demonstrated the mechanism by which exercise alleviates ischemic stroke in rats through the cGAS-STING ferroptosis signaling pathway. Notably, as a cytosolic DNA sensor, cGAS is typically activated by mitochondrial DNA (mtDNA) leaked from damaged mitochondria (Tsutomu et al. 2025). Mitochondria are not only key regulators of ferroptosis but also primary adaptive targets of exercise training. Studies have shown that exercise training can inhibit aberrant cGAS activation by maintaining mitochondrial homeostasis and reducing mtDNA leakage into the cytoplasm (Xin et al. 2025). Therefore, we speculate that exercise likely exerts its effects by preserving mitochondrial integrity and preventing mtDNA leakage. This provides a novel upstream mechanistic perspective on the neuroprotective effects of exercise, although the precise regulatory mechanisms warrant further investigation.

Overall, our study revealed for the first time that exercise inhibits ferroptosis by suppressing the cGAS-STING signaling pathway, thereby slowing the progression of IS. However, several limitations in the present study should be acknowledged. First, the sample size *per* group was relatively modest for behavioral and infarct volume assessments. Future studies with larger sample sizes and formal power calculations are needed to validate and extend our findings. Second, the optimal timing and duration of exercise intervention (acute stage or recovery stage) have not yet been determined, and further investigations are required to establish the most effective exercise protocol for ischemic stroke.

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Data availability statement. The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions. All the authors contributed substantially to this manuscript. Conceptualization YH; data curation HY, TG; formal analysis YZ, CZ; funding acquisition LY; investigation LY; methodology YZ, CZ; project administration YH; resources LY; software HY, TG; supervision LY; validation YH; visualization YH; writing – original draft YH; writing – review and editing LY. All the authors have read and approved the final manuscript.

Ethics approval. All animal experiments complied with the ARRIVE guidelines and were carried out in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals. All animal experimental protocols were approved by the Ethics Review Committee of The Second Affiliated Hospital of Kunming Medical University (kyfeyxm2024176).

Conflict of interests. The authors declare that they have no competing interests.

References

Adjetey C, Davis JC, Falck RS, Best JR, Dao E, Bennett K, Tai D, McGuire K, Eng JJ, Hsiung GR, et al. (2023): Economic evaluation of exercise or cognitive and social enrichment activities for improved cognition after stroke. JAMA Netw Open **6,** e2345687 https://doi.org/10.1001/jamanetworkopen.2023.45687

Alim I, Caulfield JT, Chen Y, Swarup V, Geschwind DH, Ivanova E, Seravalli J, Ai Y, Sansing LH, Ste Marie EJ, et al. (2019): Selenium drives a transcriptional adaptive program to block ferroptosis and treat stroke. Cell **177,** 1262-1279.e1225 https://doi.org/10.1016/j.cell.2019.03.032

Alkadhi KA (2018): Exercise as a positive modulator of brain function. Mol Neurobiol **55,** 3112-3130 https://doi.org/10.1007/s12035-017-0516-4

Bu ZQ, Yu HY, Wang J, He X, Cui YR, Feng JC, Feng J (2021): Emerging role of ferroptosis in the pathogenesis of ischemic stroke: A new therapeutic target? ASN Neuro **13,** 17590914211037505 https://doi.org/10.1177/17590914211037505

Buvarp D, Viktorisson A, Axelsson F, Lehto E, Lindgren L, Lundström E, Sunnerhagen KS (2023): Physical activity trajectories and functional recovery after acute stroke among adults in Sweden. JAMA Netw Open **6,** e2310919 https://doi.org/10.1001/jamanetworkopen.2023.10919

Cao DJ, Schiattarella GG, Villalobos E, Jiang N, May HI, Li T, Chen ZJ, Gillette TG, Hill JA (2018): Cytosolic DNA sensing promotes macrophage transformation and governs myocardial ischemic injury. Circulation **137,** 2613-2634 https://doi.org/10.1161/CIRCULATIONAHA.117.031046

Cheng J, Shen W, Jin L, Pan J, Zhou Y, Pan G, Xie Q, Hu Q, Wu S, Zhang H, Chen X (2020): Treadmill exercise promotes neurogenesis and myelin repair via upregulating Wnt/β-catenin signaling pathways in the juvenile brain following focal cerebral ischemia/reperfusion. Int J Mol Med **45,** 1447-1463 https://doi.org/10.3892/ijmm.2020.4515

Choi JW, Jo SW, Kim DE, Paik IY, Balakrishnan R (2024): Aerobic exercise attenuates LPS-induced cognitive dysfunction by reducing oxidative stress, glial activation, and neuroinflammation. Redox Biol **71,** 103101 https://doi.org/10.1016/j.redox.2024.103101

Chunchun Z, Yang L, Min X, Zhenbin Z, Kunyang T, Xiaoxue T, Zhixiao L (2025): Treadmill exercise alleviates depression in female mice induced by chronic unpredictable mild stress through the Inhibition of cGAS-STING signalling. Sci Rep **15,** 42825 https://doi.org/10.1038/s41598-025-29325-3

Collins AC, Cai H, Li T, Franco LH, Li XD, Nair VR, Scharn CR, Stamm CE, Levine B, Chen ZJ (2015): Cyclic GMP-AMP Synthase is an innate immune DNA sensor for mycobacterium tuberculosis. Cell Host Microbe **17,** 820-828 https://doi.org/10.1016/j.chom.2015.05.005

Cui J, Kim CS, Kim Y, Sohn MK, Jee S (2020): Effects of repetitive transcranial magnetic stimulation (rTMS) Combined with aerobic exercise on the recovery of motor function in ischemic stroke rat model. Brain Sci **10,** 186 https://doi.org/10.3390/brainsci10030186

Cui Y, Zhang Y, Zhao X, Shao L, Liu G, Sun C, Xu R, Zhang Z (2021): ACSL4 exacerbates ischemic stroke by promoting ferroptosis-induced brain injury and neuroinflammation. Brain Behav Immun **93,** 312-321 https://doi.org/10.1016/j.bbi.2021.01.003

Di Raimondo D, Rizzo G, Musiari G, Tuttolomondo A, Pinto A (2020): Role of regular physical activity in neuroprotection against acute ischemia. Int J Mol Sci **21,** 9086 https://doi.org/10.3390/ijms21239086

Domizio JD, Gulen MF, Saidoune F, Thacker VV, Yatim A, Sharma K, Nass T, Guenova E, Schaller M, Conrad C et al. (2022): The cGAS-STING pathway drives type I IFN immunopathology in COVID-19. Nature **603,** 145-151 https://doi.org/10.1038/s41586-022-04421-w

Fei Q, Yanan D, Songtao W, Minxiao X, Zhongwei W, Chaoyi Q, Yan Y, Jiexiu Z (2020): Maximum oxygen consumption and quantification of exercise intensity in untrained male Wistar rats. Sci Rep **10,** 11520 https://doi.org/10.1038/s41598-020-68455-8

Fricker M, Tolkovsky AM, Borutaite V, Coleman M, Brown GC (2018): Neuronal Cell Death. Physiol Rev **98,** 813-880 https://doi.org/10.1152/physrev.00011.2017

Gallego-Marin C, Schrum JE, Andrade WA, Shaffer SA, Giraldo LF, Lasso AM, Kurt-Jones EA, Fitzgerald KA, Golenbock DT (2018): Cyclic GMP-AMP synthase is the cytosolic sensor of plasmodium falciparum genomic DNA and activates type I IFN in Malaria. J Immunol **200,** 768-774 https://doi.org/10.4049/jimmunol.1701048

Gilgun-Sherki Y, Rosenbaum Z, Melamed E, Offen D (2002): Antioxidant therapy in acute central nervous system injury: current state. Pharmacol Rev **54,** 271-284 https://doi.org/10.1124/pr.54.2.271

Hribljan V, Lisjak D, Petrović DJ, Mitrečić D (2019): Necroptosis is one of the modalities of cell death accompanying ischemic brain stroke: from pathogenesis to therapeutic possibilities. Croat Med J **60,** 121-126 https://doi.org/10.3325/cmj.2019.60.121

Huang H, Liu S, Wu J, Zhu J, Xu J, Yu S, Bei L, Zhang B, Luo Y (2025): Buyang huanwu decoction alleviates ischemic stroke injury by inhibiting ferroptosis via the Nrf2/GPX4 pathway. Drug Des Devel Ther **19,** 2285-2305 https://doi.org/10.2147/DDDT.S503424

Jin L, Yu B, Wang H, Shi L, Yang J, Wu L, Gao C, Pan H, Han F, Lin W (2023): STING promotes ferroptosis through NCOA4-dependent ferritinophagy in acute kidney injury. Free Radic Biol Med **208,** 348-360 https://doi.org/10.1016/j.freeradbiomed.2023.08.025

Jin W, Zhao J, Yang E, Wang Y, Wang Q, Wu Y, Tong F, Tan Y, Zhou J, Kang C (2022): Neuronal STAT3/HIF-1α/PTRF axis-mediated bioenergetic disturbance exacerbates cerebral ischemia-reperfusion injury via PLA2G4A. Theranostics **12,** 3196-3216 https://doi.org/10.7150/thno.71029

King KR, Aguirre AD, Ye YX, Sun Y, Roh JD, Ng RP, Jr., Kohler RH, Arlauckas SP, Iwamoto Y, Savol A, et al. (2017): IRF3 and type I interferons fuel a fatal response to myocardial infarction. Nat Med **23,** 1481-1487 https://doi.org/10.1038/nm.4428

Knecht S, Hesse S, Oster P (2011): Rehabilitation after stroke. Dtsch Arztebl Int **108,** 600-606 https://doi.org/10.3238/arztebl.2011.0600

Kong C, Ni X, Wang Y, Zhang A, Zhang Y, Lin F, Li S, Lv Y, Zhu J, Yao X (2022): ICA69 aggravates ferroptosis causing septic cardiac dysfunction via STING trafficking. Cell Death Discov **8,** 187 https://doi.org/10.1038/s41420-022-00957-y

Li B, Wang W, Li Y, Wang S, Liu H, Xia Z, Gao W, Zhao B (2023): cGAS-STING pathway aggravates early cerebral ischemia-reperfusion injury in mice by activating NCOA4-mediated ferritinophagy. Exp Neurol **359,** 114269 https://doi.org/10.1016/j.expneurol.2022.114269

Li C, Liu J, Hou W, Kang R, Tang D (2021): STING1 promotes ferroptosis through MFN1/2-dependent mitochondrial fusion. Front Cell Dev Biol **9,** 698679 https://doi.org/10.3389/fcell.2021.698679

Li H, Hu L, Wang L, Wang Y, Shao M, Chen Y, Wu W, Wang L (2022): Iron activates cGAS-STING signaling and promotes hepatic inflammation. J Agric Food Chem **70,** 2211-2220 https://doi.org/10.1021/acs.jafc.1c06681

Li Q, Cao Y, Dang C, Han B, Han R, Ma H, Hao J, Wang L (2020): Inhibition of double-strand DNA-sensing cGAS ameliorates brain injury after ischemic stroke. EMBO Mol Med **12,** e11002 https://doi.org/10.15252/emmm.201911002

Li T, Chen ZJ (2018): The cGAS-cGAMP-STING pathway connects DNA damage to inflammation, senescence, and cancer. J Exp Med **215,** 1287-1299 https://doi.org/10.1084/jem.20180139

Li XN, Shang NY, Kang YY, Sheng N, Lan JQ, Tang JS, Wu L, Zhang JL, Peng Y (2024): Caffeic acid alleviates cerebral ischemic injury in rats by resisting ferroptosis via Nrf2 signaling pathway. Acta Pharmacol Sin **45,** 248-267 https://doi.org/10.1038/s41401-023-01177-5

Liu C, Wang G, Han W, Tian Q, Li M (2024): Ferroptosis: a potential therapeutic target for stroke. Neural Regen Res **19,** 988-997 https://doi.org/10.4103/1673-5374.385284

Liu M, Li Y, Han S, Wang H, Li J (2023): Activin A alleviates neuronal injury through inhibiting cGAS-STING-mediated autophagy in mice with ischemic stroke. J Cereb Blood Flow Metab **43,** 736-748 https://doi.org/10.1177/0271678X221147056

Long P, Suhua L, Qiang H, Yuxiang S, Juan S, Ting L, Yanlin W, Zhaoyong H, Weiyan L, Hui P (2025): Irisin-mediated muscle-renal crosstalk as a protective mechanism against contrast-induced acute kidney injury via cGAS-STING signalling inhibition. Clin Transl Med **15,** e70235 https://doi.org/10.1002/ctm2.70235

Ma X, Xin D, She R, Liu D, Ge J, Mei Z (2023): Novel insight into cGAS-STING pathway in ischemic stroke: from pre- to post-disease. Front Immunol **14,** 1275408 https://doi.org/10.3389/fimmu.2023.1275408

Matthew D, Raul C-P, Donna D Z (2019): NRF2 plays a critical role in mitigating lipid peroxidation and ferroptosis. Redox Biol **23,** 101107 https://doi.org/10.1016/j.redox.2019.101107

Owolabi MO, Thrift AG, Mahal A, Ishida M, Martins S, Johnson WD, Pandian J, Abd-Allah F, Yaria J, Phan HT, et al. (2022): Primary stroke prevention worldwide: translating evidence into action. Lancet Public Health **7,** e74-e85 https://doi.org/10.1016/s2468-2667(21)00230-9 https://doi.org/10.1016/S2468-2667(21)00230-9

Selim M (2010): Treatment with the iron chelator, deferoxamine mesylate, alters serum markers of oxidative stress in stroke patients. Transl Stroke Res **1,** 35-39 https://doi.org/10.1007/s12975-009-0001-0

Su W, Gao W, Zhang R, Wang Q, Li L, Bu Q, Xu Z, Liu Z, Wang M, et al. (2023): TAK1 deficiency promotes liver injury and tumorigenesis via ferroptosis and macrophage cGAS-STING signalling. JHEP Rep **5,** 100695 https://doi.org/10.1016/j.jhepr.2023.100695

Tal H, Brent RS (2018): The development of the concept of ferroptosis. Free Radic Biol Med **133,** 130-143 https://doi.org/10.1016/j.freeradbiomed.2018.09.043

Tani A, Sakakima H, Otsuka S, Mizuno K, Nakanishi K, Norimatsu K, Takada S, Matsuoka T, Matsuzaki R, Nakakogawa T, Maruyama I (2023): Stimulation of functional recovery via neurorepair mechanisms by the traditional Japanese Kampo medicine, Ninjin'yoeito, and physical exercise in a rat ischemic stroke model. J Ethnopharmacol **302,** 115927 https://doi.org/10.1016/j.jep.2022.115927

Thomsen MK, Nandakumar R, Stadler D, Malo A, Valls RM, Wang F, Reinert LS, Dagnaes-Hansen F, Hollensen AK, Mikkelsen JG, et al. (2016): Lack of immunological DNA sensing in hepatocytes facilitates hepatitis B virus infection. Hepatology **64,** 746-759 https://doi.org/10.1002/hep.28685

Tsutomu S, Yui T, Chihiro S, Riko M, Megumi S, Tatsuki K, Akira U (2025): Ionizing radiation triggers the release of mitochondrial DNA into the cytosol as a signal of mitochondrial damage. Sci Rep **15,** 23191
https://doi.org/10.1038/s41598-025-04845-0

Wang X, Zhang M, Yang SD, Li WB, Ren SQ, Zhang J, Zhang F (2014): Pre-ischemic treadmill training alleviates brain damage via GLT-1-mediated signal pathway after ischemic stroke in rats. Neuroscience **274,** 393-402
https://doi.org/10.1016/j.neuroscience.2014.05.053

Wu J, Liu Q, Zhang X, Tan M, Li X, Liu P, Wu L, Jiao F, Lin Z, Wu X, et al. (2022): The interaction between STING and NCOA4 exacerbates lethal sepsis by orchestrating ferroptosis and inflammatory responses in macrophages. Cell Death Dis **13,** 653
https://doi.org/10.1038/s41419-022-05115-x

Xin W, Jingcheng F, Xuemei D, Xinyi Z, Jianzheng B, Tan Z (2025): Mitochondrial DNA in exercise-mediated innate immune responses. Int J Mol Sci **26,** 3069
https://doi.org/10.3390/ijms26073069

Zhang J, Du J, Liu D, Zhuo J, Chu L, Li Y, Gao L, Xu M, Chen W, Huang W et al. (2024). Polystyrene microplastics induce pulmonary fibrosis by promoting alveolar epithelial cell ferroptosis through cGAS/STING signaling. Ecotoxicol Environ Saf **277,** 116357
https://doi.org/10.1016/j.ecoenv.2024.116357

Zhang P, Zhang Y, Zhang J, Wu Y, Jia J, Wu J, Hu Y (2013): Early exercise protects against cerebral ischemic injury through inhibiting neuron apoptosis in cortex in rats. Int J Mol Sci **14,** 6074-6089
https://doi.org/10.3390/ijms14036074

Zhang QW, Deng XX, Sun X, Xu JX, Sun FY (2013): Exercise promotes axon regeneration of newborn striatonigral and corticonigral projection neurons in rats after ischemic stroke. PLoS One **8,** e80139
https://doi.org/10.1371/journal.pone.0080139

Zhao Y, He X, Yang X, Hong Z, Xu Y, Xu J, Zheng H, Zhang L, Zuo Z, Hu X (2025): CircFndc3b mediates exercise-induced neuroprotection by mitigating microglial/macrophage pyroptosis via the ENO1/KLF2 axis in stroke mice. Adv Sci (Weinh) **12,** e2403818
https://doi.org/10.1002/advs.202403818

Zhao Y, Pang Q, Liu M, Pan J, Xiang B, Huang T, Tu F, Liu C, Chen X (2017): Treadmill exercise promotes neurogenesis in ischemic rat brains via caveolin-1/VEGF signaling pathways. Neurochem Res **42,** 389-397
https://doi.org/10.1007/s11064-016-2081-z

Zhu M, Chen W, Zhang J (2025): Aerobic exercise, an effective intervention for cognitive impairment after ischemic stroke. Front Aging Neurosci **17,** 1514271
https://doi.org/10.3389/fnagi.2025.1514271

Zhu Z, Lu H, Jin L, Gao Y, Qian Z, Lu P, Tong W, Lo PK, Mao Z, Shi H (2023): C-176 loaded Ce DNase nanoparticles synergistically inhibit the cGAS-STING pathway for ischemic stroke treatment. Bioact Mater **29,** 230-240
https://doi.org/10.1016/j.bioactmat.2023.07.002

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