WHIOCE PUBLISHING PTE. LTD.
PROVIDING
FIRST-CLASS SCIENTIFIC INFORMATION
FOR TOP SCHOLARS

2025 Volume 6, Issue 4
Cell Biology Research
ISSN: 2529-7627 (Online)

Application of Integrated Animal and Cell Experiment Teaching Model in Demonstrating Ferroptosis in Cerebral Ischemia-Reperfusion Injury

Guangjie Sun[1], Bingcang Huang[2]*

[1]School of Gongli Hospital Medical Technology, University of Shanghai for Science and Technology, Shanghai 200093, China

[2] Department of Radiology, Gongli Hospital of Shanghai Pudong New Area, Shanghai 200135, China

**Corresponding author: Bingcang Huang, gl_huangbc@sumhs.edu.cn*

***A*bstract:** Traditional molecular medicine experimental teaching often suffers from a disconnection between theory and practice, as well as fragmented experimental content, making it difficult for students to integrate multi-level knowledge to address complex scientific problems. To address this, we redesigned our Molecular Medicine Experimental Techniques course around the pathophysiology of cerebral ischemia-reperfusion injury (CIRI), with a focus on the mechanism of ferroptosis. Our integrated pedagogical model links an in vivo transient middle cerebral artery occlusion (tMCAO) mouse model with an in vitro oxygen-glucose deprivation/reoxygenation (OGD/R) model in HT22 neuronal cells. Within this framework, students first observe in vivo phenotypes in the tMCAO model, including increased brain iron content, downregulated GPX4 expression, and accumulation of the lipid peroxidation marker 4-HNE. They then use the OGD/R cell model to validate key ferroptosis features at the molecular and ultrastructural levels, such as enhanced lipid peroxidation, glutathione depletion, and mitochondrial damage. This "phenotype-to-mechanism" approach allows students to intuitively understand the role of ferroptosis in CIRI while systematically mastering the full research cycle, from establishing disease models and applying multi-technique assays to integrating and interpreting data. By translating a cutting-edge scientific topic into a coherent experimental teaching module, this reform effectively bridges the gap between theoretical knowledge and hands-on research practice. It fosters students' integrative scientific thinking and enhances their ability to tackle complex biomedical questions, offering a transferable paradigm for advancing high-level experimental training in molecular medicine.

Keywords: Cerebral ischemia-reperfusion injury; Ferroptosis; Molecular medicine experimental techniques; Teaching reform

Online publication: December 26, 2025

1. Introduction

Acute stroke is one of the leading causes of death and disability worldwide. The core strategy for its early treatment is to rapidly restore blood flow to the ischemic penumbra. However, this revascularization process can also induce CIRI, a common pathological process following thrombolysis or thrombectomy [1]. CIRI is a complex cascade response involving oxidative stress, inflammation, calcium overload, and multiple modes of cell death. This pathological mechanism not

only limits the benefits of reperfusion therapies but is also a key factor in poor neurological outcomes for patients [2]. Therefore, understanding the core molecular mechanisms underlying CIRI and exploring effective therapeutic targets have become critical research directions in the field of neuroscience. In recent years, ferroptosis, an iron-dependent cell death characterized by lipid peroxidation accumulation, has been identified as a crucial contributor to CIRI and has become a hotspot in neuroprotection research. Numerous studies have shown that the inhibition of GPX4 activity, the obstruction of lipid peroxidation clearance, and disruptions in iron metabolism collectively trigger ferroptosis, which exacerbates neuronal damage during CIRI. Understanding this mechanism not only provides new insights into the pathophysiology of CIRI but also opens up new opportunities for its treatment [3].

The Molecular Medicine Experimental Techniques course aims to bridge the gap between molecular-level mechanism understanding and experimental skills, enabling students to complete the full research process from theoretical deduction to experimental validation. However, ferroptosis and its relationship with cerebral ischemia-reperfusion injury belong to rapidly developing frontier research areas that involve multiple signalling pathways, complex regulatory networks of cell death, and cross-level pathological changes. Due to the lack of intuitive, systematic, and easily operable experimental scenarios, students often struggle to establish clear causal chains between abstract concepts, molecular indicators, and actual pathological events. This leads to a significant "knowledge-action separation". On the knowledge level, teaching mostly focuses on lectures and literature reading. Although students may remember key terms such as GPX4 and lipid peroxidation, they often lack an intuitive and dynamic understanding of how these molecules interact to form a complete "death signalling pathway" and how this pathway is activated and regulated in the complex in vivo microenvironment.

On the action level, the current experimental course structure faces systemic shortcomings. The course content typically consists of isolated verification experiments, such as performing Western blotting to detect a specific protein one week and cell culture the next. These techniques lack a cohesive logical thread, which results in students mastering fragmented experimental skills but struggling to integrate multiple techniques to solve a specific scientific problem. They do not know why they should choose a particular technique over another in a specific research context, nor do they know how to integrate multidimensional data, such as cell viability, molecular expression, and biochemical markers, into a comprehensive scientific picture. Traditional cell-based experiments tend to focus on specific markers, which can demonstrate changes at the molecular and cellular levels but fail to reflect the true tissue damage state in the in vivo environment. Animal experiments, while capable of presenting the overall physiological and pathological process, are time-consuming, complex, and subject to strict ethical requirements, making it difficult to conduct in-depth mechanistic exploration within limited class time. The separation between these two approaches prevents students from seeing the complete scientific logical chain from in vivo pathology to in vitro model validation, hindering their ability to form a systematic, integrated understanding of disease mechanisms.

Ultimately, this disconnection between theory and practice, between technology and scientific problems, has resulted in a passive learning habit. Students tend to follow pre-established experimental protocols mechanically, rather than actively designing experiments, analyzing data, and engaging in critical thinking. When faced with real scientific questions, such as "how to demonstrate the role of ferroptosis in CIRI", they often find themselves at a loss. Therefore, in the reform of Molecular Medicine Experimental Techniques, the urgent need is to construct a teaching model that bridges multiple biological levels and integrates theoretical depth with experimental operability. Using ferroptosis and cerebral ischemia-reperfusion injury as a teaching vehicle, the integration of animal and cell models can enable students to observe pathological phenotype changes at the in vivo level, while also validating the underlying mechanisms at the cellular and molecular levels. This approach holds the potential to achieve seamless knowledge integration from macroscopic to microscopic levels, helping students build a more comprehensive framework for understanding disease mechanisms (**Figure 1**).

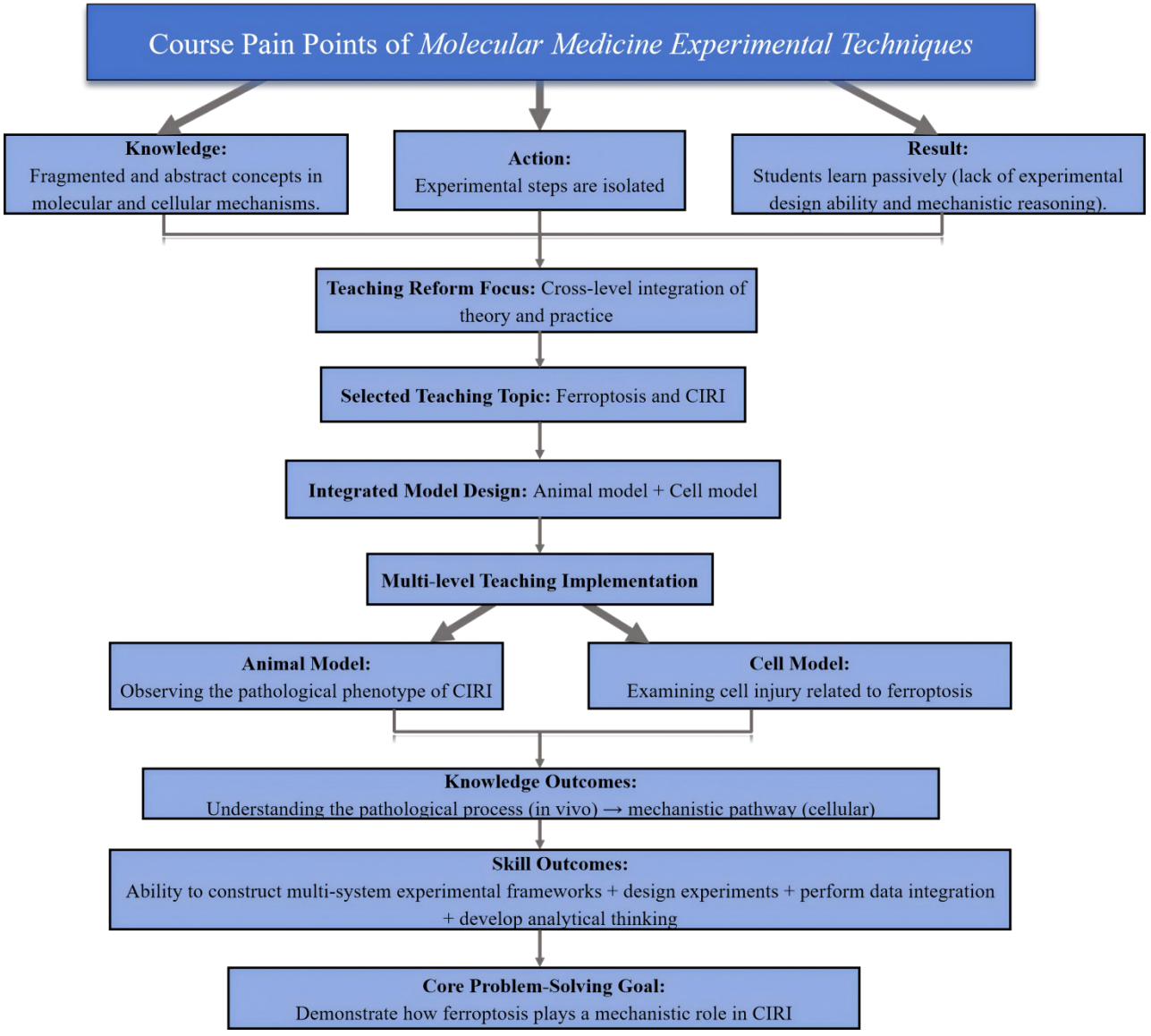

Figure 1. Teaching reform logic for the course "Molecular medicine experimental techniques".

2. Related theoretical foundation

2.1. Cerebral ischemia-reperfusion injury

Cerebral ischemia-reperfusion injury is a complex pathological process that cannot be ignored during the treatment of acute ischemic stroke. When blood supply is interrupted due to vascular blockage, brain tissue rapidly experiences energy metabolism disorders, ionic homeostasis disruption, and neuronal dysfunction [4]. Although reperfusion is crucial for salvaging the ischemic penumbra, the process itself triggers a series of secondary damage responses that further harm brain tissue. During the ischemic phase, blood flow interruption causes a loss of oxygen and glucose supply to the brain, resulting in severe disturbances in cellular energy metabolism. Reduced ATP synthesis impairs the function of the sodium-potassium pump and calcium pump, causing a massive accumulation of sodium and calcium ions. The imbalance in ionic concentrations inside and outside the cell further leads to cell swelling, acidosis, and excessive release of excitotoxic substances such as glutamate, which exacerbates neuronal injury [5]. Meanwhile, the hypoxic and low-energy environment

during ischemia leads to the accumulation of metabolic intermediates, generating a large amount of free radicals, particularly hydrogen peroxide (H_2O_2) and superoxide anions (O^{2-}), intensifying oxidative stress and activating multiple cell damage pathways, including lipid peroxidation, protein oxidation, and DNA damage. However, after blood reperfusion, a surge of oxygen floods the damaged tissues, causing a dramatic burst of oxidative stress. During the reperfusion phase, high concentrations of reactive oxygen species (ROS), including highly reactive molecules like hydroxyl radicals, are rapidly generated. These oxidative molecules not only further damage cell membranes, mitochondria, and nucleic acids but also cause mitochondrial dysfunction by opening mitochondrial permeability transition pores (mPTP), releasing a large amount of pro-apoptotic factors such as cytochrome C, which intensifies cell death [6] (**Figure 2**).

In the process of CIRI, the activation of inflammatory responses also plays a significant role. Following reperfusion, the blood-brain barrier is compromised, allowing peripheral immune cells, such as neutrophils and monocytes, to enter brain tissue and release large amounts of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukins (IL-1β, IL-6). These cytokines not only further exacerbate local inflammation but also accelerate neuronal damage by activating glial cells and astrocytes through inflammatory pathways. The excessive release of inflammatory factors can also promote excitotoxicity in neurons, leading to further neuronal death [7]. Moreover, various forms of cell death are involved in CIRI, including classical apoptosis, necrosis, pyroptosis, and ferroptosis, which has garnered increasing attention in recent years.

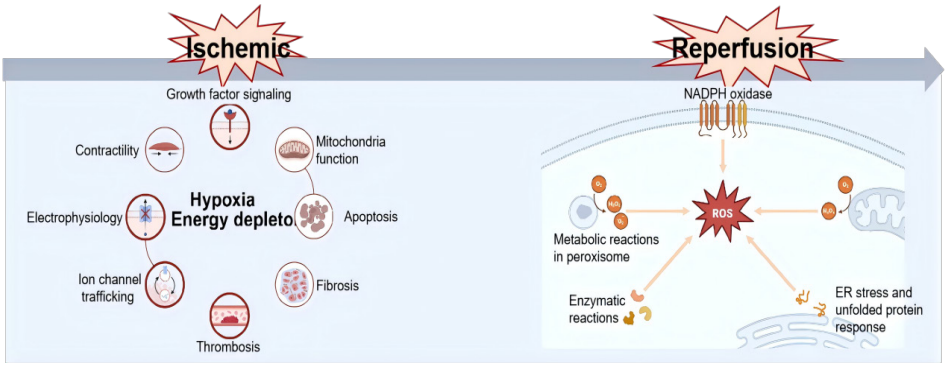

Figure 2. Schematic representation of the cellular mechanisms in ischemia and reperfusion.

2.2. Molecular mechanism of ferroptosis and its role in cerebral ischemia-reperfusion injury

Ferroptosis is a form of regulated cell death characterized by the accumulation of iron-catalyzed lipid peroxides. Unlike traditional apoptotic or necrotic pathways, ferroptosis arises from a combination of disrupted iron homeostasis, damaged membrane lipid structures, and insufficient antioxidant defences. In neurological disorders, particularly CIRI, ferroptosis has been established as one of the key mechanisms leading to neuronal dysfunction and tissue damage. The occurrence of ferroptosis involves multiple molecular regulatory axes, primarily including the following aspects (**Figure 3**):

2.2.1. Disruption of iron homeostasis leading to iron ion accumulation

During cerebral ischemia-reperfusion injury, intracellular iron homeostasis is severely disrupted. The upregulation of transferrin receptor 1 promotes iron uptake, while autophagic pathways of ferritin are activated, releasing stored iron into the cell. This results in a significant increase in the concentration of free ferrous iron (Fe^{2+}) within the cell. Excess Fe^{2+} reacts with hydrogen peroxide through the Fenton reaction, producing highly reactive hydroxyl radicals. These radicals initiate protein carbonylation, DNA damage, and lipid peroxidation, directly disrupting the structural and functional integrity of the cell [8].

2.2.2. Membrane lipid components are prone to oxidation, enhancing lipid peroxidation

Neuronal membranes are rich in polyunsaturated fatty acids, which are chemically vulnerable to oxidative stress. After

ischemia-reperfusion, the level of oxidative stress rises sharply, leading to the oxidation of polyunsaturated fatty acids in membrane lipids and the generation of a large amount of lipid peroxides. Enzymes such as ACSL4 and LPCAT3 promote the incorporation of specific fatty acids into membrane phospholipids, making them the primary substrates for oxidation. Additionally, the activation of lipoxygenases and other oxidases further drives the cascading process of lipid peroxidation. The accumulating lipid peroxides weaken the stability of membrane structures, ultimately causing membrane rupture, which is a critical step in ferroptosis execution [9].

2.2.3. Impaired antioxidant defenses and reduced GPX4 activity

Cells rely on multiple antioxidant mechanisms to limit lipid peroxidation, with GPX4 being the most crucial defense factor. GPX4 reduces phospholipid peroxides to relatively stable alcohols, thereby protecting the cell membrane from oxidative damage. Its activity depends on GSH, the production of which is reliant on cysteine uptake. Under ischemia-reperfusion conditions, oxidative stress and metabolic limitations lead to GSH depletion, while insufficient cysteine supply further reduces GPX4 activity. The collapse of the antioxidant system prevents the timely clearance of lipid peroxides, pushing the cell into an irreversible ferroptosis state. Studies have shown that ferroptosis is closely related to cell death in CIRI models, where iron ions enhance oxidative stress and lipid peroxidation, promoting neuronal death and causing irreversible brain tissue damage [10].

During the progression of CIRI, ferroptosis is not merely a form of cell death but a central process that amplifies neuronal damage. The reduction in energy production during the ischemic phase leads to a diminished ability to maintain cellular homeostasis. The oxygen influx during reperfusion drastically increases oxidative pressure, accelerating iron-dependent lipid peroxidation. The membrane damage caused by this process is irreversible, ultimately resulting in extensive neuronal death. This highlights that ferroptosis is not only a critical mechanism in CIRI but also a potential target for future therapeutic strategies.

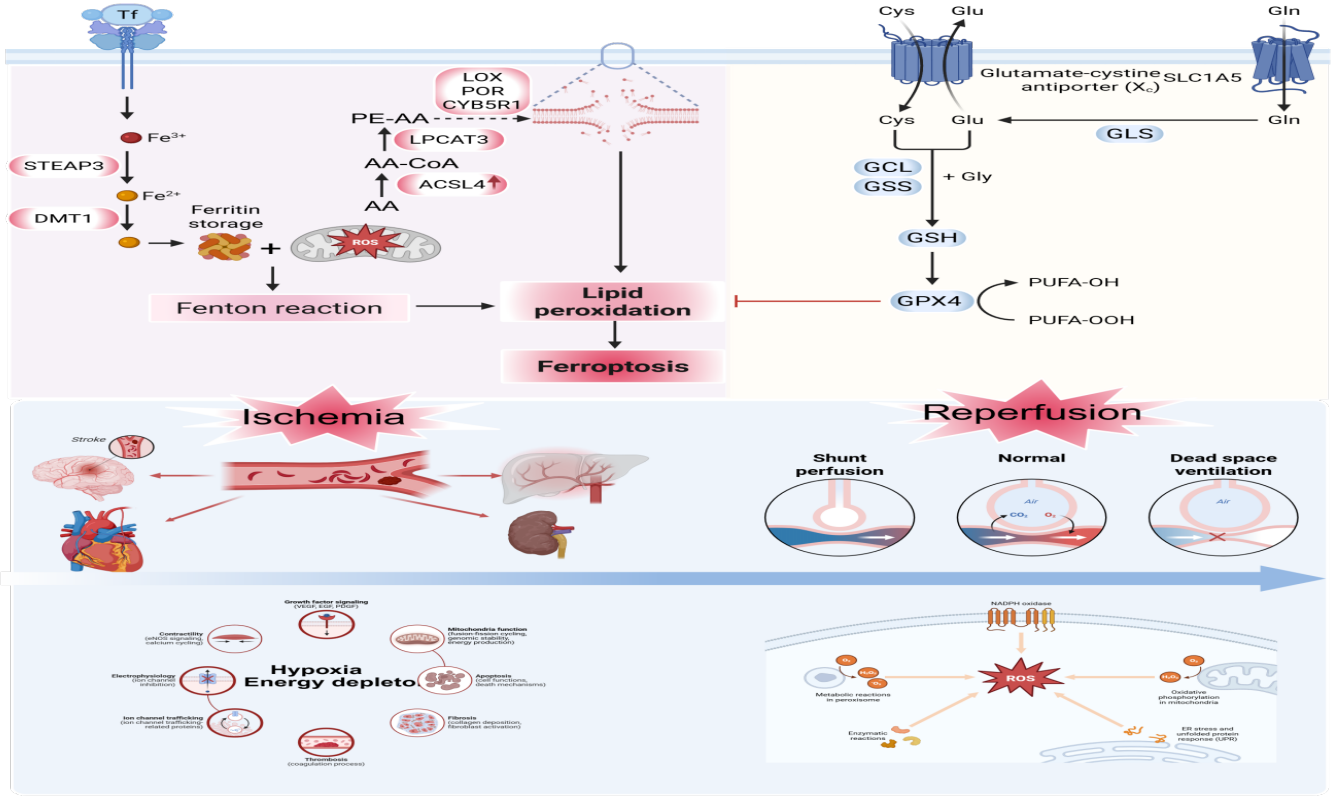

Figure 3. Molecular pathways of ferroptosis.

3. Analysis of animal and cell experiments based on the "molecular medicine experimental techniques" course

3.1. Animal experiment

In the Molecular Medicine Experimental Techniques course, to observe the dynamic changes of ferroptosis during CIRI, we established a transient middle cerebral artery occlusion/reperfusion (tMCAO/R) model using 6-8-week-old C57BL/6 mice. After anesthetizing the mice with 4% pentobarbital sodium via intraperitoneal injection, we carefully isolated the left common carotid artery, external carotid artery, and internal carotid artery under a microscope. The middle cerebral artery was then occluded for 1.5 hours using an intraluminal suture technique. After removing the suture to restore blood flow and repercussing the brain for 24 hours, brain tissue was collected to evaluate ferroptosis-related markers and to systematically assess the molecular and cellular changes induced by ischemia-reperfusion [11].

First, we measured the free iron levels in the brain tissue using an iron content assay kit. The results showed a significant increase in iron content in the tMCAO group compared to the control group (**Figure 4a**), suggesting that ischemia-reperfusion severely disrupts iron homeostasis, a key prerequisite for ferroptosis. To further assess the molecular features of ferroptosis, we examined the expression levels of the critical ferroptosis-related protein GPX4 and the lipid peroxidation product 4-HNE. Immunofluorescence results indicated a significant reduction of GPX4 signal in the brain tissue of the tMCAO group (**Figure 4b**), indicating impaired neuronal ability to clear lipid peroxides. At the same time, the fluorescence signal for 4-HNE was significantly enhanced (**Figure 4c**), indicating the accumulation of lipid peroxides, which is highly consistent with the typical process of ferroptosis. To explore the relationship between ferroptosis and inflammation, we further measured the levels of common inflammatory cytokines associated with ischemia-reperfusion injury. ELISA results showed that the expression of TNF-α, IL-1β, and IL-6 in the tMCAO group was significantly higher than in the control group (**Figure 4d-f**). These results suggest that ferroptosis is significantly activated during cerebral ischemia-reperfusion and is accompanied by a strong inflammatory response. This inflammatory amplification effect may play a crucial role in exacerbating tissue damage and advancing the pathological process.

In summary, the tMCAO/R model demonstrated a series of typical ferroptosis phenotypes, including elevated iron content, downregulation of GPX4, enhanced lipid peroxidation, and increased inflammatory cytokine levels. These findings suggest that ferroptosis may be an important pathological mechanism in cerebral ischemia-reperfusion injury.

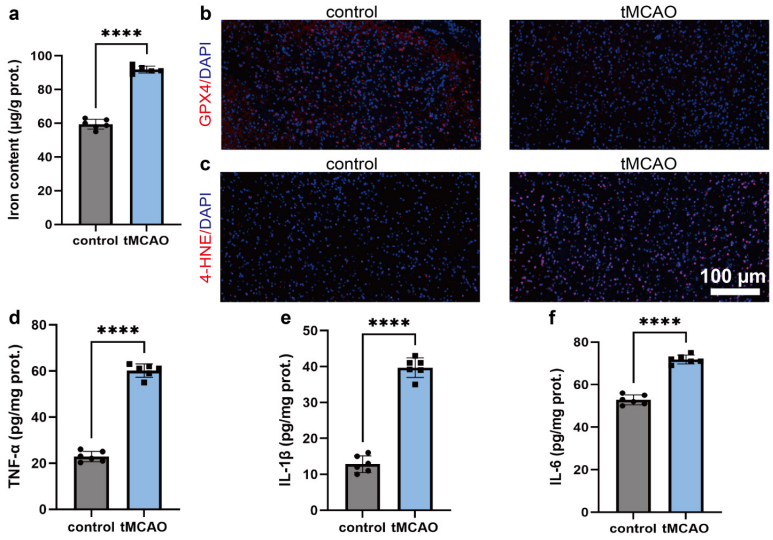

Figure 4. Assessment of ferroptosis- and inflammation-related indicators in the tMCAO/R mouse model.
(a) Quantification of total iron levels in brain tissue from control and tMCAO mice using an iron content assay. (b) Immunofluorescence staining of GPX4 in coronal brain sections, with nuclei labeled by DAPI. (c) Immunofluorescence staining of the lipid peroxidation marker 4-HNE, counterstained with DAPI. d-f) Measurement of TNF-α (d), IL-1β (e), and IL-6 (f) concentrations in brain homogenates by ELISA. All assays were performed 24 h after reperfusion following 1.5 h tMCAO. Data are presented as mean ± SEM. ****P < 0.0001.

3.2. Cell experiment

To further validate the role of ferroptosis in ischemia-reperfusion injury, we treated HT22 cells, a mouse hippocampal neuronal cell line, using an oxygen-glucose deprivation (OGD)/reperfusion (R) model in vitro. HT22 cells were chosen due to their neuronal characteristics and suitability for studying oxidative stress and neurodegenerative processes. First, HT22 cells were seeded in culture dishes and allowed to adhere for 24 hours. The cells were then switched to glucose-free MEM medium and placed in an anaerobic chamber (Mitsubishi, Japan) for 4 hours of OGD treatment. After the treatment, normal culture medium was added, and the cells were transferred to a 5 % CO_2 incubator for an additional 24 hours to simulate ischemia-reperfusion conditions.

To assess the level of lipid peroxidation in the cells, we used the BODIPY 581/591 C11 fluorescent probe. After OGD/R treatment, the oxidized (green) signal in the cells was significantly enhanced, indicating a substantial accumulation of lipid peroxides within the cells (**Figure 5a**). This change is consistent with the occurrence of ferroptosis. We then measured the levels of GSH and malondialdehyde (MDA) in the cells to further evaluate changes in the oxidative-reductive state. The GSH level in the neurons after OGD/R treatment was significantly lower than that in the control group (**Figure 5b**), suggesting impaired antioxidant capacity. Meanwhile, the MDA level in the OGD/R group was significantly higher (**Figure 5c**), further confirming the intensification of lipid peroxidation and the activation of the ferroptosis process. Finally, we used transmission electron microscopy (TEM) to observe the ultrastructure of the neurons. In the control group, the mitochondria of the neurons appeared intact, with clear inner membrane structures. However, in the neurons treated with OGD/R, the mitochondria showed signs of shrinkage, membrane densification, and loss of inner membrane structure (**Figure 5d**), which are highly consistent with mitochondrial damage associated with ferroptosis.

In summary, the characteristics of ferroptosis were confirmed both in the mouse model and in cultured HT22 neurons. The increased iron content, reduced GPX4 expression, enhanced 4-HNE levels, and elevated inflammatory factors in the mouse brain tissue all indicate that ferroptosis was significantly activated during ischemia-reperfusion. In HT22 neurons treated with OGD/R, lipid peroxidation, GSH depletion, elevated MDA levels, and changes in mitochondrial morphology further support the occurrence of ferroptosis. These results suggest that ferroptosis may play a key role in cerebral ischemia-reperfusion injury, exacerbating brain tissue damage through oxidative stress and inflammatory responses.

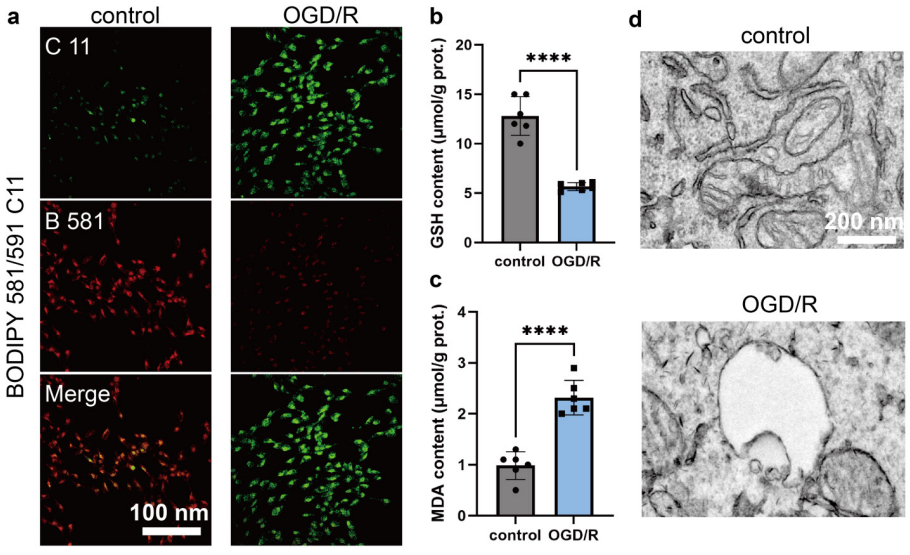

Figure 5. Ferroptosis and mitochondrial damage in HT22 cells subjected to OGD/R treatment.
(a) Lipid peroxidation assessed by BODIPY 581/591 C11 probe. (b) GSH content measurement. (c) MDA content measurement. (d) Transmission electron microscopy images showing mitochondrial morphology in control and OGD/R-treated HT22 cells. Data are presented as mean ± SEM. ****P < 0.0001.

4. Conclusions

Ferroptosis, a novel form of cell death, plays a crucial role in cerebral ischemia-reperfusion injury. By regulating iron metabolism, antioxidant responses, and lipid peroxidation, ferroptosis can be effectively mitigated, thereby protecting brain tissue and reducing the damage caused by ischemia-reperfusion. Through the experimental content in the Molecular Medicine Experimental Techniques course, students can gain a deeper understanding of the molecular mechanisms of ferroptosis and provide experimental evidence for related therapeutic strategies. As research advances, targeted treatment of ferroptosis is expected to become an important strategy in the treatment of cerebral ischemia-reperfusion injury and may lead to new breakthroughs in the treatment of neurodegenerative diseases.

This study focuses on the key pathological mechanisms of cerebral ischemia-reperfusion injury, particularly the ferroptosis process, which has garnered significant attention in recent years. We have developed a comprehensive teaching model that deeply integrates animal and cell experiments. By combining the mouse middle cerebral artery occlusion model with the HT22 cell oxygen-glucose deprivation/reperfusion model, students are able to simultaneously observe changes in ferroptosis-related molecular markers at both the in vivo and cellular levels. This approach provides an intuitive understanding of the role of ferroptosis in cerebral ischemia-reperfusion injury. Practical results demonstrate that this teaching model not only addresses the gap between theory and experiment in traditional education but also enhances students' systematic understanding of complex pathological mechanisms. It strengthens their experimental design, data analysis, and scientific thinking abilities.

Additionally, ferroptosis, as a key regulatory target in cerebral ischemia-reperfusion injury, involves disruptions in iron homeostasis, accumulation of lipid peroxides, and imbalances in antioxidant systems. These mechanisms are closely linked to neuronal structural damage and functional loss. By incorporating these cutting-edge mechanisms into experimental teaching, the model not only expands students' understanding of molecular medicine's frontier but also provides a feasible path for future innovative experimental courses.

In conclusion, the integration of animal and cell models in experimental teaching enhances the depth and breadth of the course and presents a new, replicable, and scalable paradigm for molecular medicine experimental education reform. Future teaching efforts can further incorporate multi-omics technologies, advanced imaging techniques, and explore additional novel cell death mechanisms to continuously optimize the experimental teaching system, thereby improving students' ability to understand disease mechanisms and foster scientific innovation.

5. Acknowledgements

All animal experiments were performed with the approval of the Ethics Committee of Shanghai University (Project No.: ECSHU-2021-029). This work is financially supported by the National Natural Science Foundation of China (Project No.: 82372029, 22205133), Discipline Construction of Pudong New Area Health Commission (Project No.: PWZxk2022-03), Shanghai Pudong New District Health Committee Health Industry Special Project (Project No.: PW2024E-02) and The Investigator-initiated Trial Program of Shanghai Pudong New Area Health Commission (the Cohort Study Program) (Project No.: 2025-PWDL-24).

Disclosure statement

The author declares no conflict of interest.

References

[1] Feigin VL, Brainin M, Norrving B, et al., 2025, World Stroke Organization: Global Stroke Fact Sheet 2025. *Int J Stroke*,

20(2): 132-144.

[2] Wu L, Xiong X, Wu X, et al., 2020, Targeting Oxidative Stress and Inflammation to Prevent Ischemia-Reperfusion Injury. *Front Mol Neurosci*, 13: 28.

[3] Li J, Cao F, Yin HL, et al., 2020, Ferroptosis: past, present and future. *Cell Death & Disease*, 11(2): 88.

[4] Yang M, Liu B, Chen B, et al., 2025, Cerebral ischemia-reperfusion injury: mechanisms and promising therapies. *Front Pharmacol*, 16: 1613464.

[5] Zhang M, Liu Q, Meng H, et al., 2024, Ischemia-reperfusion injury: molecular mechanisms and therapeutic targets. *Signal Transduction and Targeted Therapy*, 9(1): 12.

[6] Lee P, Chandel NS, Simon MC, 2020, Cellular adaptation to hypoxia through hypoxia inducible factors and beyond. *Nature Reviews Molecular Cell Biology*, 21(5): 268-283.

[7] Li Z, Li M, Fang Z, et al., 2025, Immunological Mechanisms and Therapeutic Strategies in Cerebral Ischemia-Reperfusion Injury: From Inflammatory Response to Neurorepair. *Int J Mol Sci*, 26(17).

[8] Ru Q, Li Y, Chen L, et al., 2024, Iron homeostasis and ferroptosis in human diseases: mechanisms and therapeutic prospects. *Signal Transduction and Targeted Therapy*, 9(1): 271.

[9] Mortensen MS, Ruiz J, Watts JL, 2023, Polyunsaturated Fatty Acids Drive Lipid Peroxidation during Ferroptosis. *Cells*, 12(5): 804.

[10] Forcina GC, Dixon SJ, 2019, GPX4 at the Crossroads of Lipid Homeostasis and Ferroptosis. *Proteomics*, 19(18): e1800311.

[11] Chaparro-Cabanillas N, Arbaizar-Rovirosa M, Salas-Perdomo A, et al., 2023, Transient Middle Cerebral Artery Occlusion Model of Stroke. *J Vis Exp*, 11(198).