Article

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IRAK1 Exacerbates Cerebral Ischemia-Reperfusion Injury by Regulating Inflammation and Apoptosis via TLR4 Activation

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Background: Ischemic stroke continues to be a leading cause of mortality globally. Interleukin-1 receptor-associated kinase 1 (IRAK1) plays a critical regulatory role in the onset and progression of stroke. Therefore, this study aimed to explore the molecular function and the underlying mechanisms of IRAK1 in cerebral ischemia-reperfusion (I-R) injury.

Methods: We established a mouse model of cerebral I-R injury to investigate the expression patterns of IRAK1 during the injury process. Its expression levels were assessed using quantitative real-time polymerase chain reaction (qRT-PCR) and Western blot analysis. Additionally, the cerebral infarcted area was evaluated through Toluidine Blue Cresyl Violet (TTC) staining. The impact of IRAK1 knockdown on cell death and inflammatory responses in mouse brain tissues was analyzed employing qRT-PCR and immunofluorescence methods. Furthermore, the effects of IRAK1 knockdown on the nuclear factor-kappa B (NF-κB) signaling pathway and Toll-like Receptor 4 (TLR4) expression levels were assessed utilizing Western blot analysis.

Results: We observed a time-dependent elevation in IRAK1 expression following the I-R injury ($p < 0.001$). Furthermore, IRAK1 knockdown significantly reduced infarcted area in mice ($p < 0.01$). Moreover, knockdown of IRAK1 alleviated cell death and inflammatory responses in mice following I-R injury ($p < 0.01$ and $p < 0.001$). However, this inhibitory effect was linked to the suppression of the Caspase-3 and NF-κB signaling pathways. Additionally, IRAK1 was found to contribute to cerebral I-R injury by regulating TLR4. We observed that suppressing TLR4 during oxygen-glucose deprivation treatment significantly mitigated the exacerbating effects of IRAK1 overexpression on the inflammatory response and cell apoptosis in microglial cells ($p < 0.01$ and $p < 0.001$).

Conclusions: These findings underscore the involvement of IRAK1 in cerebral stroke by interacting with TLR4, presenting a promising avenue for therapeutic intervention in cerebral ischemia-reperfusion injury.

Keywords: IRAK1; cerebral ischemia-reperfusion injury; TLR4; apoptosis; inflammation

Introduction

Ischemic stroke, a prevalent neurological disorder, remains a significant challenge for healthcare systems globally [1,2]. In this context, cerebral ischemia-reperfusion (I-R) injury emerges as a substantial contributor to neuronal damage and functional impairments [3,4]. Unraveling the underlying molecular mechanisms is imperative for developing innovative therapeutic strategies, and this study focuses on a crucial player in this domain—interleukin-1 receptor-associated kinase 1 (IRAK1).

IRAK1 plays a critical regulatory role in immune system signaling pathways. In the context of ischemic stroke, it is involved in modulating inflammation and cell apoptosis, thereby influencing the survival and functionality of neuronal cells [5,6]. This novel comprehension allows us to delve deeper into the multifaceted role of IRAK1 in neurological disorders.

Concurrently, our focus extends to Toll-like Receptor 4 (TLR4), a pivotal pattern recognition receptor responsible for identifying damage-associated molecular patterns

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(DAMPs) [7]. Activation of TLR4 induces a cascade of inflammatory responses [8], and recent study has unveiled that IRAK1 acts as a DAMP, enhancing inflammatory processes through its interaction with TLR4 [9]. This provides a novel perspective by integrating IRAK1 into the complex network of inflammation regulation in neurological disorders.

Our study not only examines the interaction between IRAK1 and TLR4 but also seeks to elucidate their roles in the nuclear factor-kappa B (NF-κB) and Caspase-3 pathways, two primary mechanisms mediating inflammation and cell apoptosis. By profoundly understanding the relationship between these molecules, we aim to provide a robust theoretical foundation for developing more precise and effective treatment strategies in the future.

As we continue to explore the current landscape of ischemic stroke research, we will examine the pivotal roles of inflammation and apoptosis in cerebral ischemia-reperfusion injury. Through a comprehensive review of previous studies on IRAK1 and TLR4, we aim to comprehensively explore their roles in neurological disorders. Finally, we will propose potential clinical applications of our research findings and offer insights into therapeutic paradigms for ischemic stroke. These observations will offer new perspectives for translating laboratory research into clinical practice.

Materials and Methods

Animal Experimental Model

The animal experiments involved 36 male mice aged 10–12 weeks, each weighing 30 ± 2 g. IRAK1 knockout (KO) mice (n = 18) were procured from Cyagen Biotech (Suzhou, China). Using the random number table method, both the wild-type surgery group and the IRAK1-KO surgery group were divided into the sham surgery group and the I-R surgery group. The mice of the I-R surgery group were anesthetized using 3% isoflurane. The mice were secured on the surgical table, and the surgical area was prepared by shaving and disinfection. A small cranial window was created to expose the brain surface. After this, a vascular clamp was placed on the common carotid artery to induce cerebral ischemia. After 45 minutes of occlusion, the vascular clamp was released to allow blood reperfusion to the brain. The surgical incision was closed to ensure proper wound closure. The probe of the Doppler flowmeter (Perimed, Stockholm, Sweden) was positioned on the left common carotid artery in the mouse's neck to examine the blood flow velocity in the left brain. For the sham-operated group, the filament was immediately withdrawn once a reduction in cerebral blood flow was observed. At 6 hours, 12 hours, and 24 hours post-surgery, the mice were euthanized using intraperitoneal injection of pentobarbital (3 mg/mL) at a dose of 110 mg/kg. After this, brain tissues were collected for subsequent analysis. This study was approved by the ethics committee of Hunan University of Chinese Medicine, China (Approval No.: LL2022101301).

Morris Water Maze Assay

A large water tank was filled with opaque water to conceal the bottom platform. A platform was submerged in the center of the tank, with its surface level with the water. Several training sessions were conducted to familiarize the mice with the environment. During one training session, the mice were randomly placed in the tank to observe their ability to locate the hidden platform. Once the mice found the platform, they were allowed to stay on the platform for a period to reinforce their memory. The data, such as the time taken to reach the platform and the length of the path during training, were documented. In the testing phase, the hidden platform was removed, and the mice were placed in various locations within the tank. Furthermore, their swimming paths and the duration of their stays in the tank were observed to assess their memory of the platform's location.

Cell Culture and Transfection

Human microglia (CL-0620) were obtained from Pricella, Wuhan, China. Prior to treatment, microglial cells were seeded in chamber slides at a density of 1 × 10^5 cells/well. Following a 1-hour incubation in a serum-free and glutamate-free DMEM/F12 nutrient mixture (11765054, Gibco, Waltham, MA, USA), the cells underwent a 60-minute episode of transient oxygen-glucose deprivation (OGD) and then returned to standard culture conditions for varying durations to simulate *in vitro* ischemia-reperfusion (I-R) scenarios. The cellular responses were manipulated using short interfering RNA (siRNA) and plasmids designed for IRAK1 or TLR4 overexpression (OE). The cell transfection was performed using the Lipofectamine 2000 reagent (11668027, Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. siRNAs targeting TLR4 and IRAK1, alongside plasmids tailored for IRAK1 or TLR4, were synthesized by RiboBio (Guangzhou, China). The cell lines utilized in this study underwent mycoplasma testing and STR profiling. The siRNA interfering fragments are given in Table 1.

RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from the samples using TRNzol universal reagent (DP424, TIANGEN, Beijing, China), and their concentration and purity were evaluated using a spectrophotometer or fluorometer. Subsequently, RNA was converted to complementary DNA (cDNA) using a reverse transcription kit (KR116, TIANGEN, Beijing, China). The qPCR reaction mixture included cDNA templates, gene-specific primers, and fluorescent probes (FP205, TIANGEN, Beijing, China). The reaction mixture was then distributed into wells of a qPCR plate, and PCR

Table 1. siRNA fragments used in cell transfection.

Name	Sequences (5′-3′)
si-NC-sense	UUCUCCGAACGUGUCACGUTT
si-NC-antisense	ACGUGACACGUUCGGAGAATT
si-*TLR4*-sense	GCAUGAAGCUGACUUCAUATT
si-*TLR4*-antisense	UAUGAAGUCAGCUUCAUGCTT
OE-NC-sense	GACGAGCGGCTGCGGTTATT
OE-NC-antisense	AATCCGCAGCCGCTCGTCTT
OE-*IRAK1*-sense	ATGGACCGAGGAGGAGACGA
OE-*IRAK1*-antisense	TCAGGTGCCACTGAGAAACC

siRNA, short interfering RNA; *TLR4*, Toll-like Receptor 4; *IRAK1*, interleukin-1 receptor-associated kinase 1; OE, overexpression; NC, negative control.

Table 2. A list of primers used in qRT-PCR.

Primer names	Primer sequences (5′-3′)
IRAK1-F	CGGTGCCAGGACCAAGTATCT
IRAK1-R	CCTCTCGTACACCTGGGTCATAG
TNF-α-F	CCATCGGACGGGCTGTACCTC
TNF-α-R	CTCGGCGCTGAGTCGGTCTC
IL-1β-F	CTGAAAGCTCTCCACCTC
IL-1β-R	CTTTGAACAGAATGTGCC
Mouse-*IL-6*-F	GGCCTTCCCTACTTCACAAG
Mouse-*IL-6*-R	ATTTCCACGATTTCCCAGAG
Human-*IL-6*-F	ACTCACCTCTTCAGAACGAATTG
Human-*IL-6*-R	CCATCTTTGGAAGGTTCAGGTTG
β-*actin*-F	GCACCACACCTTCTACAATGAG
β-*actin*-R	ATAGCACAGCCTGGATAGCAAC

qRT-PCR, quantitative real-time polymerase chain reaction; *TNF*-α, tumor necrosis factor-α; *IL*, interleukin.

amplification was performed on a real-time PCR instrument (LightCycler 480, Roche, Basel, Switzerland). The relative gene expression levels of the target genes were assessed by calculating the Ct value. Moreover, the $2^{-\Delta\Delta Ct}$ method was used for quantitative analysis of the data, interpretation of results, and presentation of graphical and statistical data. β-*actin* was used as the internal reference gene. The primer sequences used in this study are listed in Table 2.

Western Blot Analysis

Total protein was extracted from cell or tissue samples and separated through sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). After this, proteins were transferred onto a poly (vinylidene fluoride) (PVDF) membrane, which was then blocked to inhibit nonspecific binding. Subsequently, the membrane underwent overnight incubation with primary antibodies, including anti-IRAK1 (1:2000 dilution; cat no. Ab302554, Abcam, Cambridge, UK), anti-p-Inhibitor of nuclear factor kappa-B kinase subunit alpha (IkBα) (1:2000 dilution; cat no. Ab133462, Abcam, Cambridge, UK), anti-IkBα (1:2000 dilution; cat no. Ab32518, Abcam, Cambridge, UK), anti-p-p65 (1:2000 dilution; cat no. Ab31624, Abcam, Cambridge, UK), anti-NF-κB p65 (1:2000 dilution; cat no. Ab32536, Abcam, Cambridge, UK), anti-Cleaved Caspase-3 (1:2000 dilution; cat no. ab2302, Abcam, Cambridge, UK), anti-B-cell lymphoma-2 (Bcl-2) (1:2000 dilution; cat no. ab182858, Abcam, Cambridge, UK), anti-Bcl-2 associated X protein (Bax) (1:2000 dilution; cat no. ab182733, Abcam, Cambridge, UK), anti-Myeloid differentiation primary response 88 (MyD88) (1:2000 dilution; cat no. Ab133739, Abcam, Cambridge, UK), anti-TLR4 (1:2000 dilution; cat no. Ab22048, Abcam, Cambridge, UK), and anti-Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:2000 dilution; cat no. Ab9485, Abcam, Cambridge, UK). The next day, the membrane was washed and incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (1:2000 dilution; cat no. ZB-2305, ZB-2301, ZSGB-BIO, Beijing, China). After washing the membrane, protein bands were visualized using a chromogenic substrate, and images were recorded either in a darkroom or by scanning the membrane. Finally, quantitative analysis of protein bands was performed using image analysis software (version 1.54f, NIH, Bethesda, MD, USA).

TUNEL Assay

The cell apoptosis rate was assessed using the Terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL) assay (T2130, Solarbio, Beijing, China). The first step in this assay involves enhancing the permeability of cell membranes to reagents by fixing the cells and permeabilizing their membranes. Subsequently, the TUNEL reaction is conducted, introducing terminal transferase, and labeled dUTP into cells to mark the ends of DNA fragments produced during apoptosis. After washing, the samples underwent nuclear staining, followed by mounting onto slides. Finally, the degree of apoptosis was assessed by observing TUNEL-labeled cells using a fluorescence microscope (CK31, Olympus, Tokyo, Japan).

Fluoro-Jade B Experiment

In the Fluoro-Jade B experiment (32160702, Sigma-Aldrich, St. Louis, MO, USA), tissue sections containing areas of neuronal damage were first prepared. Following dehydration and Fluoro-Jade B staining, the damaged neurons were labeled. Subsequently, the sections were washed in a clearing solution, mounted onto slides, and observed using a fluorescence microscope (CK31, Olympus, Tokyo, Japan) to evaluate the degree of neuronal damage.

Analysis of Infarct Size

Initially, a physiological saline solution containing 2% Toluidine Blue Cresyl Violet (TTC) (17779, Sigma-Aldrich, St. Louis, MO, USA) was prepared. Then, mice brain tissue samples were sliced, and subsequently immersed in the TTC solution for approximately 15–30 min-

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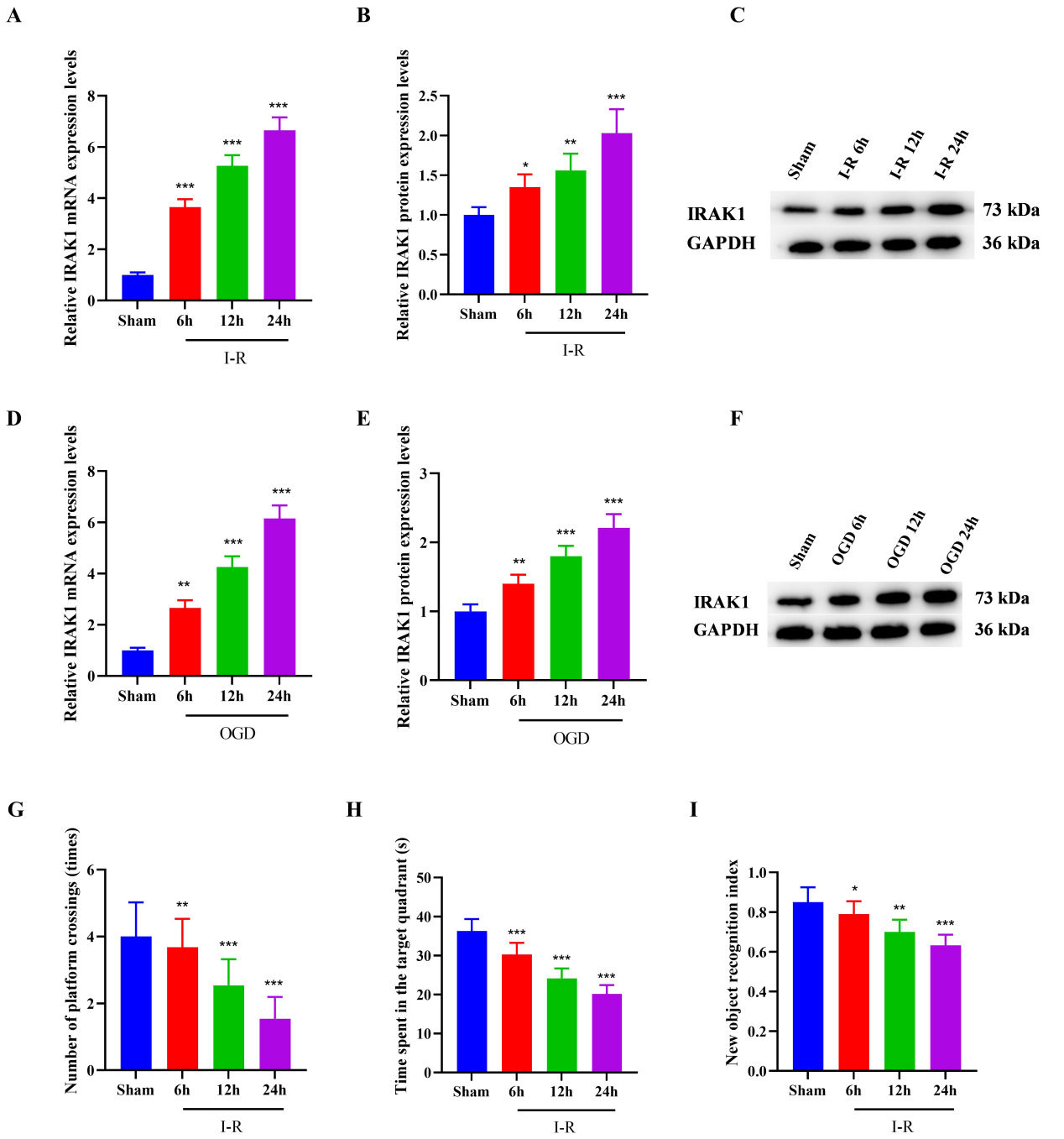

Fig. 1. IRAK1 expression levels were upregulated following exposure to cerebral I-R injury in both *in vivo* and *in vitro* settings. (A–C) The levels of IRAK1 expression in mice experiencing cerebral ischemia-reperfusion injury at designated time intervals were evaluated using RT-qPCR and Western blot analysis. (D–F) The expression levels of IRAK1 in microglial cells exposed to oxygen-glucose deprivation (OGD) at specified time points were determined using RT-qPCR and protein immunoblotting. (G–I) Behavioral testing in mice, including platform crossings, time spent in the target quadrant, and novel object recognition index (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. I-R, ischemia-reperfusion; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase.

utes. After staining, the slices were removed, and images were captured, typically with the infarcted areas appearing white and viable tissue appearing red. The images were quantitatively analyzed using ImageJ software (version 1.54f, NIH, Bethesda, MD, USA) to measure the infracted area, and the data were recorded for statistical analysis.

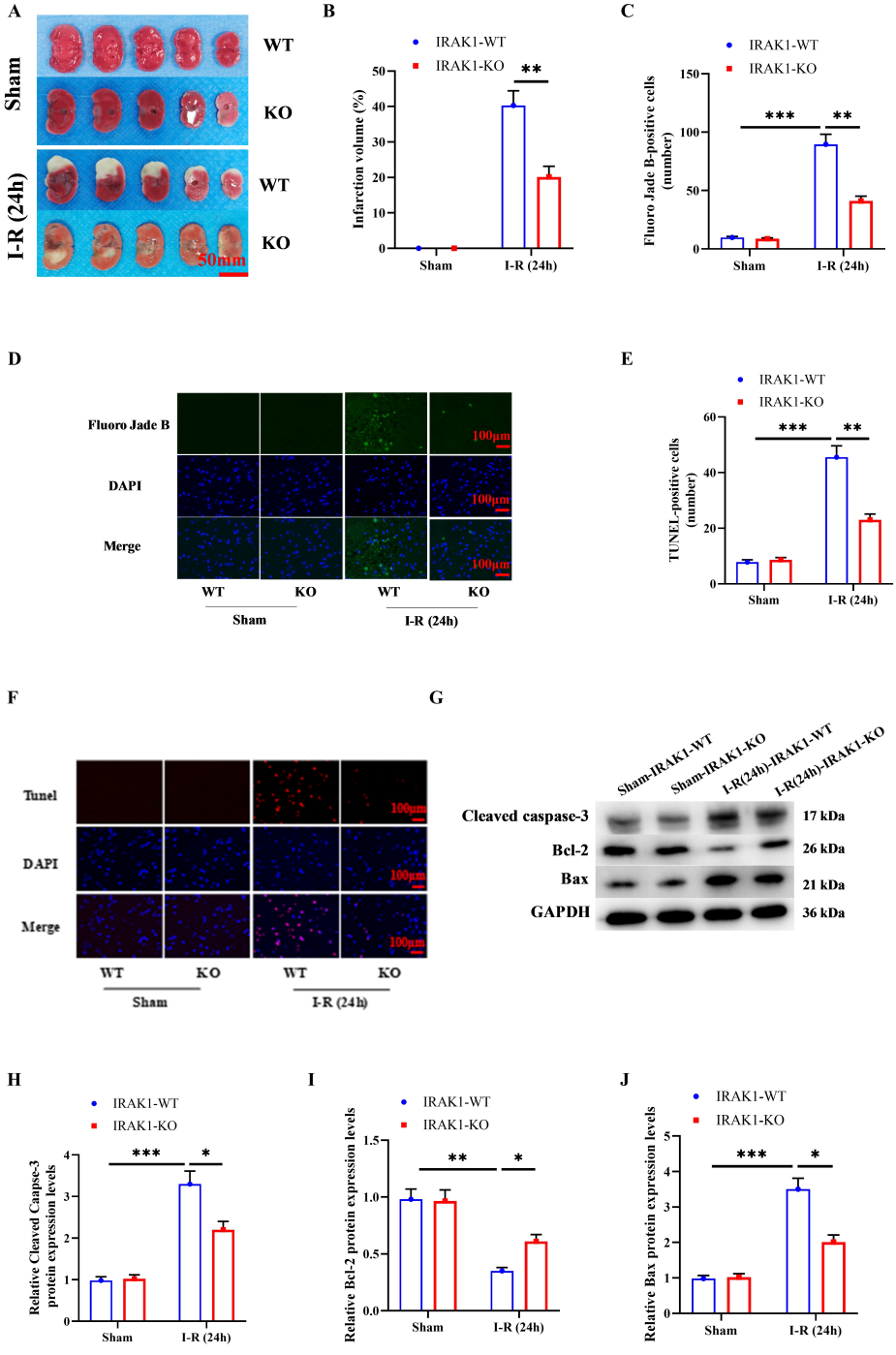

Fig. 2. The deletion of IRAK1 mitigated the damage caused by ischemia-reperfusion injury. (A) Illustrative images of Toluidine Blue Cresyl Violet (TTC) staining in brain sections from the WT and IRAK1-KO groups 24 hours post-reperfusion. (B) Quantitative assessment of infarct volume in the designated mouse groups. (C–F) Fluoro-Jade B and TUNEL staining of brain sections in mice. Quantitative results present the number of Fluoro-Jade B (C) and TUNEL (D) positive cells. (G–J) Immunoblot analysis of protein expression levels for B-cell lymphoma-2 (Bcl-2), Bcl-2 associated X protein (Bax), Bid, and Cleaved Caspase-3 in mouse brain tissue (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. TUNEL, Terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling; KO, knockout; WT, wild type.

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Statistical Analyses

Statistical analyses were performed utilizing GraphPad Prism software (version 8.0, GraphPad Inc., San Diego, CA, USA). The data were expressed as mean ± standard deviation. The comparison between the two groups was conducted using the *t*-test, while multiple group comparisons were performed using Analysis of Variance (ANOVA), followed by Tukey's post hoc test. The statistical significance was determined at a p-value < 0.05.

Results

The Expression Level of IRAK1 was Increased Following Cerebral I-R Injury in Vivo and in Vitro

A significant increase in the levels of IRAK1 mRNA and protein was observed over time following cerebral ischemia-reperfusion ($p < 0.001$, Fig. 1A–C). Similarly, in an *in vitro* setting, human astrocytes exposed to OGD showed a time-dependent increase in IRAK1 expression ($p < 0.01$ and $p < 0.001$, Fig. 1D–F). Furthermore, the Morris water maze test revealed that mice subjected to ischemia-reperfusion (I-R) demonstrated a substantial reduction in platform crossings and spent less time in the target quadrant compared to the Sham group ($p < 0.01$ and $p < 0.001$), indicating a time-dependent effect (Fig. 1G,H). Additionally, the I-R group of mice showed a significant alleviation in the new object recognition index compared to the Sham group ($p < 0.05$, $p < 0.01$, and $p < 0.001$, Fig. 1I), with the reduction becoming more pronounced as the duration of I-R increased.

IRAK1 Knockout Alleviated Ischemia-Reperfusion Injury

To examine the role of IRAK1 in regulating cerebral ischemia-reperfusion injury, we used IRAK1-KO mice. In the middle cerebral artery occlusion (MCAO) model, the infarct size was significantly smaller in IRAK1-KO mice ($p < 0.01$, Fig. 2A,B). Fluorescent staining indicated a substantial reduction in Fluoro-Jade B and TUNEL-positive cells in IRAK1-KO mice post-MCAO surgery compared to wild type (WT) mice ($p < 0.01$ and $p < 0.001$, Fig. 2C–F). At 24 hours post-MCAO, IRAK1-KO mice exhibited lower levels of pro-apoptotic proteins, such as Bax and Cleaved Caspase-3, than WT mice ($p < 0.01$ and $p < 0.001$, Fig. 2G–J). In contrast, the anti-apoptotic protein Bcl-2 level was significantly higher in IRAK1-KO mice following ischemia-reperfusion injury compared to the WT group ($p < 0.05$ and $p < 0.01$, Fig. 2G–J).

IRAK1 Knockout Attenuated the Inflammatory Response Following Ischemia-Reperfusion Injury

After MCAO surgery, the IRAK1 knockout (IRAK1-KO) group exhibited significant reductions in mRNA levels of pro-inflammatory regulators tumor necrosis factor-α (*TNF-*α), interleukin (*IL*)-*1*β, and *IL-6* ($p < 0.05$, $p < 0.01$, and $p < 0.001$, Fig. 3A–C). Moreover, protein immunoblotting demonstrated a substantial alleviation in the phosphorylation of IκBα and NF-κB (p65) in IRAK1-KO mice ($p < 0.05$ and $p < 0.01$, Fig. 3D–F). The NF-κB pathway, critical for inflammatory responses involving TLR4, was further investigated. Western blot analysis indicated that IRAK1 knockout significantly attenuated MCAO-induced TLR4 expression ($p < 0.05$ and $p < 0.001$, Fig. 3G–I). Additionally, IRAK1-KO mice showed a substantial decrease in the expression of the downstream TLR4 signaling mediator MyD88 after MCAO ($p < 0.05$ and $p < 0.001$, Fig. 3G–I).

The I-R Injury Induced by IRAK1 was Directly Contingent on the Activation of TLR4

We investigated the interaction between IRAK1 and TLR4 to examine the molecular mechanisms through which the absence of IRAK1 influences TLR4 in cerebral ischemia-reperfusion injury. Our hypothesis suggested that IRAK1 might directly interact with TLR4. To elucidate whether IRAK1-induced brain injury is TLR4-dependent, we established glial cells with IRAK1 overexpression and TLR4 knockdown. The expression levels of IRAK1 and TLR4 were validated employing Western blot analysis ($p < 0.01$ and $p < 0.001$, Fig. 4A–C). Following 24 hours of exposure to OGD, TLR4 knockdown significantly suppressed the expression of TNF-α, IL-1β, and IL-6 compared to the si-negative control (NC) group. However, co-transfection with OE-IRAK1 significantly elevated the expression of TNF-α, IL-1β, and IL-6 ($p < 0.01$ and $p < 0.001$, Fig. 4D–F). Additionally, TLR4 knockdown significantly suppressed cell apoptosis compared to the si-NC group, and IRAK1 overexpression substantially counteracted the reduced apoptosis caused by si-TLR4 ($p < 0.01$, Fig. 4G,H).

Discussion

Ischemic stroke is a leading cause of death and disability worldwide [10]. Despite various pathological processes being targeted to improve ischemic brain injury, further investigation is needed to translate these findings into effective clinical treatments and uncover potential molecular mechanisms [11,12]. Our study identifies IRAK1 as a plausible upstream regulator of inflammation and cell apoptosis following MCAO. Significantly, IRAK1/TLR4 pathway appears to play a crucial role in regulating brain ischemia-reperfusion injury.

Our findings underscore the significant interaction between IRAK1 and TLR4, which influences NF-κB and cellular apoptosis, contributing to cerebral ischemia-reperfusion injury. IRAK1, a protein kinase, is primarily involved in immune system signaling pathways [13]. IRAK1 has been identified as crucial in inflammation and apoptosis processes associated with ischemic injury [14,15]. As a

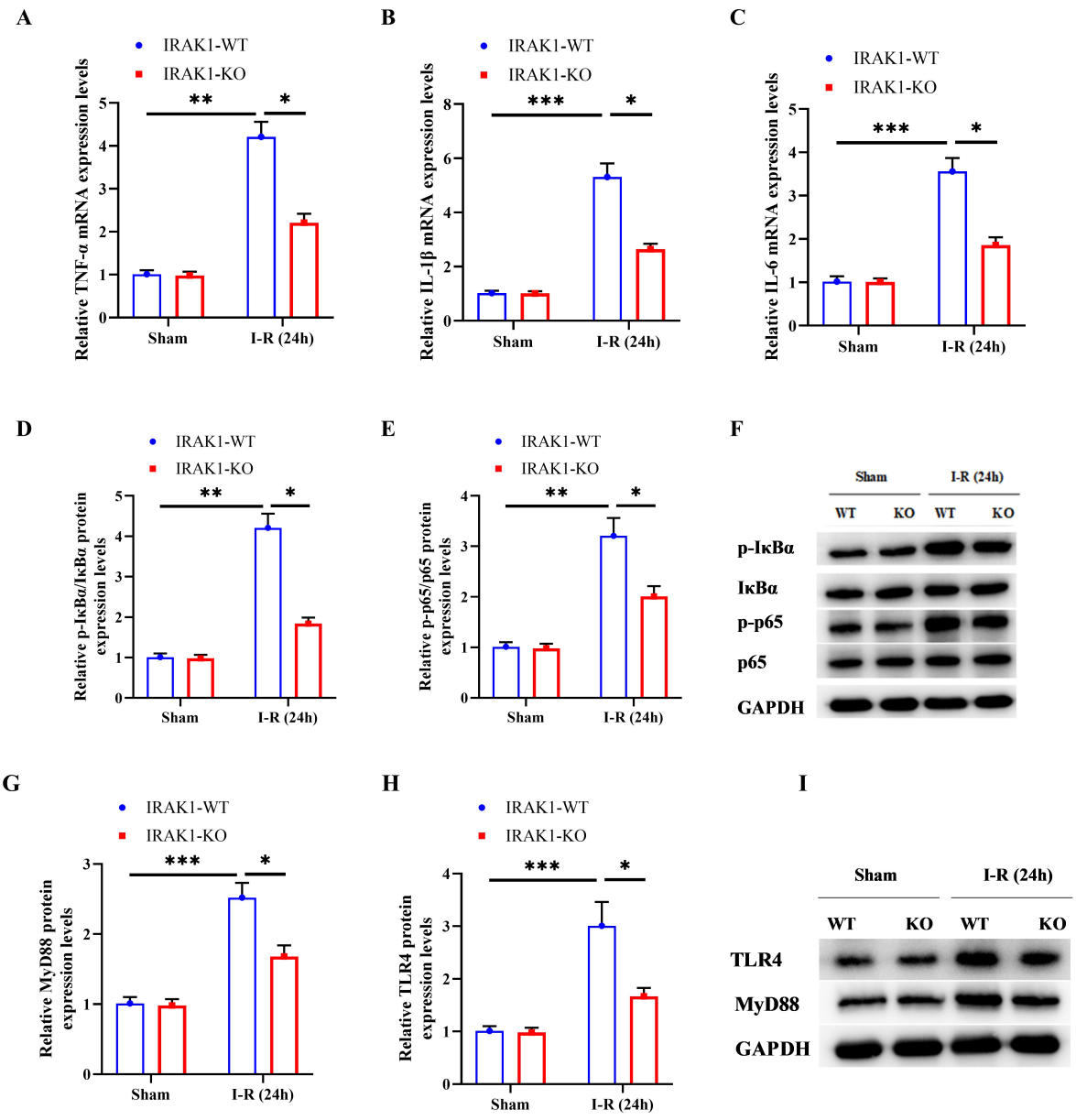

Fig. 3. The deletion of IRAK1 alleviated the inflammatory response following I-R injury. (A–C) RT-qPCR outcomes depicting the levels of TNF-α, IL-1β, and IL-6 in the brain tissue of mice. (D–F) Western blot analysis illustrating the phosphorylation of IκBα and NF-κB in the brain tissue of mice. (G–I) Western blot analysis of Myeloid differentiation primary response 88 (MyD88) and TLR4 expression in the brain tissue of mice (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. NF-κB, nuclear factor-kappa B; IkBα, Inhibitor of nuclear factor kappa-B kinase subunit alpha.

vital component of the Interleukin-1 Receptor (IL-1R) signaling pathway [16], IRAK1 is activated upon IL-1R stimulation and induces a series of signaling events, thereby leading to inflammation and immune responses [17,18]. Ischemic injury results from insufficient blood supply, particularly in conditions like stroke, which leads to tissue damage [19]. IRAK1 has been found to be involved in regulating inflammation and apoptosis during this process, closely associated with the body's physiological response to ischemic injury [20]. Research indicates that IRAK1 may interact with TLR4, thereby modulating the NF-κB signaling pathway and enhancing apoptosis [21]. In the context of cerebral I-R injury, this interaction may be critical for the survival and function of neuronal cells [22].

In the aftermath of cerebral ischemia, inflammation assumes a pivotal role. The occurrence of brain injury triggers the activation of resident microglial cells and the infiltration of immune cells like neutrophils, T cells, and

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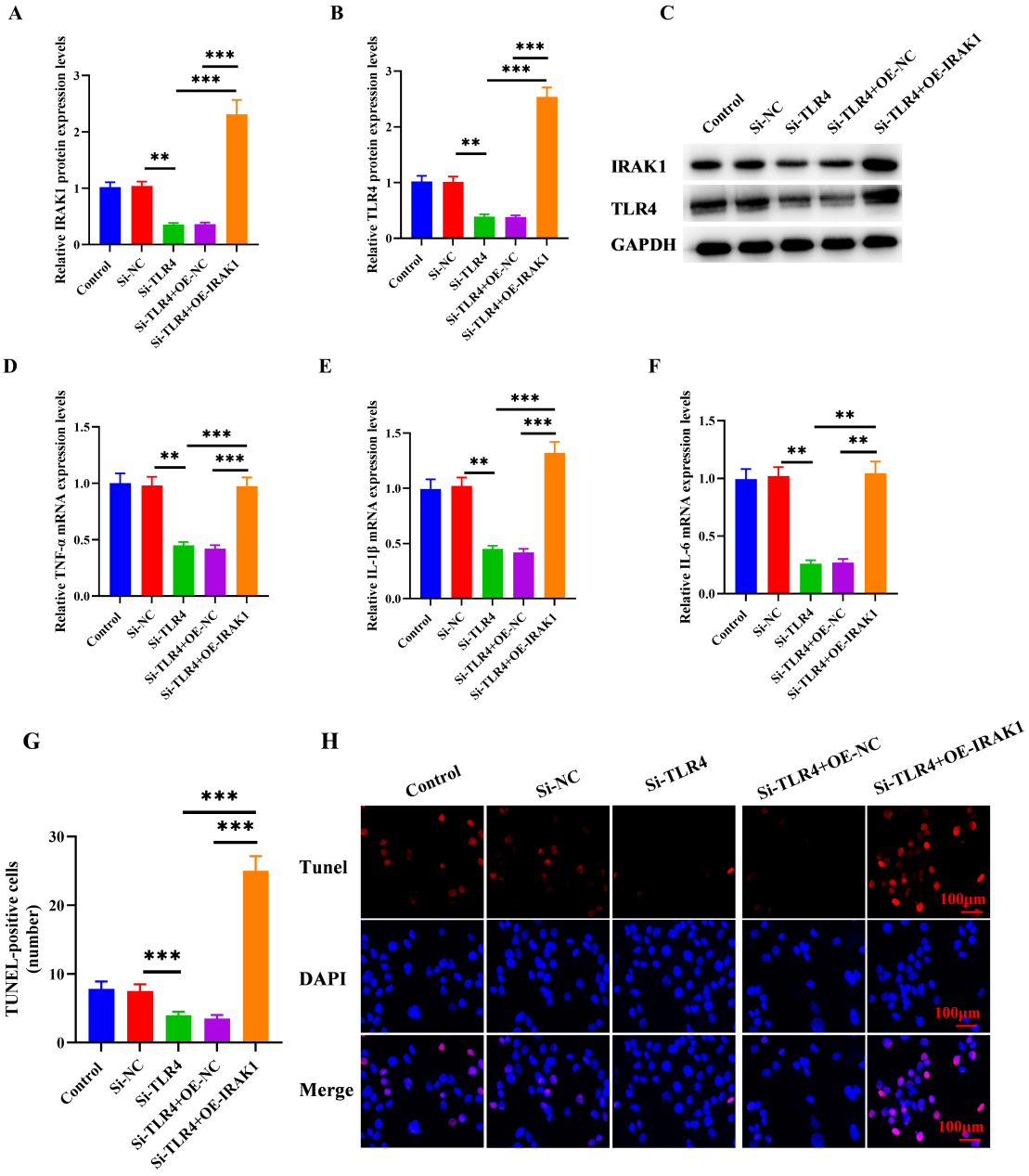

Fig. 4. IRAK1 induced I-R injury through TLR4 activation. Following the transfection of microglial cells with si-TLR4, OE-IRAK1, or their combination for 24 hours, these cells underwent an additional 24-hour exposure to oxygen-glucose deprivation (OGD) treatment. Subsequently, (A–C) the protein expression levels of IRAK1 and TLR4 were determined. (D–F) qRT-PCR analysis was employed to assess the mRNA levels of *TNF-α*, *IL-1β*, and *IL-6* in the cells. (G,H) Cell apoptosis was evaluated using the TUNEL immunofluorescence assay (n = 6). $^{**}p < 0.01$, $^{***}p < 0.001$.

macrophages, leading to the production of diverse pro-inflammatory mediators. These pro-inflammatory mediators, including TNF-α, IL-1β, and IL-6, exacerbate brain damage [23]. Therefore, targeting the suppression of microglial cell activation could be a promising therapeutic strategy to mitigate acute cerebral ischemia [24].

Apoptosis is a pivotal factor in the pathophysiology of cerebral I-R injury [25]. In this study, we confirmed that MCAO induces apoptosis in affected brain tissues, supported by a decrease in Bcl-2 expression and an increase in Bax and Cleaved Caspase-3 levels. Notably, knockout of IRAK1 significantly attenuated this pro-apoptotic process, thereby alleviating cerebral ischemia-reperfusion injury.

TLR4 is a critical Pattern Recognition Receptor (PRR) that mediates inflammation through receptor dimerization and downstream interaction with NF-κB [26]. Inhibiting the activation of TLR4 signaling is emerging as a promising therapeutic strategy against inflammation and holds potential for mitigating ischemic strokes [27]. Surprisingly, extracellular IRAK1 has recently been identified as a novel DAMP owing to its inflammatory properties resulting from its interaction with TLR4 [28]. Although the mouse model of cerebral ischemia-reperfusion injury is widely used in research, it may not entirely replicate the complexity of human stroke pathology. Further validation in other animal models or clinical studies is required to confirm these findings. This study primarily explores the role of IRAK1 in cerebral ischemia-reperfusion injury. However, stroke is a multifactorial condition involving various molecular pathways and cellular processes. Other genes and pathways may also play a substantial role in its pathogenesis. Furthermore, the experimental conditions, such as the duration and severity of ischemia-reperfusion injury, may not fully replicate the clinical scenario of human stroke. Variability in experimental conditions could affect the reproducibility and generalizability of the findings.

Furthermore, we elucidated the role of IRAK1 in regulating TLR4 expression and the ensuing production of inflammatory cytokines during I-R injury. Moreover, we also demonstrated that IRAK1 facilitates the activation of the NF-κB signaling pathway and apoptosis through its interaction with and activation of TLR4. These findings offer insights into the therapeutic prospects of targeting the regulatory axis involving IRAK1 and TLR4 in the development of brain ischemia-reperfusion injury.

Conclusions

In summary, this study reveals a novel role of IRAK1 in influencing ischemic stroke through its interaction with TLR4, triggering inflammation and cellular apoptosis via the NF-κB and Caspase-3 pathways, respectively. Targeting IRAK1 emerges as a promising avenue for developing novel therapies for individuals with ischemic stroke.

Availability of Data and Materials

The corresponding authors will provide the data that underpin the study's conclusions with a reasonable application.

Author Contributions

JD, SH, JG, HS, JL, and RW designed the study. All authors conducted the study. XS, CL, SL, and WG collected and analyzed the data. XS and CL participated in drafting the manuscript, and all authors contributed to the critical revision of important intellectual content. All authors gave final approval of the version to be published. All authors participated fully in the work, took public responsibility for appropriate portions of the content, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or completeness of any part of the work were appropriately investigated and resolved.

Ethics Approval and Consent to Participate

This study was approved by the ethics committee of Hunan University of Chinese Medicine, China (Approval No.: LL2022101301).

Acknowledgment

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Conflict of Interest

The authors declare no conflict of interest.

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