Knockout of Sirt2 alleviates traumatic brain injury in mice

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Abstract

Sirtuin 2 (SIRT2) inhibition or Sirt2 knockout in animal models protects against the development of neurodegenerative diseases and cerebral ischemia. However, the role of SIRT2 in traumatic brain injury (TBI) remains unclear. In this study, we found that knockout of Sirt2 in a mouse model of TBI reduced brain edema, attenuated disruption of the blood-brain barrier, decreased expression of the nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) inflammasome, reduced the activity of the effector caspase-1, reduced neuroinflammation and neuronal pyroptosis, and improved neurological function. Knockout of Sirt2 in a mechanical stretch injury cell model in vitro also decreased expression of the NLRP3 inflammasome and pyroptosis. Our findings suggest that knockout of Sirt2 is neuroprotective against TBI; therefore, Sirt2 could be a novel target for TBI treatment.

Key Words: blood-brain barrier; caspase-1; cerebral edema; neuroinflammation; neuroprotection; NLRP3; pyroptosis; Sirt2; tight junction protein; traumatic brain injury

Introduction

Traumatic brain injury (TBI) is a complex disease process that includes primary injury and secondary injury (Morgant-Kossmann et al., 2019). Primary injury is caused by an initial direct mechanical force; secondary injury is caused by a series of pathophysiological changes induced by the primary injury, including neuroinflammation, blood-brain barrier (BBB) disruption, oxidative stress, and excitotoxicity and etc. (Ma et al., 2017b; Sweeney et al., 2019; Jing et al., 2020).

Neuroinflammation is a crucial biological process in central nervous system injury that leads to continuous neurological impairment that is characterized by the accumulation of immune cells and the abnormal production of proinflammatory cytokines (O’Brien et al., 2020; Kelley et al., 2019). Activated caspase-1 cleaves the precursors of IL-1β and IL-18 to form IL-1β and IL-18. IL-1β and IL-18 are the most potent proinflammatory cytokines (O’Brien et al., 2020; Henry and Loane, 2021; Shaheen et al., 2021; Li et al., 2022). Among the proinflammatory cytokines induced by TBI, interleukin (IL)-1β plays a crucial role in triggering the inflammatory cascade and is predominantly regulated by the nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) inflammasome (Irrera et al., 2020).

The NLRP3 inflammasome is a multiprotein complex with three protein domains: NACHT, LRR, and PYD domains-containing protein 3 (NLRP3; sensor), apoptosis-associated speck-like protein containing a CARD (ASC; adapter), and caspase-1 (effector) (Sutterwala et al., 2006). When a cell is stimulated by damage-associated molecular pattern molecules, the NLRP3 protein oligomerizes and recruits ASC and procaspase-1, forming the NLRP3 inflammasome, which leads to the activation of procaspase-1 (Ma et al., 2017a; Kelly et al., 2019). Activated caspase-1 cleaves the precursors of IL-1β and IL-18 to form IL-1β and IL-18. IL-1β and IL-18 are the most potent cytokines that initiate inflammation; the increased inflammatory response leads to tissue damage, causing pyroptosis (Jha et al., 2010; Yang et al., 2019b; Chang et al., 2020).

Pyroptosis is an inflammatory form of programmed cell death mediated by caspase-1 activation and IL-1β and IL-18 release (Cookson and Brennan, 2001; Ding et al., 2016). A variety of cell types in multiple central nervous system diseases can die by pyroptosis, such as microglia, neurons, and astrocytes (de Rivera Vaccari et al., 2014; Gustin et al., 2015; Zhi et al., 2021). Gene knockout or inhibition of key molecules in the pyroptosis pathway, such as NLRP3, absent in melanoma 2, and caspase-1, can inhibit TBI damage and sequelae in animal models (Adamczak et al., 2014; Ge et al., 2018; Liu et al., 2018a).

Sirt2 (SIRT2) is an NAD⁺-dependent deacetylase involved in energy metabolism, oxidative stress, inflammation, cell apoptosis, and cell cycle regulation (Harting and Knoll, 2010). The role of SIRT2 in neurological diseases remains controversial. Some studies have shown that SIRT2 inhibition or Sirt2 knockout has a protective effect on neurodegenerative diseases and cerebral ischemia (Xie et al., 2017; Fourcade et al., 2018; Wu et al., 2018), whereas others showed the opposite effect (Eshun-Wilson et al., 2019; Wang et al., 2019). However, few TBI studies have investigated the relationship between Sirt2 and NLRP3. Therefore, we aimed to investigate whether Sirt2 can affect the NLRP3 inflammasome, which affects neuroinflammation and pyroptosis of nerve cells in TBI, and to explore mechanisms of action.

According to 2017 statistics from the TBI Model Systems National Database (National Institute on Disability, Independent Living and Rehabilitation Research, U.S. Department of Health and Human Services), cases of TBI in men greatly outnumbered cases in women and accounted for more than 73% of all TBI reported (Capizzi et al., 2020). Estrogen also has a neuroprotective effect on TBI (Brotfain et al., 2016). Therefore, female mice were excluded from the present study to reduce data variability.

Methods

Animals and experimental design

Adult male Sirt2 knockout mice (Sirt2−/−; RRID: IMSR_JAX:012772; 8–10 weeks; Shanghai Model Organisms Center, Inc., Shanghai, China) and adult male wild type (WT) C57BL/6J mice (8–10 weeks, 20–25 g; Shanghai SLAC Laboratory Animal Corp., Shanghai, China) were used for our in vivo study. We also used 12 pregnant Sirt2−/− mice and 12 pregnant WT C57BL/6J mice. The mice were housed under a standard 12-hour light/dark cycle at 23 ± 2°C.
with controlled humidity. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Shanghai Sixth People’s Hospital affiliated to Shanghai Jiao Tong University, Shanghai, China (approved on March 2, 2016). All experiments were designed and reported according to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines (Percie du Sert et al., 2020). Every necessary effort was taken to minimize the suffering and pain of the mice.

The Sirt2–/– and WT mice were randomly assigned to four groups: sham-WT, sham-Sirt2–/–, TBI-WT, and TBI-Sirt2–/–. A total of 244 mice were used in our study, and the experimental design is given in Figure 1. The mice were anesthetized intraperitoneally with xylazine (75 mg/kg; Sigma-Aldrich, St. Louis, MO, USA) and ketamine (10 mg/kg; Aladdin Biochemical Technology Co., Ltd., Shanghai, China) both for operations and for euthanasia in this study.

Neurobehavioral tests
The modified neurological severity score was used to evaluate neurological deficits (Yang et al., 2019a) before TBI and at 1, 3, 7, and 14 days post-TBI. A score of 0 indicates normal behavior, and a score of 14 indicates maximal neurological deficit.

The rotarod test was used to evaluate the motor coordination of mice (Yang et al., 2019a). Briefly, each mouse was trained for 3 days with 3 trials per day at 5 minutes per trial. The speed of the rod was accelerated to 40 revolutions/minute. Mice that could not stay on the rod for 5 minutes were excluded from data analysis. The latency of mice that stayed on the rod was recorded before TBI and at 1, 3, 7, and 14 days post-TBI.

The Morris water maze test was used to assess spatial memory (Xu et al., 2018). A pool was divided into four equal quadrants, and a 10-cm-diameter circular platform was placed in a quadrant 1 cm below the water. On days 15–19 post-TBI, each mouse was given 60 seconds to locate the hidden platform. If the mouse failed to find the platform within 60 seconds, it was guided onto the platform and kept on it for 15 seconds. On day 20, the platform was removed and each mouse was placed into the water in the quadrant diagonal to the platform’s prior location. The number of crossings at the platform location and the time spent in the target quadrant were recorded.

Brain water content measurement
The wet–dry weight method (Yang et al., 2019a) was used to determine brain edema at 3 days post-TBI. Briefly, the mouse brain was removed immediately after euthanasia without heart perfusion, and the ipsilateral injured cerebral tissue was separated and weighed using a precise analytical balance (Mettler Toledo, Switzerland). The tissues were immediately weighed, homogenized in 50% trichloroacetic acid (Sigma-Aldrich) solution, and then centrifuged at 12,000 × g for 20 minutes. The supernatant was transferred into a new tube for precipitation with 3 volumes of ethanol. EB extravasation was determined as follows: (weight wet – dry weight)/weight wet × 100.

Blood-brain barrier integrity measurement
BBB integrity was determined by Evans blue (EB) dye extravasation. At 70 hours post-TBI, the mice were anesthetized, and 2% EB (Sigma-Aldrich) was injected intravenously through the tail vein. The entire circulation for the brain was euthanized and then transcardially perfused with normal saline to remove all dye. The brain was removed from each mouse and divided into the two hemispheres. The tissues were immediately weighed, homogenized in 50% trichloroacetic acid (Sigma-Aldrich) solution, and then centrifuged at 12,000 × g for 20 minutes. The supernatant was used to determine the concentration of EB dye using a microplate reader (Agsient Technologies, Santa Clara, CA, USA).

Nissl and terminal deoxynucleotidyl transferase dUTP nick-end labeling staining
Samples were collected at 3 days post-operation for terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining and at 14 days post-TBI or post-sham operation for Nissl staining.

Nissl staining was used to determine the brain lesion volume. After euthanasia and transcardial perfusion with normal saline, brain tissues were fixed with 4% paraformaldehyde and sectioned at 4 μm. Sections were stained with Nissl stain, and the lesion volume was calculated using ImageJ (National Institutes of Health, Bethesda, MD, USA) (Schneider et al., 2012). The TUNEL assay was used to detect pyroptosis. TUNEL staining was performed using the Invitrogen Cell Death Detection Kit (Roche, Basel, Switzerland) in accordance with the manufacturer’s instructions. A fluorescence microscope (Leica, Wetzlar, Germany) was used to view and record images. For each mouse, three equally spaced coronal sections were selected from the injury site, and the number of pyroptotic cells was counted in each section in five random fields of view.

Immunofluorescence staining
Glia fibrillary acidic protein (GFAP), neuronal nuclei antigen (NeuN), and ionized calcium-binding adapter molecule 1 (Iba-1) were used to identify astrocytes, neurons, and microglia at 3 days post-TBI, respectively. The brain samples were fixed in 4% paraformaldehyde (4% PFA) and stored at 4 °C overnight as follows: goat anti-CD31 (1:200, R&D Systems, Minneapolis, MN, USA), rabbit anti-α-smooth muscle actin (α-SMA) (1:500, Abcam, Cambridge, MA, USA, Cat#: ab182878), or rabbit anti-IL-1β (1:500, R&D Systems, Minneapolis, MN, USA, Cat#: AF314). The primary antibodies were incubated at 4 °C overnight in 1% bovine serum albumin (BSA) in PBS. The samples were washed and blocked in 5% BSA for 30 min. The samples were incubated with the following secondary antibodies for 2 h at room temperature following rinsing in PBS: donkey anti-rabbit Alexa Fluor 488 (1:500, Thermo Fisher Scientific, Cat# A-21202), donkey anti-mouse Alexa Fluor 594 (1:500, Thermo Fisher Scientific, Cat# A-21207), donkey anti-rabbit Alexa Fluor 594 (1:500, Thermo Fisher Scientific, Cat# A-21207), donkey anti-mouse Alexa Fluor 488 (1:500, Thermo Fisher Scientific, Cat# A-21202, RRID:AB_303983), or goat anti-rabbit IgG-Alexa Fluor 594 (1:500, Thermo Fisher Scientific, Cat# A-21207, RRID:AB_141637), or donkey anti-mouse IgG-Alexa Fluor 488 (1:500, Thermo Fisher Scientific, Cat# A-21202, RRID:AB_141607). The samples were incubated with 4’,6-diamidino-2-phenylindole dihydrochloride (1:2000, Thermo Fisher Scientific) for 5 minutes at room temperature in the dark. Images were captured with a fluorescence microscope (Leica). Fluorescence intensity was quantified by LAS AF 2.8.0 software (Leica).

Western blot assay
Proteins from brain tissue at 3 days post-TBI and primary cortical neurons at 3 days post-operation were extracted using radioimmunoprecipitation assay (RIPA) buffer with protease inhibitor (10 mM phenylmethylsulfonyl fluoride (PMSF), 10 mg/ml aprotinin, 100 mg/ml sodium orthovanadate) and separated on a 10% SDS-PAGE gel. The proteins were transferred to a nitrocellulose membrane, blocked, and then incubated with primary antibodies against β-actin (1:1000, Sigma-Aldrich, Cat#: A5441, RRID: AB_227013), Sirt2 (1:200, Abcam, Cat#: ab108980, RRID: AB_2298772), mouse anti-NLRP3 (1:200, Abcam, Cat#: ab214185, RRID: AB_2298772), mouse anti-GFAP (1:200, MilliporeSigma, Cat#: MAB3402, RRID: AB_227013), or rabbit anti-zonula occludens 1 (ZO-1) (1:500, Abcam, Cat#: ab16008, RRID: AB_2161028). After washing, the membranes were incubated with secondary antibodies conjugated to horseradish peroxidase (1:10000) and washed again before exposure to an enhanced chemiluminescence detection kit (Thermo Fisher Scientific, Cat# A-21202, RRID: AB_141637). Western blot images were captured with a fluorescence microscope (Leica).
24 hours post-SI were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes. The membranes were blocked with 5% nonfat milk for 1 hour and incubated in primary antibodies at 4°C overnight. Secondary antibodies were used at a 1:1000 concentration for 2 hours at room temperature. The antibodies were as follows: rabbit anti-ZO-1 (1:1000, Thermo Fisher Scientific, Cat# 61-7300, RRID: AB_2533938), rabbit anti-NLRP3 (1:500, Abcam, Cat# ab214185, RRID: AB_2184793), mouse anti-ZO-1 (1:1000, Santa Cruz Biotechnology, Dallas, TX, USA, Cat# sc-22514-R, RRID: AB_2184784), rabbit anti-caspase-1 p15, p20 (1:1000, Cell Signaling Technology, Danvers, MA, USA, Cat# 24232, RRID: AB_2890194), rabbit anti-caspase-1 p20 (1:1000, www.antibodies-online.com), mouse anti-b-actin (1:100, Cell Signaling Technology, Cat# 3700, RRID: AB_2242334), mouse anti-β-tubulin (1:1000, Cell Signaling Technology, Cat# 6829R, RRID: AB_2715541), anti-mouse iGg-horseradish peroxidase (1:5000, Cell Signaling Technology, Cat# 7076, RRID: AB_3109224), or anti-rabbit IgG-horseradish peroxidase (1-5000, Cell Signaling Technology, Cat# 7074, RRID: AB_2099233). Protein was visualized using an electrochemiluminescence reagent (Cat# WBL5S1000, MilliporeSigma) and a chemiluminescence instrument (Tanon, Shanghai, China). The relative protein levels were expressed by optical density ratio to β-actin.

Real-time reverse transcription-polymerase chain reaction
Total RNA was prepared from primary cultured neurons at 24 hours post-SI or brain tissue at 3 days post-TBI using TRIzol reagent (Thermo Fisher Scientific), and the RNA was reverse transcribed into complementary DNA template using a PrimeScript® Reverse Transcription Reagent Kit (TaKaRa Biotechnology) with a SYBR Premix Ex Taq Kit (TaKaRa Bio) on an ABI 7900HT PCR instrument (Thermo Fisher Scientific). The following primers were used: NLRP-3 (forward: 5'-ATC AAG AGC GAC GCT CGA TCG-3' and reverse: 5'-GTC CTC CGT CAA GAT GAC GAA-3'), caspase-1 (forward: 5'-ACA AGG ACC GGA CCT ATG-3' and reverse: 5'-TCC CAG TCA CTG GCA ATG AGT-3'), IL-1β (forward: 5'-GAC ACT GGC GAC GAG AGG C-3' and reverse: 5'-CGA CTC CAG ATG GTC GGA TCG C-3'), caspase-1 (forward: 5'-ACA AGG ACC GGA CCT ATG-3' and reverse: 5'-TCC CAG TCA CTG GCA ATG AGT-3'), IL-1β (forward: 5'-GAC ACT GGC GAC GAG AGG C-3' and reverse: 5'-CGA CTC CAG ATG GTC GGA TCG C-3'). The PCR conditions were as follows: denaturation at 95°C for 30 seconds, followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing and extension at 60°C for 30 seconds, for a total of 40 cycles. We used the comparative Ct (threshold cycle) method (Zhu et al., 2019) normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) for each reaction.

Statistical analysis
No statistical methods were used to predetermine sample sizes; however, our sample sizes are similar to those reported in previous publications (Xu et al., 2017a, b). No animals or data points were excluded from the analysis. The evaluators were blinded to the assignment. All data were indicated as mean ± standard deviation. The comparisons between two groups were performed by Student’s t-test. *P < 0.05 was considered statistically significant. SPSS (Version 21.0, IBM, Armonk, NY, USA) and GraphPad Prism 5.01 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com) were used for statistical analysis and visualization, respectively.

Results

The NLRP3 inflammasome is upregulated in the pericontusional area post-TBI
Western blot and PCR were performed to examine the NLRP3, ASC, and caspase-1 expression levels, respectively. The numbers of TUNEL-positive primary neuron cells were counted for each group at 4°C for 1 hour to compare with the corresponding secondary antibody for 2 hours at room temperature. The antibodies were as follows: rabbit anti-ZO-1 (1:1000, Thermo Fisher Scientific, Cat# 61-7300, RRID: AB_2533938), rabbit anti-NLRP3 (1:500, Abcam, Cat# ab214185, RRID: AB_2184793), mouse anti-ZO-1 (1:1000, Santa Cruz Biotechnology, Dallas, TX, USA, Cat# sc-22514-R, RRID: AB_2184784), rabbit anti-caspase-1 p15, p20 (1:1000, Cell Signaling Technology, Danvers, MA, USA, Cat# 24232, RRID: AB_2890194), rabbit anti-caspase-1 p20 (1:1000, www.antibodies-online.com), mouse anti-b-actin (1:100, Cell Signaling Technology, Cat# 3700, RRID: AB_2242334), mouse anti-β-tubulin (1:1000, Cell Signaling Technology, Cat# 6829R, RRID: AB_2715541), anti-mouse iGg-horseradish peroxidase (1:5000, Cell Signaling Technology, Cat# 7076, RRID: AB_3109224), or anti-rabbit IgG-horseradish peroxidase (1-5000, Cell Signaling Technology, Cat# 7074, RRID: AB_2099233). Protein was visualized using an electrochemiluminescence reagent (Cat# WBL5S1000, MilliporeSigma) and a chemiluminescence instrument (Tanon, Shanghai, China). The relative protein levels were expressed by optical density ratio to β-actin.

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Statistical analysis
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Research Article
**Discussion**

This study demonstrates that Sirt2 knockout reduces the expression of the components of the NLRP3 inflammasome and the activity of caspase-1, which alleviates neuroinflammation and reduces pyroptosis, leading to reversal of neurological dysfunction and reductions in cerebral edema and BBB disruption.

Secondary injury post-TBI is a gradual process that causes chemical and metabolic changes in nerve cells and leads to a series of pathological changes, such as apoptosis, neuroinflammation, and BBB disruption (Kulbe and Hall, 2017). The processes behind these pathological changes may be vital targets for TBI therapy (Jha et al., 2019). The BBB maintains brain homeostasis by transporting nutrients, removing toxic substances, and inhibiting the entry of peripheral immune cells. The BBB is composed of specialized endothelial cells closely joined by tight junction proteins. The loss of tight junction proteins, such as ZO-1 and occludin, allows typically restricted molecules, such as peripheral immune cells, to pass through the BBB, leading to vasogenic brain edema (Wang et al., 2018). We found that Sirt2 knockout reduced EB extravasation and brain edema, indicating that Sirt2 knockout partially reversed the BBB disruption induced by TBI. Furthermore, the results of ZO-1 immunofluorescence staining and western blot analysis showed that Sirt2 knockout reduced the loss of the tight junction protein ZO-1. Therefore, we hypothesize that SIRT2 improves the integrity of the BBB by reducing the loss of tight junction proteins, thereby alleviating cerebral edema.

Widely used TBI animal models can be generated by fluid percussion injury, CCI injury, weight drop impact injury, blast injury, or penetrating ballistic-like injury (Xiong et al., 2013). We chose to use the CCI model, as it is easy to control the mechanical factors, such as time, speed, and impact depth (Saatman et al., 2006), and it has a wide range of simulation scenarios. We used the parameters of the moderate-TBI CCI model (Liu et al., 2018b). When pyroptosis occurs, the cells swell, pores are formed in the cell membrane, and the integrity of the cell membrane is lost. The resultant release of IL-1β and IL-18 causes an inflammatory response (Cookson and Brennan, 2001; Ding et al., 2016). Pyroptosis is driven by canonical and noncanonical inflammasomes. In the canonical pathway, the caspase-1 precursor combines with NLRP3 protein and adapter protein ASC to form the NLRP3 inflammasome, which activates caspase-1 and cleaves and activates IL-1β and IL-18 precursors; IL-1β and IL-18 then cleave gasdermin D, which causes formation of holes in the cell membrane, causing cell pyroptosis (Kovacs et al., 2017; Sun et al., 2020).
Figure 4 | Knockout of Sirt2 alleviates blood-brain barrier disruption and brain edema in mice after traumatic brain injury.
(A) Representative costained immunofluorescence images of ZO-1 (green, stained with Alexa Fluor 488) and CD31 (red, stained with Alexa Fluor 555) at 3 days post-TBI. ZO-1 gaps are indicated by arrows. Scale bar: 10 μm. (B) Representative western blot bands of ZO-1 3 days post-TBI. (C) Quantification of ZO-1 protein expression normalized to β-tubulin. (D) Representative images of Nissl staining of brain tissue of mice 14 days post-TBI. Scale bar: 3 mm. (E) Quantification of brain lesion volume. (F) Representative images of the amount of Evans blue dye exudation at 3 days post-TBI. (G) Quantification of the amount of Evans blue dye exudation at 3 days post-TBI. (H) Quantification of brain water content. Data are expressed as mean ± SD (n = 6 per group). *P < 0.05, **P < 0.01 (Student’s t-test). Sirt2: Sirtuin 2; TBI: traumatic brain injury; WT: wild type; ZO-1: zonula occludens-1.

Figure 5 | Knockout of Sirt2 reduces the expression of the NLRP3 inflammasome and nerve cell pyroptosis after traumatic brain injury in vivo.
(A) Representative western blot bands of the NLRP3 inflammasome components at 3 days post-TBI. (B–F) Quantification of protein expression of NLRP3, ASC, caspase-1 p45, caspase-1 p20, and caspase-1 p10 at 3 days post-TBI. The target protein expression was normalized to the sham-WT group. (G–J) Quantification of mRNA expression of NLRP3, ASC, caspase-1, IL-1β, and IL-1β at 3 days post-TBI by PCR. The target mRNA expression was normalized to the sham-WT group. (K) IL-1β secretion in blood by ELISA. (L) Caspase-1 activity at 3 days post-TBI. (M, N) Representative images and quantification of TUNEL-positive cells (red) at 3 days post-TBI. Double-positive TUNEL (red) and DAPI staining (blue) indicates the pyroptotic cells. Scale bar: 50 μm. Data are expressed as mean ± SD (n = 6 per group). *P < 0.05, **P < 0.01 (Student’s t-test). ASC: Apoptosis-associated speck-like protein containing CARD; DAPI: 4′,6-diamidino-2-phenylindole; ELISA: enzyme-linked immunosorbent assay; IL: interleukin; NLRP3: nucleotide binding oligomerization domain-like receptor protein 3; PCR: polymerase chain reaction; Sirt2: Sirtuin 2; TBI: traumatic brain injury; TUNEL: terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling; WT: wild type.

Caspase-1

Caspase-1 activity

Relative mRNA level

Relative protein level

Relative protein level

Relative protein level

Relative protein level

Relative protein level

Relative protein level
The role of the NLRP3 inflammasome post-TBI has been confirmed in experimental TBI models and patients with moderate or severe TBI. Liu et al. (2013) reported that NLRP3, ASC, and caspase-1 mRNA increased 6 hours after fluid percussion injury in rats, and the protein levels of NLRP3 and caspase-1 remained increased 24 hours after injury. Chen et al. (2019) showed that NLRP3 mRNA began to increase within 6 hours after TBI and exhibited peaks at 24 and 72 hours post-TBI in mice; on day 7, the expression of NLRP3 decreased to a level that remained higher than the control group. TBI can activate the NLRP3 inflammasome, so we hypothesized that targeting this pathway may effectively alleviate neuroinflammation and promote the recovery from TBI. In animal models of other neuroinflammatory diseases, such as Alzheimer's disease, NLRP3 knockout has been shown to reduce neuroinflammation and improve functional prognosis (Feng et al., 2020). MCC950 is a highly selective and potent NLRP3 inhibitor. A study found that intraperitoneal injection of MCC950 in mice reduced the expression of NLRP3, caspase-1, ASC, and IL-1β 24 hours after CCI and decreased IL-1β levels 72 hours post-TBI (Ismael et al., 2018). In our study, expression of the components of the NLRP3 inflammasome increased post-TBI, peaked on day 3, and lasted at least 7 days; these components were expressed in neurons and microglia 3 days post-TBI.

Caspase-1 activity determines the inflammatory response mediated by the NLRP3 inflammasome. Caspase-1 knockout mice are partially resistant to stroke, and intracerebroventricular administration of caspase-1 inhibitors provides protective effects in experimental stroke models (Ross et al., 2007). A study showed that caspase-1 and ASC levels were elevated in the serum of patients post-TBI and were related to poor prognosis post-TBI (Kerr et al., 2018). Sun et al. (2020) demonstrated that VX765, a caspase-1 inhibitor, inhibits IL-1β and IL-1β production, which decreases pyroptosis in the TBI acute phase, decreases LDH release, reduces neuroinflammation, and inhibits microglial death. Thus, the NLRP3 inflammasome plays a key role in regulating neuroinflammation and pyroptosis. Activation of the NLRP3 inflammasome is a two-step process of priming and activation (Swanson et al., 2019). Priming upregulates the expression of the NLRP3 inflammasome and is induced by various signals, such as damage-associated molecular patterns, Toll-like receptors, and tumor necrosis factor, that result in nuclear factor-κB activation (Bauernfeind et al., 2009; Xing et al., 2017). In our study, Sirt2−/− mice post-TBI had decreased expression of the NLRP3 inflammasome components and decreased caspase-1 activity. We suggest that knockdown of Sirt2 may alleviate neuroinflammation and reduce pyroptosis via the NLRP3/caspase-1 pathway.

The role of SIRT2 in neurological diseases is still controversial. The Parkinson's disease (PD) model generated by long-term administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine can replicate most of the clinical features of PD and produce reliable and reproducible PD. The SIRT2 inhibitor AK7 can prevent dopamine depletion and dopaminergic neuron loss caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in vivo (Chopra et al., 2012). Sirt2−/− mice had reduced neurodegeneration caused by long-term administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, which was caused by a reduction in apoptosis by an increase in FOXO3a acetylation and a reduction in Bcl-2-like protein 11 levels (Liu et al., 2014). In contrast, in the PD model induced by 6-hydroxydopamine treatment, the decrease in SIRT2 activity lead to an increase in acetylated α-tubulin, and the function of tubulin deacetylase was improved (Patel and Chu, 2014). AK7 treatment can improve motor function, prolong survival, reduce brain atrophy, and significantly reduce the accumulation of mutant huntingtin protein and improve Huntington's disease symptoms (Chopra et al., 2012); however, another study reached the opposite conclusion (Capizzi et al., 2020). Sirt2 knockout or use of the SIRT2 inhibitor AGK2 showed neuroprotection against cerebral ischemia (Wang et al., 2016). Another study showed that SIRT2 determines the level of α-tubulin acetylation, which regulates microtubule assembly. Acetylated α-tubulin are necessary for the activation of the NLRP3 inflammasome (Misawa et al., 2013). Thus, the relationship between SIRT2 and NLRP3 in TBI needs to be further investigated.

There are several limitations to this study. First, the role of SIRT2 in microglial cells was not investigated. Second, we did not use overexpression technology or rescue experiments to validate our hypothesis. Thus, further research needs to be performed. In future studies, we plan to investigate gender differences in the response to Sirt2 knockout post-TBI.

In conclusion, Sirt2 knockout can improve the neurological dysfunction of mice post-TBI, improve memory, reduce the loss of tight junction proteins, reduce the disruption of the BBB, and reduce cerebral edema. Sirt2 knockout may reduce neuroinflammation and pyroptosis by reducing the expression of the NLRP3 inflammasome and the activity of caspase-1.

Author contributions: Study design: WW, HLT; manuscript writing: WW. All authors participated in experiment implementation, analyzed data, and approved the final version of manuscript for publication.

Conflicts of interest: No competing financial interests exist.

Availability of data and materials: All data generated or analyzed during this study are included in this published article and its supplementary information files.

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