LncRNA Tug1 Contributes Post-stroke NLRP3 Inflammasome-Dependent Pyroptosis via miR-145a-5p/Tlr4 Axis

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Abstract
Pyroptosis, a type of programmed cell death illuminated by inflammasomes and active caspases, is implicated in post-stroke inflammation. Our previous study showed that lncRNA taurine upregulated gene 1 (Tug1) sponging miR-145a-5p modulated microglial activation after oxygen-glucose deprivation (OGD). However, the role and mechanism of Tug1 on post-stroke pyroptosis is not fully clear. Photo-thrombosis stroke mice and OGD-treated BV-2 microglia were established respectively. Tug1 knockdown or overexpression was achieved by intraventricular infusion of AAV-shTug1 in vivo, or transfection of siTug1 and pcDNA3.1-Tug1 in vitro. Neurological function and infarction volume were evaluated. Meanwhile, pyroptosis-associated proteins (IL-1β, IL-18, NLRP3, ASC, cleaved-caspase-1, and GSDMD-N), TLR4, and p-p65/p65 as well as Tug1 and miR-145a-5p were detected 24 h after photo-thrombosis or 4 h after OGD by qRT-PCR, western blot, and ELISA. The correlation between Tug1/miR-145a-5p/Tlr4 axis and pyroptosis was explored by dual-luciferase reporter assay and functional gain-and-loss experiments. Photo-thrombosis or OGD caused neural injury and upregulated pyroptosis-associated proteins, Tug1, TLR4, and p-p65 as well as downregulated miR-145a-5p, which was prevented by Tug1 knockdown in vivo and in vitro. Tlr4 gene, putatively binding with miR-145a-5p by bioinformatics analysis, was found to be a direct target of miR-145a-5p with negative interactions. Furthermore, miR-145a-5p inhibitor abolished the inhibitive effects of siTug1 on TLR4 and p-p65 as well as pyroptosis-associated proteins, whereas miR-145a-5p mimics abrogated the enhanced effects of pcDNA3.1-Tug1 on that, suggesting an involvement of Tug1/miR-145a-5p/Tlr4 axis on pyroptosis. Tug1 contributes NLRP3 inflammasome-dependent pyroptosis through miR-145a-5p/Tlr4 axis post-stroke, providing a promising therapeutic strategy against inflammatory injury.

Keywords Pyroptosis · Tug1 · miR-145a-5p · Tlr4 · NLRP3 inflammasome · Cerebral ischemia

Introduction
Stroke is a leading cause of death and long-term disability worldwide, about 87% of which can be attributed to ischemic type [1, 2]. At present, thrombolysis within 4.5 h and mechanical thrombectomy at most 24 h are both approved therapies for acute ischemic stroke, nonetheless, the therapies are unpractical to stroke patients who are outside the time-window [3, 4]. Ischemic stroke generates sterile inflammation characterized by the activation of microglia and the release of inflammatory cytokines at the early stage, in which microglial pyroptosis plays an important role to cell death and brain injury [5–9]. Pyroptosis is a novel type of programmed cell death illuminated by inflammasomes and requires active caspases [10]. Typically, NOD-like receptor protein 3 (NLRP3) inflammasome-dependent pyroptosis is triggered by toll-like receptors (TLRs), a kind of pattern recognition receptors in respond to both pathogen-related molecule patterns and damage-related molecular patterns, and then facilitates the activation of caspase-1 (casp-1) and subsequent release of proinflammatory cytokines interleukins-1β (IL-1β) and interleukins-18 (IL-18) [11,
We recorded 120 consecutive speckle images of mice skull and the selected region of interest (ROI) area was 1 in vivo. A laser probe was positioned about 12 cm above the used to record the real-time changes of cortical blood flow system (PeriCam PSI HD system, Stockholm, Sweden) was green light at 540 nm through the irradiation window at 2 CA, USA) via the tail vein, the mice were illuminated with on the exposed skull. Immediately after an injection of pho- was centered 1 mm lateral and right posterior to the bregma was fixed on a stereotaxic instrument. An irradiation window [17]. In brief, adult male C57BL/6 mice were anesthetized in accordance with the ARRIVE guidelines. The animal was carried out the procedure, respiratory status was in a smooth condition and body temperature was kept at 37.5 °C with a heating pad.

Neurological function was blindly assessed in each group before and 24 h after PT with a modified neurologic severity score (mNSS) [18]. The mNSS consists of a comprehensive test of motor, sensory, and reflex activities, with a score range of 0–14 points (1–4 as mild injury, 5–9 as moderate injury, 10–14 as severe injury).

Tug1 Knockdown In Vivo

Three weeks before PT, 2.5 μl of AAV-shTug1 (1 × 10^{12} v.g./ml, Gene Chem, Shanghai, China) was continuously infused into the lateral ventricle at a rate of 0.2 μl/min to knock down Tug1. The infusion was made via a microliter syringe (Hamilton Co., NV, USA) at the following stereotaxic coordinates: 0.3 mm posterior to the bregma, 1 mm right lateral to the midline, 2 mm beneath the surface of the skull. After the infusion was completed, the needle was kept in position for 15 min and then slowly pulled out. The wound was closed by bone cement and skin glue. AAV-NC (Gene Chem) was used as the control following the same operation.

Cortex Tissue Collection and Staining

Twenty-four hours after PT or sham PT, 18 mice from each group were anesthetized and sacrificed. For immunofluorescence, the brains from six mice were perfused and postfixed with 4% paraformaldehyde at 4 °C for 8 h. Series of adjacent 10-μm coronal frozen sections were collected at the infarcted level. For western blot and real-time quantitative PCR (qRT-PCR), the brains from the other six mice were perfused with pre-cooling heparinized saline. Subsequently, the peri-infarction cortex tissue was quickly obtained and stored at −80 °C.

An anti-NeuN antibody (Abcam, Cambridge, UK) and an anti-Iba-1 antibody (WKAO, Tokyo, Japan) were used to mark cortical neurons and microglial cells, respectively. Sections were first incubated with the NeuN antibody or the Iba-1 antibody at 4 °C overnight followed by species-specific Alexa Fluor plus 555- or 488-conjugated antibody (Thermo Fisher Scientific, MA, USA) at room temperature for 1 h. Thereafter, sections were counterstained with DAPI and analyzed under a fluorescence microscope (Nikon DS-Ri2, Japan). To observe the gross infarcted zone, small 2-mm coronal blocks from the brain were stained with 2,3,5-triphenyltetrazolium chloride (TTC, Solarbio, Beijing, China) at room temperature for 30 min, then photographed and
calculated using the ImageJ software (National Institutes of Health, MD, USA).

**Cell Culture and OGD**

BV-2 microglial cells (Kunming Cell Bank, Chinese Academy of Sciences) were cultured in Dulbecco’s modified eagle medium (Gibco, CA, USA) containing 10% fetal bovine serum (Thermo Fisher Scientific) and 1% antibiotics (TransGen Biotech, Beijing, China) in a humidified incubator under 5% CO2 at 37 °C. Cells were split at 70–80% confluence. OGD was induced to mimic ischemic conditions in vitro [16]. Briefly, cells were cultured in glucose-free Dulbecco’s modified eagle medium and flushed with 95% N2/5% CO2 gas mixture at a flow rate of 4 l/min for 10 min to create an anaerobic condition. A gas analyzer (Coy Laboratory, MI, USA) was used to monitor the anaerobic conditions. Cells were then transferred to normal culture medium under 5% CO2 for reoxygenation. Our previous study showed that the regimen of OGD for 4 h and reoxygenation for 24 h was able to induce pivotal signaling events in cells without causing excessive death [16]. Control cells were treated without OGD.

**Cell Transfection**

_Tug1_ small interfering RNA (si_Tug1), pcDNA3.1+_Tug1_ vector, miR-145a-5p mimics, miR-145a-5p inhibitor as well as their NCs were constructed by Tsigke (Guangzhou, China) and GenePharma (Guangzhou, China), respectively. The corresponding sequences are listed in Table 1. A lipofectamine 3000 reagent kit (Invitrogen, CA, USA) was used to transfect the cells (3 × 10⁶/well) in a 6-well plate according to the manufacturer’s protocols. The amount of transfection was 2.5 μg for pcDNA3.1+_Tug1_ vector and its control, or 150 nmol for the others. Transfected cells were incubated 24 h and then subjected to OGD treatment. The efficiency of transfection was confirmed by qRT-PCR.

**qRT-PCR**

Total RNAs were extracted from BV-2 cells or peri-infarction cortex tissue using a RNAzol RT reagent (MRC, OH, USA), followed by the reverse-transcription to cDNA using a PrimeScript® RT reagent kit (Takara, Japan) according to the manufacturer’s instructions. The relative level of _Tug1_ or miR-145a-5p to _Gapdh_ or _U6_ was quantitatively measured in 2^-ΔΔCt method using a TB Green® Premix Ex Taq™ kit (Takara) on ABI 7500 real-time system (Applied Biosystems, CA, USA). The primer sequences were synthesized by Tsigke (Guangzhou) and are listed in Table 1. All samples were performed at least three parallel reactions.

**Western Blot**

Proteins were extracted from peri-infarction cortex tissue or cultured cells using RIPA lysis buffer plus 10 μl/ml protease inhibitor (Thermo Fisher Scientific). The protein

| Name                  | Primer       | Sequences                                                                 |
|-----------------------|--------------|---------------------------------------------------------------------------|
| si_Tug1               | Sense        | 5'-CCAUCUCACAAGGCUCUAAAT-3'                                               |
|                       | Anti-sense   | 5'-UUGAAGCCUGAGAUGGT-3'                                                   |
| si_Tug1 NC            | Sense        | 5'-UUCUGACGGUGUCACTT-3'                                                   |
|                       | Anti-sense   | 5'-TTAGAGCGCUCAAGCUGA-3'                                                  |
| mmu-miR-145a-5p mimics| Sense        | 5'-GUCCAGUUUCACAGAAUCCC-3'                                                |
|                       | Anti-sense   | 5'-GGAAUCCUGGAAACUGCAGUCU-3'                                             |
| mimics NC             | Sense        | 5'-UUCUGACGGUGUACAGUTT-3'                                                 |
|                       | Anti-sense   | 5'-ACGUAGACCGUAGGAAATT-3'                                                 |
| mmu-miR-145a-5p inhibitor NC | Sense | 5'-AGGGAUACUGGAAACUGGAC-3'                                               |
|                       | Anti-sense   | 5'-CAGUACUUUGUGUAGCAAC-3'                                                |
| _Gapdh_               | Forward      | 5'-TGCCCAATTCACCAAGGAA-3'                                                |
|                       | Reverse      | 5'-CTGCAAACATTCTATACGCCT-3'                                              |
| _Tug1_                | Forward      | 5'-CAAACCTGCTACTAATCTATT-3'                                              |
|                       | Reverse      | 5'-CATTCAAGCATAAGGAC-3'                                                  |
| _U6_                  | Forward      | 5'-CTGCTTGGCCGACATACTT-3'                                                |
|                       | Reverse      | 5'-ACGCTTACGATTTTCGTC-3'                                                 |
| miR-145a-5p           | miR-145a-5p RT | 5'-CTCAACTTGGTGTCGTGGAGTCGGCAATTCAGTGGGAGATCC-3'                          |
|                       | Forward      | 5'-ACACTCCAGCTGGGGTCCAGTTTCCCAGG-3'                                     |
|                       | Reverse      | 5'-GGTGTCGTGGAGATC-3'                                                    |
concentration was quantified using a BCA protein assay kit (Thermo Fisher Scientific). Equivalent amount of proteins from each sample was resolved by SDS-PAGE and transferred to polyvinylidene fluoride membranes. Blots were incubated at 4 °C overnight with the following primary antibodies: TLR4, p-p65, p65, gasederin D (GSDMD), apoptosis-associated speck-like protein containing CARD (ASC), IL-1β, IL-18, and GAPDH (all 1:1000, Affinity Biosciences, OH, USA); NLRP3 (1:1000, Abcam); casp-1 (1:1000, AdipoGen, Liestal, Switzerland). Antibodies binding to blots were visualized using an HRP-linked secondary antibody kit (1:3000, CST, MA, USA). The relative levels of proteins to GAPDH were measured using ImageJ software.

**ELISA**

Blood samples from the ocular vein of mice and cultured supernatant from BV-2 cells in different treatment groups were carefully collected and centrifuged to remove cell debris and concretionary stuff. The concentration of IL-1β and IL-18 in the serum and the cultured supernatant were quantified using specific ELISA kits (Elabscience, Wuhan, China) following the manufacturer’s instructions. A standard curve by using a standard solution was made to normalize the measurement.

**Dual Luciferase Reporter Gene Assay**

The putative binding sites between miR-145a-5p and Tlr4 are predicted by searching an online database (http://www.targetscan.org). The 3′UTRs of both wild type and mutated Tlr4 (Tlr4 3′-UTR-WT or 3′-UTR-MUT) were constructed by Tsigke (Guangzhou) and separately inserted into the pmirGLO vector (Promega, Madison, USA). The Tlr4 3′-UTR-WT or 3′-UTR-MUT vector, as well as miR-145a-5p mimics or mimics NC were co-transfected into BV-2 cells using a lipofectamine 3000 reagent kit (Invitrogen). Transfected cells were harvested 48 h later and the luciferase activity was detected using a dual-luciferase reporter assay kit (TransGen Biotech, Beijing, China) according to the manufacturer’s instructions.

**Statistical Analysis**

All data were analyzed with GraphPad Prism 8.0 software (GraphPad Inc., CA, USA) in a blinded manner without knowledge of the treatment assignment. Numerical data were presented as the mean ± standard deviation. Student’s t-test or a general linear model with Bonferroni correction for analysis of variance was used to detect any intergroup differences when appropriate. A two-tailed P value of 0.05 or less inferred statistical significance.

**Results**

**PT Resulted in Local Cortex Infarction with Upregulated Tug1 and Downregulated miR-145a-5p, Relieved by Tug1 Knockdown**

PT conducted a consistent infarction in the right cerebral cortex, shown by decreased local cortical blood flow using laser speckle imaging and TTC staining (Fig. 1A and B, P < 0.001). In the meantime, decreased number of NeuN-positive neurons and increased Iba-1-positive microglia in the peri-infarction cortex as well as impaired neurological function were observed 24 h after PT (Fig. 1C and D, P < 0.05). When compared with vehicle treatment, Tug1 knockdown by intraventricular infusion of AAV-shTug1 salvaged cortex neurons, reduced microglia accumulation and infarct volume with improved neurological function (Fig. 1B–D, P < 0.05), demonstrating a detrimental role of Tug1 to neuronal survival post-stroke. In addition, the levels of Tug1 and miR-145a-5p were measured by RT-qPCR. Tug1 was upregulated while miR-145a-5p was downregulated 24 h after PT. By contrast, AAV-shTug1 infusion obviously lowered Tug1 and raised miR-145a-5p, implying that the role of Tug1 could be probably through suppressing miR-145a-5p (Fig. 1E, P < 0.01). The level of Tug1 was effectively knocked down by AAV-shTug1 was shown in Supplementary Fig. S1.

**Tug1 Knockdown Inhibited Ischemia-Induced Pyroptosis In Vivo and In Vitro**

The in vivo activity of pyroptosis was evaluated 24 h after PT by detecting pyroptosis-associated proteins in both the serum and the ipsilateral cortex using ELISA and western blot. The protein levels of IL-1β, IL-18, NLRP3, ASC, cleaved-casp-1, and GSDMD-N were notably increased. Tug1 knockdown by AAV-shTug1 intraventricular infusion reduced the levels of these proteins compared with the vehicle treatment. The differences were statistically significant (Fig. 2A and B, P < 0.05). Similarly, the levels of all these pyroptosis-associated proteins were also enhanced in OGD-treated BV-2 cells in vitro, together with upregulated Tug1 and downregulated miR-145a-5p. The changes after OGD treatment were sufficiently suppressed by siTug1 transfection, suggesting that Tug1 could promote ischemia-induced pyroptosis (Fig. 3A–E, P < 0.05).

**Tlr4 Gene Was a Direct Target of miR-145a-5p**

Online bioinformatics analysis reveals putative binding sites of the 3′UTR region of Tlr4 with miR-145a-5p (Fig. 4A). The direct binding between them was further
**Fig. 1** Tug1 knockdown protecting neurons against ischemic injury with probable involvement of miR-145a-5p after PT-induced stroke in mice. PT stroke model was successfully established in the present regiment, represented by decreased blood flow in the right cortical ROIs (laser speckle imaging) (A) and consistent infarcted zone (TTC staining) (B) in the cortex. Ischemia destructed NeuN-immunostaining cortical neurons, caused Iba-1-positive microglia accumulation and neurological deficit with upregulated Tug1 and downregulated miR-145a-5p, reversed by intraventricular infusion of AAV-shTug1 (C, D, E). *P < 0.05, **P < 0.01, ***P < 0.001, n = 6 per each group.
confirmed in the present study. After the verification of miR-145a-5p mimics increasing miR-145a-5p while miR-145a-5p inhibitor decreasing miR-145a-5p in vitro (Fig. 4B and C, \( P < 0.01 \)), the targeting relationship between 3'UTR region of Tlr4 and miR-145a-5p was detailedly explored by constructing Tlr4 3'UTR-WT and western blot. Intraventricular infusion of AAV-shTug1 significantly suppressed the ischemia-reinforced pyroptosis. \( * P < 0.05, ** P < 0.01, *** P < 0.001, n = 6 \) per each group.
miR-145a-5p directly targeting to Tlr4 gene with negative interaction. Putative binding sites of the 3′ UTR region of Tlr4 with miR-145a-5p bioinformatics analysis (A). On condition of the efficient function to miR-145a-5p by miR-145a-5p mimics (B) and miR-145a-5p inhibitor (C) by qPCR, the luciferase activities of wild type Tlr4 (Tlr4-WT) and Tlr4 with mutant miR-145a-5p binding site (Tlr4-MUT) were determined by dual luciferase reporter gene assay, showing a direct binding between Tlr4 and miR-145a-5p (D). The protein level of TLR4 was reduced by miR-145a-5p mimics but raised by miR-145a-5p inhibitor in BV-2 cells by western blot, indicative of negative interaction between them (E, F). *P < 0.05, **P < 0.01, ***P < 0.001, n = 3 independent experiments per each group.

Tug1 Contributed Ischemia-Induced Pyroptosis Through miR-145a-5p/Tlr4 Axis

Our previous study found that Tug1 sponged miR-145a-5p with negative interactions [16]. In the present study, the levels of TLR4 and p-p65 were distinctly incremental 24 h after PT and 4-h OGD. By contrast, Tug1 knockdown by using AAV-shTug1 or siTug1 significantly lowered the levels of TLR4 and p-p65 in vivo and in vitro (Fig. 5A–C, P < 0.05). We further explored whether Tug1 regulated...
**Fig. 5** Tug1 contributing ischemia-induced pyroptosis through miR-145a-5p/Tlr4 axis. PT- and OGD-induced elevation of TLR4 and p-p65 was inhibited by Tug1 knockdown using AAV-shTug1 (A) or siTug1 (B, C). On condition of the efficient knockdown to Tug1 by siTug1 or overexpression by pcDNA3.1-Tug1 (D), functional gain- and-loss experiments revealed that miR-145a-5p inhibitor abolished the inhibitive effect of siTug1 on TLR4 and p-p65 as well as pyroptosis-associated proteins, whereas miR-145a-5p mimics abrogated the enhanced effect of pcDNA3.1-Tug1 on that (E). There was the same trend on the expression of IL-18 and IL-1β in cultured supernatant by ELISA (F), suggesting the involvement of Tug1/miR-145a-5p/Tlr4/NF-kB axis on pyroptosis. *\(P < 0.05\), **\(P < 0.01\), ***\(P < 0.001\), \(n = 6\) per each group for A, \(n = 3\) independent experiments per each group for B–F.
ischemia-induced pyroptosis through miR-145a-5p/Tlr4 axis in OGD-treated BV-2 cells by functional gain-and-loss experiments. Tug1 knockdown or overexpression was efficiently achieved by siTug1 or pcDNA3.1-Tug1 transfection (Fig. 5D, P < 0.01). Western blot analysis showed that siTug1 inhibited the levels of TLR4 and p-p65 as well as pyroptosis-associated proteins (IL-18, IL-1β, NLRP3, ASC, cleaved-caspase-1, and GSDMD-N), which was turned over by miR-145a-5p inhibitor. In comparison, pcDNA3.1-Tug1 increased the levels of TLR4 and p-p65 as well as pyroptosis-associated proteins, which was reversed by miR-145a-5p mimics (Fig. 5E, P < 0.05). Likewise, ELISA results also indicated that siTug1-induced downregulation of IL-18 and IL-1β was abolished by miR-145a-5p inhibitor, whereas pcDNA3.1-Tug1-induced upregulation of that was abrogated by miR-145a-5p mimics (Fig. 5F, P < 0.05), indicating that pyroptosis was regulated by Tug1/miR-145a-5p/Tlr4 axis involving NF-kb signaling.

Discussion

Pyroptosis is morphologically characterized by cell swelling and eventually membrane rupture, distinctly different from other programmed cell deaths [19, 20]. In the canonical inflammasome pathway of pyroptosis, NLRP3 inflammasome, which is composed of NLRP3, ASC, and pro-casp-1, is assembled from the recognition of disadvantaged stimulation by TLRs [21–23]. Upon the activation of casp-1, pro-IL-1β and pro-IL-18 are cleaved to their mature forms and GSDMD is to its GSDMD-N form. GSDMD-N binds to the plasma membrane and forms open pores across the plasma membrane, causing the release of mature IL-1β and IL-18 through the membrane pores into the extracellular matrix with a result of pyroptotic inflammation [24, 25]. In the present study, we observed that Iba-1-positive microglia was accumulated in the peri-infarction zone at 24 h after PT. It is believed for now that microglial pyroptosis is an important mediator of the innate immune response to ischemic injury and is considered as markers of neuroinflammation [26–30]. After ischemic stroke, all or part of these pyroptosis-associated proteins were upregulated in microglia at the early stage, showing enhanced pyroptosis activity [29, 31]. We also observed Iba-1 positive microglia specifically accumulated in the cortex of peri-infarct zone at 24 h after PT. Meanwhile, NLRP3 knockout markedly reduced infarction volume and preserved permeability of blood-brain barrier with improved neurological function, indicative of a potential therapeutic strategy for stroke by inhibiting pyroptosis [31, 32]. Previous experimental studies demonstrated that TLR4/NF-kb was an important signaling pathway to reinforce NLRP3 inflammasome-dependent pyroptosis after ischemia [33], nonetheless, the upstream regulator on TLR4/NF-kb pathway need to be further clarified.

LncRNAs have been found to modulate gene expressions at epigenetic, transcriptional, and post-transcriptional levels, in which the lncRNA-miRNA-mRNA ceRNA network is implicated in multiple biological processes and development of diseases [34, 35]. Tug1 was originally thought to be an oncogenic lncRNA, an upregulation of which closely contributed to tumorigenesis [36, 37]. Recent studies revealed that Tug1 was abnormally upregulated after ischemic injury in various tissue, inhibiting the expression of antioxidant genes and participating in post-ischemia inflammation [38, 39]. Our team also found that Tug1, acting as a ceRNA sponging miR145a-5p with negative interactions, strengthened microglial polarization and inflammatory response after OGD [16]. Nevertheless, the role and mechanism of Tug1 on post-stroke pyroptosis is not fully clear. By using in vivo PT stroke model, we observed that Tug1 was upregulated while miR-145a-5p was downregulated in the ipsilateral cortex 24 h after PT, in parallel with impaired neurological function and lessened cortex neurons. Meanwhile, the activity of pyroptosis was elevated, represented by higher levels of pyroptosis-associated proteins (IL-1β, IL-18, NLRP3, ASC, cleaved-casp-1, and GSDMD-N). It can be deduced that Tug1 and miR-145a-5p were drawn into post-stroke pyroptosis. Indeed, we further found that Tug1 knockdown by AAV-shTug1 infusion increased the level of miR-145a-5p and the number of cortical neurons, simultaneously, decreased the levels of these pyroptosis-associated proteins with better neurological function. All above post-stroke changes were also reconfirmed in OGD-treated BV-2 cells in vitro. Altogether, in the context of Tug1 directly sponging miR-145a-5p, the data indicated that Tug1 contributed to post-stroke pyroptosis through miR-145a-5p.

MiR-145a-5p has been proved to function as an inflammatory regulator of cell survival by specifically targeting specific gene transcription [40]. On condition of a predicted correlation between miR-145a-5p and Tlr4 by bioinformatics analysis, we confirmed that miR-145a-5p was a negative regulator directly binding to Tlr4 gene by dual luciferase reporter gene assay in the present study. With the administration of AAV-shTug1 or siTug1, the elevation of TLR4 and p-p65 induced by PT and OGD was inhibited. Thus, it is reasonable to speculate that Tug1/miR-145a-5p could guide post-stroke pyroptosis ignited by TLR4/NF-kb. For further verification, functional gain-and-loss experiments were performed in OGD-treated BV-2 cells and the results showed that miR-145a-5p inhibitor prevented the inhibitive effects of siTug1 to pyroptosis-associated proteins and TLR4/p-p65, whereas miR-145a-5p mimics abrogated the positive effects of pcDNA3.1-Tug1 to that. Taken together, the data demonstrated that Tug1/miR-145a-5p/Tlr4 axis facilitated ischemia-induced pyroptosis involving NF-kb signaling.
There were several limitations in our study. First, we used BV-2 microglia as a substitute for primary microglia. Primary microglia are undoubtedly the best candidate for microglial study. However, BV-2 microglia have also been widely used in many microglial studies due to the most reservation of major characteristics and function of primary microglia [41, 42]. Second, pyroptosis can be induced by non-canonical inflammasome pathway, in which caspase-4/5/11 straightly cleave GSDMD excluding assembly of inflammasome. We did not detect the levels of these caspases and cannot evaluate the role of Tug1 on non-canonical inflammasome pathway. Future studies are needed to clarify these issues.

**Fig. 6** Skeleton diagram depicting the role and mechanism of Tug1 on post-stroke pyroptosis. miR-145a-5p targets Tlr4 mRNA and inhibits its protein expression. Tug1 contributes NLRP3 inflammasome-dependent pyroptosis through miR-145a-5p/Tlr4 axis involving NF-κB signaling.

**Conclusion**

In conclusion, the present study supports the results of lncRNA Tug1 contributes NLRP3 inflammasome-dependent pyroptosis through miR-145a-5p/Tlr4 axis involving NF-κB signaling post-stroke (Fig. 6), providing a promising therapeutic strategy against inflammatory injury.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s12035-022-03000-4.
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