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Deficiency of unc-51 like kinase 1 (Ulk1) protects against mice traumatic brain injury (TBI) by suppression of p38 and JNK pathway

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ABSTRACT

Unc-51 like autophagy activating kinase 1 (Ulk1) is a serine/threonine kinase that plays a key role in regulating autophagy processes. We attempted to investigate the effects of Ulk1 on traumatic brain injury (TBI) progression by using wild type (WT) mice and Ulk1-knockout (KO) mice suffered with or not TBI. The results were verified using LPS-treated primary astrocyte (AST). Here, Ulk1 was over-expressed in hippocampus of WT mice after TBI, as well as in lipopolysaccharide (LPS)-stimulated AST. Ulk1-deletion improved cognitive ability and hippocampus histological changes in TBI mice. Nissl and neuronal nuclei (NeuN) staining indicated that Ulk1-deletion increased the number of surviving neurons in hippocampus of TBI mice. Ulk1-ablation alleviated neuroinflammation, as evidenced by the reduced expression of hippocampus pro-inflammatory cytokines in TBI mice. TBI-induced apoptosis was also ameliorated by Ulk1-ablation, as proved by the reduced number of TUNEL-staining cells, and cleaved Caspase-3 and poly (ADP-ribose) polymerase (PARP) expressions. Moreover, Ulk1-knockout suppressed TBI-stimulated activation of astrocytes and microglia cells. Additionally, hippocampus autophagy induced by TBI was attenuated by Ulk1-knockout. Further, TBI-activated p38/c-Jun N-terminal Kinase (JNK) pathway was repressed by Ulk1-deletion in hippocampus of mice. The findings above were confirmed in LPS-stimulated AST with or without Ulk1 siRNA transfection. Intriguingly, pre-treatment of p38 or JNK activator markedly abolished the anti-inflammation, anti-apoptosis and anti-autophagy effects of Ulk1-knockdown on LPS-incubated AST. In conclusion, our results demonstrated that Ulk1 might be a potential target for developing therapeutic strategy against TBI in future.

1. Introduction

Traumatic brain injury (TBI), a major cause of death and disability worldwide, is characterized by a direct injury to the head, resulting in tissue damage [1]. The function of brain is abnormal in patients with TBI, exhibiting neurological dysfunction, which is mainly caused by various pathological processes, such as inflammatory response, apoptosis and autophagy [2,3]. Despite the improved diagnosis and treatment methods, further study is still necessary to reveal the underlying mechanism by which TBI progresses and thus to develop effective therapeutic strategies.

Unc-51 like autophagy activating kinase 1 (Ulk1), as a serine/threonine kinase, plays a key role in autophagy induction, integrating signals from up-streaming sensors and transducing them to the down-streaming autophagy pathway [4]. In several cell lines, Ulk1 knockdown inhibits autophagy [5]. In addition, liver-specific deficiency of Ulk1 alleviates liver injury in animals through reducing autophagy under the regulation of JNK pathway [6]. Ulk1 is of great importance in the initial stages of autophagy and it is possible that genetic variation in this gene may lead to autophagy-mediated control of cellular processes, such as inflammation and apoptosis [7,8]. As reported previously, stress kinase p38 MAPK in microglia senses inflammatory cue LPS, directly activates Ulk1, reliving the autophagic inhibition on the inflammatory machinery, and therefore allowing for a full immune response [9]. Therefore, we hypothesized that Ulk1 might be also involved in TBI progression through regulating various cellular processes. And up until now, the effects of Ulk1 in TBI have not been reported.

In the present study, the wild type (WT) and Ulk1-knockout (KO) mice were employed to explore the role of Ulk1 in TBI development. We first found that Ulk1 was over-expressed in the hippocampus of WT mice. Ulk1-KO mice showed improved

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cognitive ability after TBI, accompanied with alleviated histological alterations of hippocampus. Ulk1-deletion reduced hippocampus inflammation and apoptosis in TBI mice. Further, the activation of astrocytes and microglial cells stimulated by TBI was also inhibited by Ulk1-KO. Autophagy, triggered by TBI, in hippocampus of mice was diminished in Ulk1-KO mice. The in vitro study using LPS-stimulated primary astrocytes confirmed that Ulk1 deficiency protected mice from TBI through p38/JNK pathway. These results supplied that Ulk1, at least partially, could be considered as an effective candidate for developing novel therapeutic strategies to suppress TBI progression.

2. Materials and methods

2.1. Animals and treatment

Adult wild type (WT) male C57/BL6 and Ulk1-knockout (KO) male mice (6–8 weeks old), weighing approximately 20 ± 2 g, were purchased from Laboratory Animal Center of Shandong University (Shandong, China) and Cyagen Biosciences (Guangzhou, China), respectively. Animals were cultured in a pathogen free feeding center under controlled conditions (temperature 25 ± 2 °C and humidity 70 ± 5%) and a 12:12 h light: dark cycle. Mice were randomly divided into four groups (n = 8/group): WT/Sham, WT/TBI, KO/Sham and KO/TBI groups. The study was performed in strict line with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Liaocheng People's Hospital Institutional Animal Care and Use Committee (Shandong, China). TBI procedures were conducted according to previous study [10].

2.2. Astrocytes isolation and treatment

Astrocytes (AST) were isolated from the cortex of postnatal day 4 C57BL6 mice as previously described [11]. AST were incubated in plates and cultured in DMEM (Gibco, USA) supplemented with 10% fetal bovine serum (Gibco) and 100 KU/L penicillin and 100 mg/L streptomycin at 37 °C, 5% CO_2. Ulk1 siRNA sequences and negative control (NC) siRNA sequences were designed and obtained from Generay Biotech (Shanghai, China). Cells were transfected with siRNAs using Lipofectamine 2000 reagent (Invitrogen, USA) for 24 h. Dehydrocorydaline chloride (DHC) and Anisomycin (ANS) were purchased from MedChemExpress (USA) to elevate p38 and JNK activation.

2.3. Morris water maze (MWM) analysis

After one day for TBI, adult mice were subjected to MWM as previously described [12]. Spatial memory ability was determined by the time spent in the target quadrant. The latency time to find the platform, run across the target quadrant, time spent in the target quadrant and distance in the target quadrant were recorded.

2.4. RNA isolation, reverse transcription and quantitative real-time PCR (qRT-PCR)

Total RNA were isolated from tissues or cells using Trizol reagent (Invitrogen, USA). The procedures used for RT-qPCR analysis was as previously described [13]. The forward and reverse primer sequences were listed in Supplementary Table 1.

2.5. Western blot

Proteins were extracted from hippocampus or AST using ice-cold lysis buffer (KeyGen Biotech). Mitochondrial and cytoplasmic proteins were extracted using Mitochondria Protein Extraction Kit (EnoGene™, Nanjing, China). Total lysates (40 μg) were subjected to SDS-PAGE, transferred to a PVDF membrane (Millipore, USA), and probed with specific antibodies (Supplementary Table 2). GAPDH was used as a loading control.

2.6. Enzyme-linked immunosorbent assay (ELISA) analysis

Commercial kits purchased from Roche System (USA) were used for hippocampus interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) and IL-18 measurements following the manufacturer's protocols.

2.7. Immunohistochemical (IHC) analysis

The fixed brain tissue was subjected to gradient hydration, wax dripping, embedding, and section (5 μm of thickness). H&E staining was conducted. For Nissl staining, brain sections were then immersed in 0.1% cresyl violet for 20 min at 37 °C. After washing with distilled water, sections were dehydrated, fitted with coverslips and examined using a light microscope. The IHC staining was conducted using primary NeuN antibody (1:250, Santa Cruz, USA). The stained images were acquired with a light microscope.

2.8. Fluorescence staining

The immunofluorescent (IF) analysis of hippocampus and primary AST was performed using GFAP and/or Iba1 (1:250, Abcam) as described previously [13]. One Step TUNEL Apoptosis Assay Kit (Beyotime, Nantong, China) was used to determine TUNEL-positive cells in tissues following the manufacturer's instructions. Staining patterns of the fluorescence were examined with a confocal laser-scanning microscope.

2.9. Flow cytometry analysis

Flow cytometric analyses using fluorescein isothiocyanate (FITC)-labelled annexin V and propidium iodide (PI) (Sigma-Aldrich) were performed following the manufacturer's protocol to analyze apoptosis.

2.10. Statistical analysis

The differences of index were analyzed by mean ± SEM in all experiments. Statistical differences were determined using Student's t-test for two-group comparisons or analysis of variance (ANOVA) followed by Dunnett's test for multiple comparisons in more than two groups. P value less than 0.05 was considered significant.

3. Results

3.1. Ulk1 is highly expressed in hippocampus of mice after TBI and Ulk1-deficiency improves MWM performance in TBI mice

RT-qPCR and western blot analysis showed an evidently increased expression of Ulk1 in hippocampus of WT mice after TBI at D1, D3, D5 and D7 (Fig. 1A). Consistently, LPS treatment stimulated Ulk1 mRNA and protein expressions in a time-dependent manner (Fig. 1B). To further reveal the effects of Ulk1 in TBI, the WT mice and Ulk1-null mice (Ulk1-knockout/KO) mice were used for further research. MWM analysis was performed after TBI. The performance in probe trial of MWM on day 6 was determined through calculating the percentage of time spent swimming toward the platform. WT mice after TBI spent longer time to find the

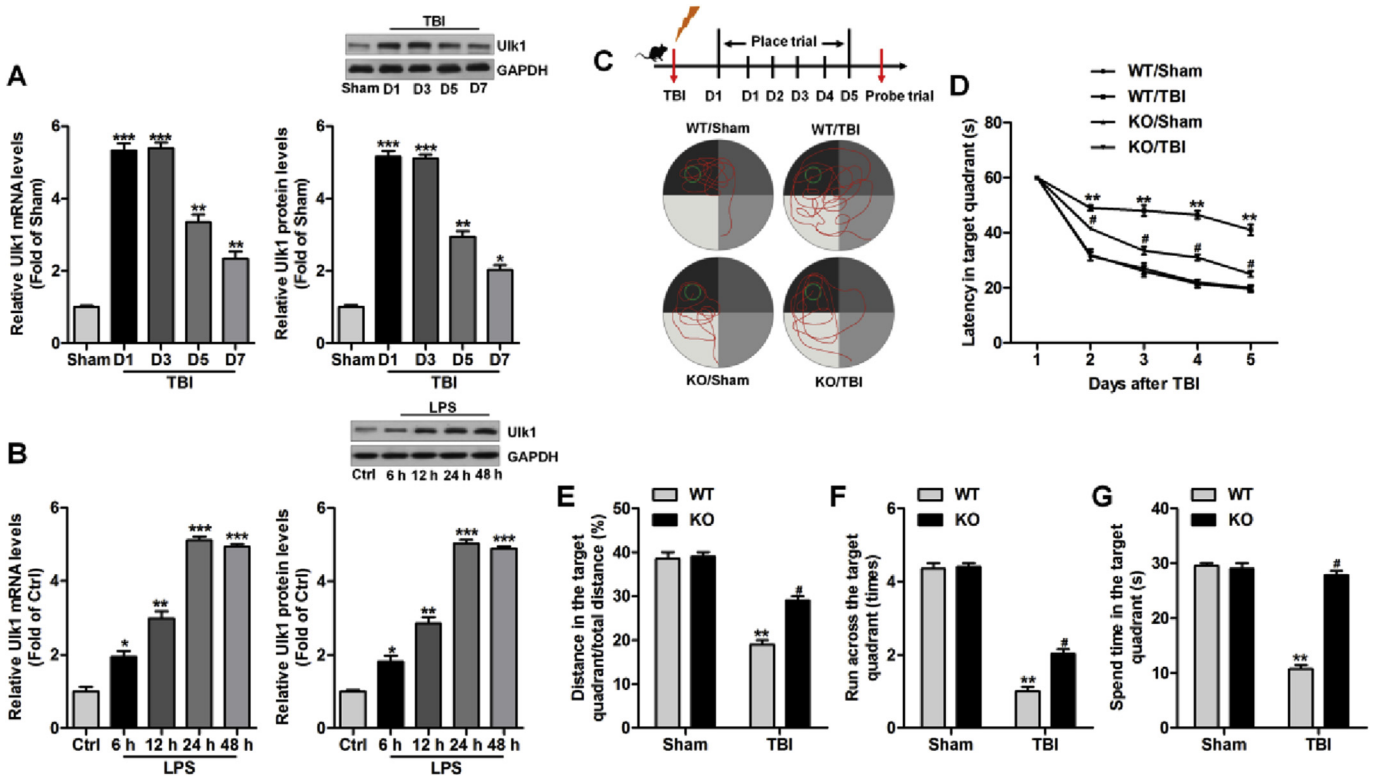

Fig. 1. Ulk1 is highly expressed in hippocampus of mice after TBI and Ulk1-deficiency improves MWM performance in TBI mice. (A) WT mice were subjected to TBI. At days 1, 3, 5, and 7, respectively, hippocampus Ulk1 expression levels were measured using RT-qPCR and western blot analysis. (B) Astrocytes isolated from WT mice were stimulated with 100 ng/ml of LPS for the indicated time, followed by RT-qPCR and western blot analysis of Ulk1. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs Sham or Ctrl group. (C) MWM experiment flow chart and representative images of the path chart of mice in MWM were exhibited. (D) The escape latency of mice at the time as indicated after TBI. (E) Distances swam in the target quadrant were evaluated in the probe trial. (F) The number of platform crossings in the probe trial was measured. (G) Time spent in the target quadrant during the probe trial was determined. Data are expressed as the means ± SEM (n = 8). $^{**}p < 0.01$ vs WT/Sham group.

target quadrant and exhibited lower accuracy compared with untreated WT mice and Ulk1-KO mice, suggesting an improved memory in Ulk1-KO mice after TBI (Fig. 1C and D). In addition, the Ulk1-KO mice displayed better behaviors, supported by the reduced ratio of distance (Fig. 1E), run across (Fig. 1F) and spending time in the target quadrant (Fig. 1G) after TBI, which were comparable to WT/TBI group.

3.2. Ulk1-KO mice show reduced inflammation and apoptosis in hippocampus after TBI

H&E staining showed no significant pathological change in neurons of hippocampal CA1 region of the mice from the WT/Sham and KO/Sham groups. However, the reduced number, irregular arrangement and expended gap of neurons in WT/TBI mice were observed, which were obviously attenuated in mice from Ulk1-KO group after TBI (Fig. 2A). WT/TBI mice exhibited the reduced number of Nissl bodies with blue staining, color-reducing cytoplasm and vacuoles. On the other, Ulk1-deletion could rescue the damage of neurons in CA1 with more blue-stained Nissl bodies (Fig. 2B). Similarly, significantly reduced number of NeuN positive cells was observed in CA1 of WT/TBI mice, while being reversed by Ulk1-deficiency (Fig. 2C). ELISA and RT-qPCR analysis suggested that IL-1β, TNF-α and IL-18 expression levels were elevated in WT mice suffered from TBI. Importantly, Ulk1-deletion decreased the release of these pro-inflammatory cytokines (Fig. 2D and E). The positive number of TUNEL staining cells was up-regulated in response to TBI. Ulk1-ablation markedly reduced the number of TUNEL staining cells in CA1 of TBI mice (Fig. 2F). Consistently, the levels of Bax, Cyto-c, cleaved Caspase-3 and PARP in hippocampus of WT/TBI mice were evidently enhanced, which were reduced in Ulk1-KO mice after TBI. Inversely, Bcl-2 down-regulated by TBI in hippocampus of WT mice was up-regulated by Ulk1-knockout (Fig. 2G).

3.3. Ulk1-KO mice alleviated glial cells activation and autophagy in hippocampus of mice after TBI

IF and RT-qPCR analysis suggested that the expressions of GFAP and Iba1 in the hippocampus were significantly enhanced in the WT/TBI mice, and Ulk1-deletion reduced GFAP and Iba1 expression levels (Fig. 3A–C). TBI resulted in a significant increase of LC3BII and Beclin-1, and a decrease of p62 in hippocampus of WT mice, which were reversed by Ulk1-knockout (Fig. 3D). In addition, the increase of p-p38 and p-JNK induced by TBI was also decreased by Ulk1-deletion in the hippocampus of mice (Fig. 3E).

3.4. Ulk1 inhibition reduces inflammatory response, apoptosis and autophagy in LPS-stimulated astrocytes (AST) via p38/JNK pathway

The effects of Ulk1 on TBI were also investigated in vitro using AST with LPS stimulation. Western blot analysis suggested that Ulk1 expression was suppressed by transfection of its siRNAs (Fig. 4A). Following, LPS-stimulated GFAP activation and expression were decreased by inhibiting Ulk1 (Fig. 4B and C). High mRNA levels of IL-1β, TNF-α and IL-18 triggered by LPS were restrained in Ulk1-knockdown AST (Fig. 4D). LPS-treatment led to up-regulation of Bax, cleaved Caspase-3 and PARP, and down-regulation of Bcl-2,

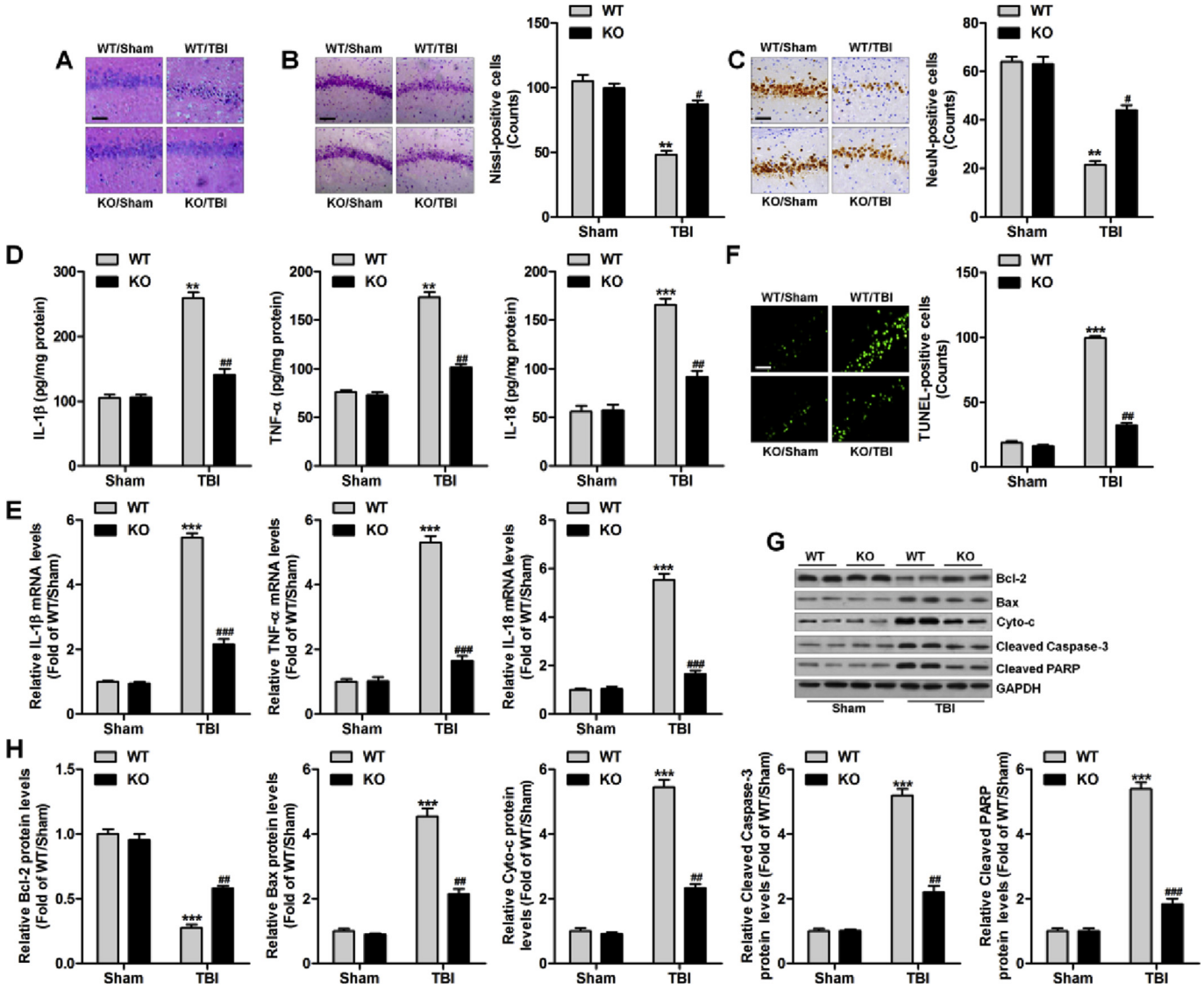

Fig. 2. Ulk1-KO mice show reduced inflammation and apoptosis in hippocampus after TBI. All mice were sacrificed 3 days post trauma, brain tissue samples were removed for following studies. (A) H&E staining of CA1 area of hippocampus. (B) Nissl staining of CA1 area. (C) IHC analysis of NeuN expressions in CA1 area. Scale: 50 μm. (D,E) IL-1β, TNF-α and IL-18 expressions in hippocampus were measured using ELISA and RT-qPCR analysis, respectively. (F) TUNEL staining of hippocampus CA1 area. Scale: 50 μm. (G,H) Apoptosis-associated signals were measured using western blot analysis. Data are expressed as the means ± SEM (n = 8). $^{**}p < 0.01$, $^{***}p < 0.001$ vs WT/Sham group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs KO/Sham group.

which were inverted by Ulk1-silence (Fig. 4E). Further, protein expression levels of Cyto-c in cytoplasm were highly induced by LPS incubation, and Ulk1-knockdown reversed the process. However, contrary expressed trends of Cyto-c were observed in mitochondria of LPS-treated AST with or without Ulk1 expressions (Fig. 4F). Similarly, LPS-induced apoptosis was abrogated by Ulk1-inhibition in AST (Fig. 4G). Moreover, LPS-incubated AST showed higher expression levels of LC3BII and Beclin-1, while lower levels of p62, while being reversed by Ulk1-knockdown (Fig. 4H). The highly phosphorylated p38 and JNK was observed in LPS-treated AST, and Ulk1-suppression markedly reduced p-p38 and p-JNK (Fig. 4I). Ulk1 has been suggested to b important in regulating MAPKs [8,9]. Thus, the activators of p38 and JNK were used to further reveal the underlying molecular mechanism. As shown in Fig. 4J and K, Ulk1 suppression reduced IL-1β, TNF-α and IL-18, and GFAP mRNA levels in LPS-stimulated AST, which were markedly abolished by DHC and ANS. Also, Ulk1 knockdown-attenuated apoptosis rate was eliminated in DHC- or ANS-pretreated cells after LPS incubation (Fig. 4L). Similarly, the increase of Bcl-2 and decrease of Bax, cleaved Caspase-3 and PARP induced by Ulk1-inhibition in LPS-exposed AST were abrogated by the DHC or ANS pre-treatment (Fig. 4M). Cyto-c expressions were down-regulated in cytoplasm by Ulk1-knockdown, while being up-regulated in mitochondria in LPS-stimulated AST, which were again reversed by DHC or ANS pre-treatment (Fig. 4N). Ulk1 suppression-induced reduction of LC3BII and Beclin-1 was rescued in DHC- or ANS-pre-treated AST with LPS exposure. However, contrast results were observed in p62 expressions (Fig. 4O). Finally, silencing Ulk1 expressions reduced p-p38 and p-JNK in LPS-stimulated cells, which were significantly attenuated by DHC or ANS pre-treatment (Fig. 4P).

4. Discussion

Various molecular mechanisms or signaling pathways are involved in TBI, such as oxidative stress, inflammation, neuronal

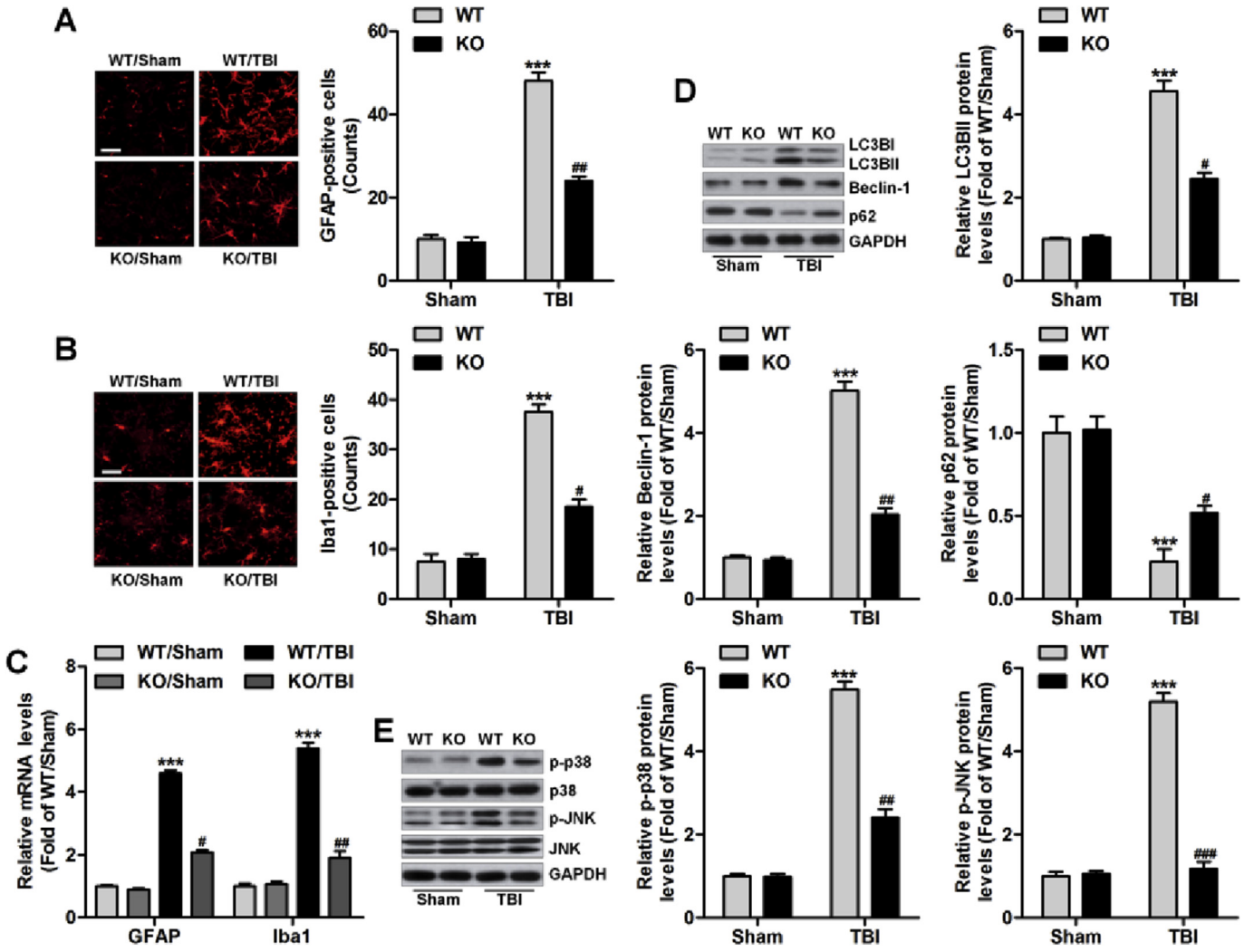

Fig. 3. Ulk1-KO mice exhibits alleviated glial cells activation and autophagy in hippocampus of mice after TBI. All mice were sacrificed 3 days post trauma, brain tissue samples were isolated for further experiments. (A,B) IF analysis of GFAP and Iba1 expressions in CA1 area. Scale: 50 μm. (C) GFAP and Iba1 mRNA expressions were measured using RT-qPCR analysis. (D) Western blot analysis of LC3BII, Beclin-1 and p52 in hippocampus of mice. (E) p-p38 and p-JNK protein expression levels were calculated using western blot analysis. Data are expressed as the means ± SEM (n = 8). $^{***}p < 0.001$ vs WT/Sham group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs KO/Sham group.

cell apoptosis and autophagy [1–3]. However, it's still not fully demonstrated. Ulk1 is reported to have a vital contribution in regulating autophagy [4–6]. And Ulk1 has been shown to be associated with brain injury; however, its role in TBI has never been elucidated. The present study evaluated the effects of Ulk1 on a mouse model of TBI. The major findings of the current study are 1) Ulk1 was over-expressed in hippocampus of WT mice with TBI, as well as in LPS-stimulated primary astrocytes. 2) Ulk1-KO mice exhibited improved cognitive dysfunction induced by TBI using MWM analysis; 3) Histological changes of hippocampus and increased NeuN-positive cells were ameliorated by Ulk1-deletion in mice after TBI. 3) Ulk1-ablation reduced the activation of glial cells, inflammation and apoptosis in TBI-induced mice. 4) Ulk1-knockout down-regulated autophagy and p38/JNK activity in the hippocampus of TBI mice. 5) Ulk1-suppression-reduced inflammation, apoptosis and autophagy was associated with p38/JNK activation.

Inflammation, especially within the central nervous system (CNS) after brain injury, could cause secondary injury following the initial damage [14]. TBI has been well-known to give rise to acute classical secondary neurogenic inflammation linked to inflammatory cytokine secretion [15]. Excessive release of pro-inflammatory cytokines could result in a decline in learning ability, degraded memory and cognitive function in brain [16]. In addition, activation of glia in CNS compartment represents the cornerstone of the CNS neuroinflammatory response, which is tightly associated with the release of inflammatory cytokines [17]. Previous study also reported that Ulk1 alters inflammatory response via regulating AMPK [18]. Ulk1 activation could target STING to sustain the production of inflammatory cytokines [19]. Therefore, Ulk1 plays a crucial role in modulating inflammatory response. In this study, we found that WT/TBI mice showed a significant increase of pro-inflammatory cytokines in hippocampus, including IL-1β, TNF-α and IL-18, as well as GFAP and Iba1, demonstrating the activity of astrocytes and glial cells. Loss of Ulk1 mice exhibited a remarkable down-regulation of IL-1β, TNF-α, IL-18, GFAP and Iba1 induced by TBI. Therefore, Ulk1-deficiency-alleviated TBI might be partially attributed to the suppression of inflammatory response.

Neuronal or other brain cell death after TBI is considered to be comprised of primary cell death, attributed to mechanical destruction, and secondary cell death, which is caused by several

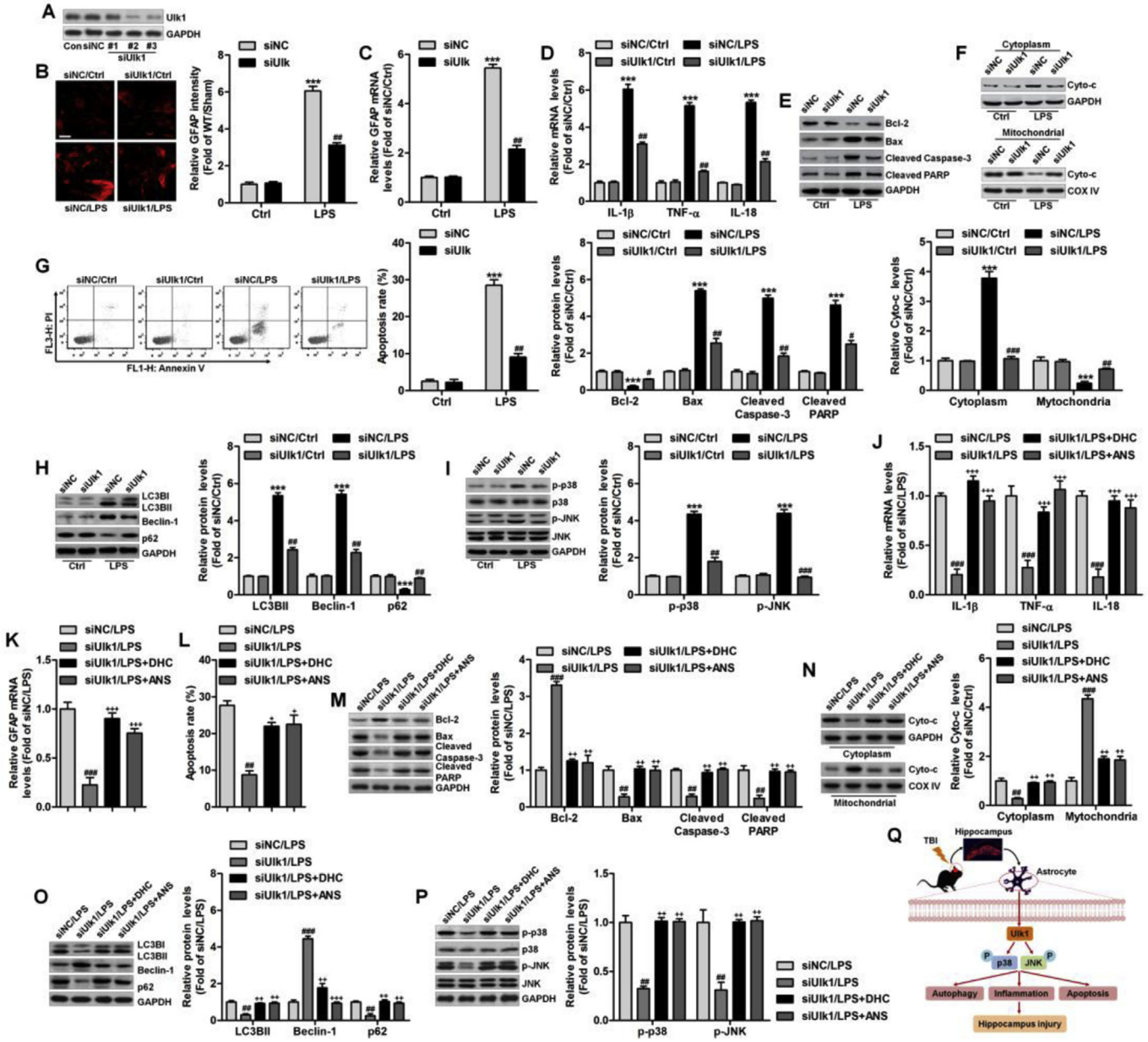

Fig. 4. Ulk1 inhibition reduces inflammatory response, apoptosis and autophagy in LPS-stimulated astrocytes (AST) via p38/JNK pathway. (A) Ulk1 siRNAs or negative control siRNA (siNC) were transfected into AST for 24 h, followed by western blot analysis of Ulk1. #2siUlk1 siRNA was selected and transfected into AST for 24 h, and then cells were challenged with 100 ng/ml of LPS for another 24 h. Subsequent experiments were performed. (B) IF staining of GFAP in AST. Scale: 30 μm. (C) RT-qPCR analysis of GFAP in AST. (D) IL-1β, TNF-α and IL-18 mRNA levels were measured using RT-qPCR analysis. (E) Western blot analysis of apoptosis related molecules. (F) Cyto-c expressions in cytoplasm or mitochondria were calculated using western blot analysis. (G) Flow cytometry analysis was used to calculate apoptosis in AST. Western blot analysis of (H) LC3BII, Beclin-1 and p62, and (I) p-p38 and p-JNK in cells. $^{***}p < 0.001$ vs siNC/Ctrl group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs siNC/LPS group. AST were pre-treated with p38 activator (DHC, 500 nM) and JNK activator (ANS, 10 μM) for 2 h, followed by #2siRNA of Ulk1 transfection for 24 h. Then, all cells were incubated with LPS (100 ng/ml) for an addition 24 h. The subsequent studies were performed. RT-qPCR assays of (J) IL-1β, TNF-α and IL-18, and (K) GFAP in AST. (L) Apoptosis rate of cells was assessed using flow cytometry analysis. (M) Western blot analysis of apoptosis-associated molecules. (N) Cyto-c expressions in cytoplasm or mitochondria were determined by western blotting analysis. (O) LC3BII, Beclin-1 and p62, and (P) p-p38 and p-JNK protein expression levels were calculated using western blot analysis. (Q) Schematic showing the proposed model of Ulk1-regulated TBI. $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs siNC/LPS group; $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$ vs siUlk1/LPS group. Data are expressed as the means ± SEM (n = 6).

pathologic cascades initiated by trauma [20]. Apoptosis is a tightly modulated, homeostatic process essential in embryologic progression and in tissues with cell turnover. But, dysregulation of apoptosis could result in various human disease processes [21]. Extracellular release of Cyto-c has been reported to be vital for apoptosis induction. Caspase-3 is believed to function primarily in apoptosis [22]. Caspase-3 regulates apoptosis after experimental TBI, and pharmacologic suppression of Caspase-3 leads to improved outcome [23]. Moreover, Ulk1 enhances mitochondrial apoptosis via reactive oxygen species. Over-expressed Ulk1 potentiated apoptosis in vitro [24]. In this study, TBI caused a significant increase of Bax, cleaved Caspase-3 and PARP, as well as Cyto-c in hippocampus of mice. In contrast to Bax, a key pro-apoptotic signal, Bcl-2, as an essential anti-apoptotic molecule, was found to be markedly down-regulated by TBI. Intriguingly, loss of Ulk1 attenuated apoptosis by reversing the expression of these molecules. And similar results were observed in LPS-stimulated AST in the absence of Ulk1. Thus, ablation of Ulk1 could improve TBI through

inhibiting apoptosis.

As reported, TBI activated autophagy, and increased microtubule-associated protein 1 light chain 3 (LC3) immunostaining in neurons or glial cells [25]. Previous studies using Beclin-1 and LC3 as autophagic biomarkers suggested that autophagy could be detected in human brain after trauma and critical illness. Presently, p62 is identified as one of the specific substrates that are degraded via the autophagy pathway [26]. Here, TBI-induced autophagy was reversed by Ulk1-deletion in hippocampus of mice, as evidenced by the reduced LC3BII and Beclin-1, while the increased p62. The process above was reversed by Ulk1-deletion. MAPKs, including p38 and JNK, are essential in autophagy induction [27]. Increased activation of p38 and JNK was observed in CNS under various injured conditions [28]. Here, WT/TBI mice exhibited higher p-p38 and p-JNK expressions. Ulk1 has been indicated to play an essential role in autophagy induction through elevating MAPKs pathway [29]. Recently, a study demonstrated that Ulk1 is a novel up-streaming signal of JNK, inducing autophagy in hepatotoxicity [30]. Here, we also found that Ulk1-knockout reduced p-p38 MAPK in hippocampus of TBI mice. Of note, the in vitro results indicated that Ulk1 knockdown-reduced inflammation, apoptosis and autophagy stimulated by LPS were abrogated by the activation of p38/JNK pathway. The results above demonstrated that loss of Ulk1-alleviated TBI was attributed to the suppression of inflammation, apoptosis and autophagy via regulating p38/JNK pathway.

In summary, Ulk1-deficiency protected mice from TBI through reducing inflammation, apoptosis and autophagy, which was at least partially dependent on the inactivation of p38/JNK (Fig. 4Q). Together, our findings provided the potential value of Ulk1 to alleviate TBI. However, the exact mechanisms involved are yet to be elucidated in future.

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.bbrc.2018.04.154.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.bbrc.2018.04.154.

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