OPEN

Curcumin protects from LPS-induced activation of astrocytes via AMPK pathway

Jing Cheng[a*], Yu Zhou[b*], Haowen Qiao[c], Hongxiang Jiang[a] and Yanqin Fan[d]

Curcumin, a phenolic pigment, plays an inhibitory role in astrocytes activation which are involved in the pathogenesis of neurological diseases and inflammatory responses. The present study aimed to investigate the underlying regulatory mechanism behind the therapeutic effect of curcumin on the lipopolysaccharide (LPS)-activated astrocytes in vitro. Specifically, we investigated the inhibitory effect of curcumin on LPS-induced astrocyte's proliferation. Additionally, we investigated whether the adenosine-monophosphate-activated protein kinase signaling (AMPK) pathway was involved in this process. Our data demonstrated that curcumin significantly increased the level of phosphorylated AMPK protein in LPS-activated astrocytes. In addition, our data demonstrated that curcumin play an inhibitory role on the migration, autophagy, the pro-inflammatory mediators by the AMPK signaling pathway in LPS-activated astrocytes. These results could shed light on understanding of molecular mechanism for the inhibition of curcumin on migration, autophagy, and the pro-inflammatory mediators during the process of astrocyte activation, and might contribute to a promising therapeutic intervention in the neurological diseases-related astrocytes activation.

NeuroReport 2023, **34**:748–758

Keywords: activated astrocytes, adenosine-monophosphate-activated protein kinase signaling pathway, curcumin

[a]Department of Neurosurgery, Renmin Hospital of Wuhan University, Wuhan, [b]Department of Neurosurgery, The Second Xiangya Hospital of Central South University, Changsha, Hunan, [c]Department of Physiology, School of Medicine, Wuhan University and [d]Division of Nephrology, Renmin Hospital of Wuhan University, Wuhan, Hubei, China

Correspondence to Yanqin Fan, MD, PhD, Division of Nephrology, Renmin Hospital of Wuhan University, 238 Jiefang Rd, Wuhan, Hubei 430060, China
Tel: +0086 18207133361; e-mail: 18207133361@163.com

*Jing Cheng and Yu Zhou contributed equally to the writing of this article.

Received 2 August 2023 **Accepted** 7 August 2023.

Introduction

Astrocytes play a central role in the maintenance of the blood-brain barrier, nervous system stability, and protection from nervous system damage [1,2]. As the most abundant cell type in the central nervous system (CNS), astrocytes are thought to be involved in the pathogenesis of neurological diseases and inflammatory responses, which provides essential support for the homeostasis of CNS. However, during the pathogenesis and progression of nervous system diseases [3,4], astrocytes undergo morphological and phenotypic changes, such as the upregulation of glial fibrillary acidic protein (GFAP) expression [5]. Along with the gene expression changes, the reactive astrocytes exhibit a series of functional changes, including promoted proliferation. Astrocytes are mostly quiescent under normal physiological conditions in the adult brain but become migratory after injury [6,7]. Besides, the accumulating evidence suggests that these activated astrocytes produce diverse inflammatory factors and neuro-toxic cytokines, such as pro-inflammatory cytokines, interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, and nitric oxide [8,9].

Additionally, multiple reports [10,11] have demonstrated that astrocyte-mediated neuroinflammation plays a central role in the pathogenesis and progress of brain injuries and neurodegenerative diseases including Parkinson's disease. Furthermore, the astrocyte-derived pro-inflammatory factors also induce microglial activation, thereby leading to neuronal dysfunction [12]. Therefore, the understanding of functional changes in activated astrocytes, including proliferation, neuroinflammation, and migration, may provide insight into inflammation-related neuronal diseases and offer a pathway to target astrocytes for neuroprotection.

Curcumin [13], as a phenolic pigment extracted from curcuma longa, has been shown to suppress inflammatory response and anti-oxidant against the pathological changes in various neurological disorders [14]. It is reported that curcumin could inhibit the inflammatory cytokines such as TNF-α, IL-1β, IL-6, and monocyte chemoattractant protein 1 (MCP-1) [15]. Additionally, curcumin exerts the anti-oxidant effect through nuclear factor erythroid 2-related factor 2 (Nrf2) pathways [16]. Recent studies [17,18] have revealed that curcumin acts as direct inhibition of astrocyte activity in neurodegenerative diseases and ischemic stroke. However, the underlying mechanisms of migration and neuroinflammation in the activated astrocytes are not yet fully understood. Further explorations are required to better understand how curcumin regulates the activation of astrocytes.

0959-4965 Copyright © 2023 The Author(s). Published by Wolters Kluwer Health, Inc. DOI: 10.1097/WNR.0000000000001950

Lipopolysaccharide (LPS) [19] is often used as a proinflammogen to stimulate astrocytes and induce an inflammatory response. In this study, curcumin was evaluated for its effectiveness on LPS-activated astrocytes. We intended to investigate the underlying regulatory mechanism of curcumin on the LPS-activated astrocytes, focusing on its role in the signaling pathway which mediated the effect of curcumin on the LPS-activated astrocytes. Overall, we found that curcumin inhibited LPS-activated astrocytes' proliferation in vitro. In addition, we reported that curcumin modulated migration, autophagy, and the pro-inflammatory mediators by targeting the adenosine-monophosphate-activated protein kinase signaling (AMPK) signaling pathway in LPS-activated astrocytes.

Materials and methods

Reagents and antibodies

The Compound C (a specific AMPK inhibitor), AICAR (a specific AMPK activator), and Cell Counting Kit-8 were from Sigma-Aldrich (St. Louis, MO, USA). Compound C and AICAR were dissolved in DMSO. Enhanced BCA Protein Assay Kit and Molecular Probes EdU-Alexa imaging detection kit were from Life Technologies. The primary antibodies including phospho-AMPKα (Cell Signaling Technology, 2535), AMPKα (Cell Signaling Technology, 2532), LC3I/II (Sigma, ABC929), Beclin-1 (Cell Signaling Technology, 3728), P62 (Cell Signaling Technology, 5114), GFAP (Cell Signaling Technology, 80788), MMP2 (Cell Signaling Technology, 40994), MMP9 (Cell Signaling Technology, 13667), and β-actin (Cell Signaling Technology, 3077) were purchased. The iTaqTM Universal SYBR Green was purchased from Bio-Rad Laboratories (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Cell culture and treatment

Primary cortical astrocytes were isolated and cultured as previously described. In brief, the cortices were harvested from the cerebrum of 0-24h-old SD rats. Hemispheres were then mechanically dissociated into tissue pieces of 1 ~3 mm in size under sterile conditions. This was followed by digesting in 0.25% trypsin for 20 min at 37 °C. The dissociated cells were seeded in Dulbecco's modified Eagle's medium-F12 nutrient mixture (DMEM-F12) with 10% fetal bovine serum, 2 mmol/L glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. The medium was replaced with fresh medium every 3 days. On days 12–14, confluent cultures were shaken at 37 °C and 200 rpm overnight to minimize microglial contamination. The purified astrocytes were reseeded at a density of 2.5×10^4 cells/cm^2 into 96- or 6-well plates for subsequent experiments. Astrocytes were pretreated with or without curcumin for 24 h and then exposed to 1 μg/ml LPS for an additional 24 h. To study the role of AMPK pathway, astrocytes were pretreated with AICAR, or Compound C for 1 h before curcumin treatment.

Cell proliferation assay

The Cell Counting Kit-8 (CCK-8; Sigma-Aldrich) was performed to evaluate cell viability. In current experiment, the astrocytes were treated in accordance with the experimental design. Briefly, the astrocytes in 96-well plates at a density of $1X10^4$ cells were stimulated with LPS (1 μg/ml) in the presence or absence of Curcumin (40μM). The astrocytes were pretreated with were incubated with curcumin for 24 h. Samples were collected after culturing for 12, 24, or 48 h after stimulation with LPS. 20 μL of MTT solution (5 mg/ml) was added to each well and incubated for another 4 h at 37 °C. The cell viability was measured at 570 nm using a microplate (Molecular Devices, CA, USA). Each experiment was performed at least three times.

Cell migration by Transwell assay

For migration, a Transwell assay was performed using chambers with polycarbonate filters (24-mm diameter, 0.4-mm pore size; Corning). The invasion of astrocytes was determined using a commercial cell invasion assay kit. Astrocytes were pretreated with or without Curcumin for 24 h and then exposed to 1 μg/ml LPS for an additional 24 h. To study the roles of AMPK pathway in LPS-induced migration, astrocytes were pretreated with AICAR, or Compound C for 1 h before curcumin exposure. The primary astrocytes (4×10^5 per well) were plated onto the top side of the upper chamber of the migration chamber. Next, the astrocytes were resuspended and added to the upper components of the migration chamber. The lower chamber was filled with 10% FBS medium (600 μl). After 24 h of incubation, the cells on the top side were removed with cotton swabs. Cells migrated to the opposite side were fixed with 4% paraformaldehyde for 15 min and stained with 0.1% crystal violet for another 30 min. Five images were randomly captured for each well by a phase-contrast microscope (Nikon TS100, Japan). The assays were performed in triplicate.

Immunofluorescence

Immunofluorescence staining was carried out to detect GFAP expression in the LPS-activated astrocytes. The astrocytes were fixed with 4% paraformaldehyde at room temperature for 30 min, and washed three times in PBS. After blocking with a blocking solution (5% FBS) for 30 min at 37 °C, they were incubated with the anti-GFAP overnight at 4 °C. Next day, cells were incubated with a mixture of Alexa-Fluor 488-conjugated secondary antibodies for 2 h at room temperature. Then cells were washed with PBS three times and stained with DAPI at 15 min at 37 °C.

EdU cell proliferation assay was performed following the Molecular Probes EdU-Alexa imaging detection kit (Life Technologies). While astrocytes were incubated with 10 μM EdU for 2 h, fixed with 4% paraformaldehyde, permeabilized

with 1% Triton X-100, and stained with the Alexa-Fluor 594 reaction cocktail for EdU and DAPI. Images were observed using a fluorescence microscope (Olympus, Japan). All assays were repeated at least three times.

Western blot analysis

Western blot analysis was performed as described previously [20]. Briefly, The total protein was extracted from astrocytes with ice-cold RIPA buffer (Roche, Mannheim, Germany) and the protein content was estimated by using an Enhanced BCA Protein Assay Kit (Beyo-time Biotechnology, Haimen, China) according to the manufacturer's protocol. A total of astrocytes lysate (25 μg/lane) was electrophoresed by 15% SDS-PAGE, and the protein bands were transferred onto PVDF membrane (Millipore, Burlington, MA). The following antibodies were used: phospho-AMPKα, AMPKα, LC3, Beclin, P62, GFAP, MMP2, MMP9, and β-actin. The membrane was washed and incubated with HRP conjugated secondary antibodies anti rabbit (1:5000) and anti-mouse (1:5000), anti-goat secondary antibody (1: 2000) at room temperature for 2 h. The proteins were visualized by chemiluminescence reagents (ECL, Amersham).

ELISA assay

Levels of TNF-α, IL-1β, IL-6, and PEG_2 were quantified using commercial ELISA kits according to the instructions. Intensity of color was measured at 450 nm using a microplate reader (TECAN, Mannedorf, Switzerland). The concentration of protein was determined by using standard curve.

Measurement of oxidative stress

The amount of reactive oxygen species (ROS) and malondialdehyde (MDA), and the activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were performed according to manufacturer's instructions. The fluorescent probe DCFH-DA was used to monitor the intracellular accumulation of ROS and the fluorescence of samples was measured using a microplate reader with 488nm excitation. The level of MDA was expressed as (pmol/mg protein) (λ=523 nm). The activity of SOD (U/mg protein) was expressed at 450 nm and the activity of GSH-Px (U/mg protein) was read for its absorbance at 412 nm.

RNA isolation and real-time PCR

Total RNA was isolated from cells using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA). RNA was reverse-transcribed into cDNA using a cDNA reverse transcription kit (Takara Biotechnology Co, Ltd.) at 37 °C for 15 min, 85 °C for 5 s, and stored at –20 °C. The products were used for two-step qPCR. The cycle threshold (Ct) values were normalized against the housekeeping gene GAPDH and analyzed by using the ΔΔCt method. The values were expressed as fold change in mRNA level of target gene relative to GAPDH.

Statistical analysis

The results are obtained as the mean ± SD of at least three assays and the differences between means were evaluated by using an unpaired two-sided Student's *t*-test. For multiple comparisons between more than two groups, analysis of variance with post hoc analysis was used. $P < 0.05$ was considered as significant difference.

Results

The inhibitory effects of curcumin on proliferation of LPS-activated astrocytes

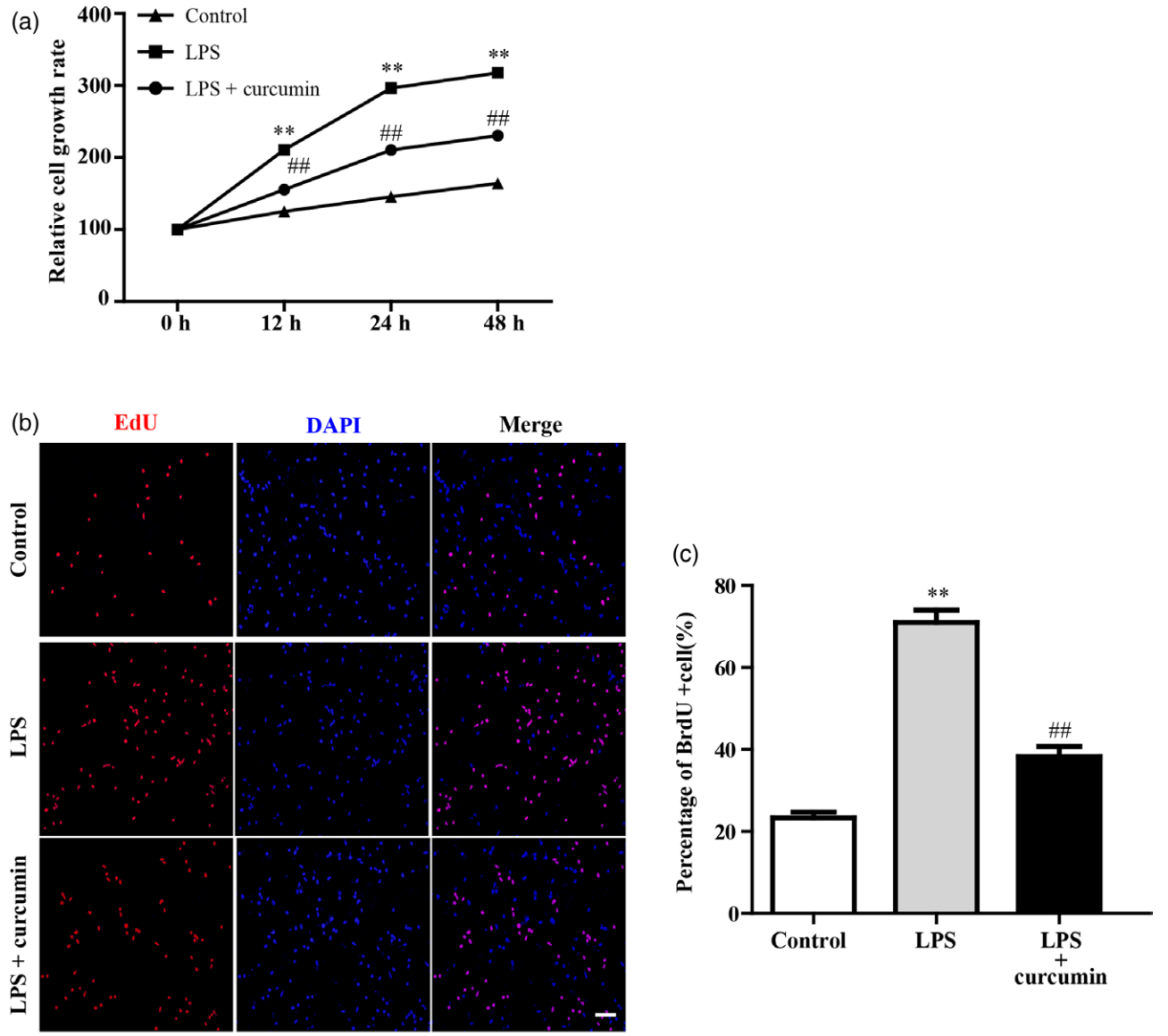
The CCK-8 assays were performed to measure the astrocytes proliferation at indicated time-points. The results in Fig. 1a showed that proliferation of astrocytes was significantly increased by the effect of LPS at, 12h, 24 h, and 48 h, while treatment with curcumin significantly inhibited the proliferation. EDU cell proliferation assay was also used in the present study to detect cell proliferation of astrocytes. As shown in Fig. 1b and c, we found that the percentage of EDU-positive astrocytes was significantly decreased in the curcumin-treated group in the LPS-activated astrocytes.

AMPK activation was required for the inhibitory effect of curcumin on LPS-activated astrocytes

Increasing studies [21] have shown that AMPK phosphorylation will protect astrocytes from permanent hypoxia and glucose deprivation. In our study, we hypothesized that the inhibitory effect of curcumin in LPS-activated astrocytes was through the AMPK signaling pathway. To verify this hypothesis, western blot was performed to examine the effect of curcumin on the AMPK activation in LPS-activated astrocytes. As displayed in Fig. 2a and b, the LPS decreased the level of phosphorylated AMPK protein at 24 h, the inhibitory effect of LPS on the level of phosphorylated AMPK protein was partially counteracted by curcumin.

To further confirm the role that the AMPK signaling pathway played the inhibitory effect of curcumin in LPS-activated astrocytes, we used Compound C (the specific AMPK inhibitor) and AICAR (the specific AMPK activator) to examine the expression levels of GFAP, the specific marker of astrocytes. The astrocytes were pretreated with or without Curcumin for 24 h and then exposed with AICAR, or Compound C for 1 h before LPS treatment. As compared to the control, the results in Fig. 2c showed that LPS significantly increased the number of activated astrocytes (GFAP-reactive cells), and curcumin treatment attenuated the expression of GFAP-reactive cells compared to the LPS-treated group. These findings also demonstrated that AICAR plus curcumin could further suppress GFAP-reactive cells, whereas the inhibition effect of the GFAP expression by curcumin was significantly blocked with Compound C. In addition, Fig. 2d revealed that LPS increased the GFAP protein expression and Curcumin reduced GFAP protein expression in the LPS-activated astrocytes, while AICAR plus curcumin showed a significant decrease in the levels of GFAP expression in LPS-activated astrocytes and the inhibition

Fig. 1



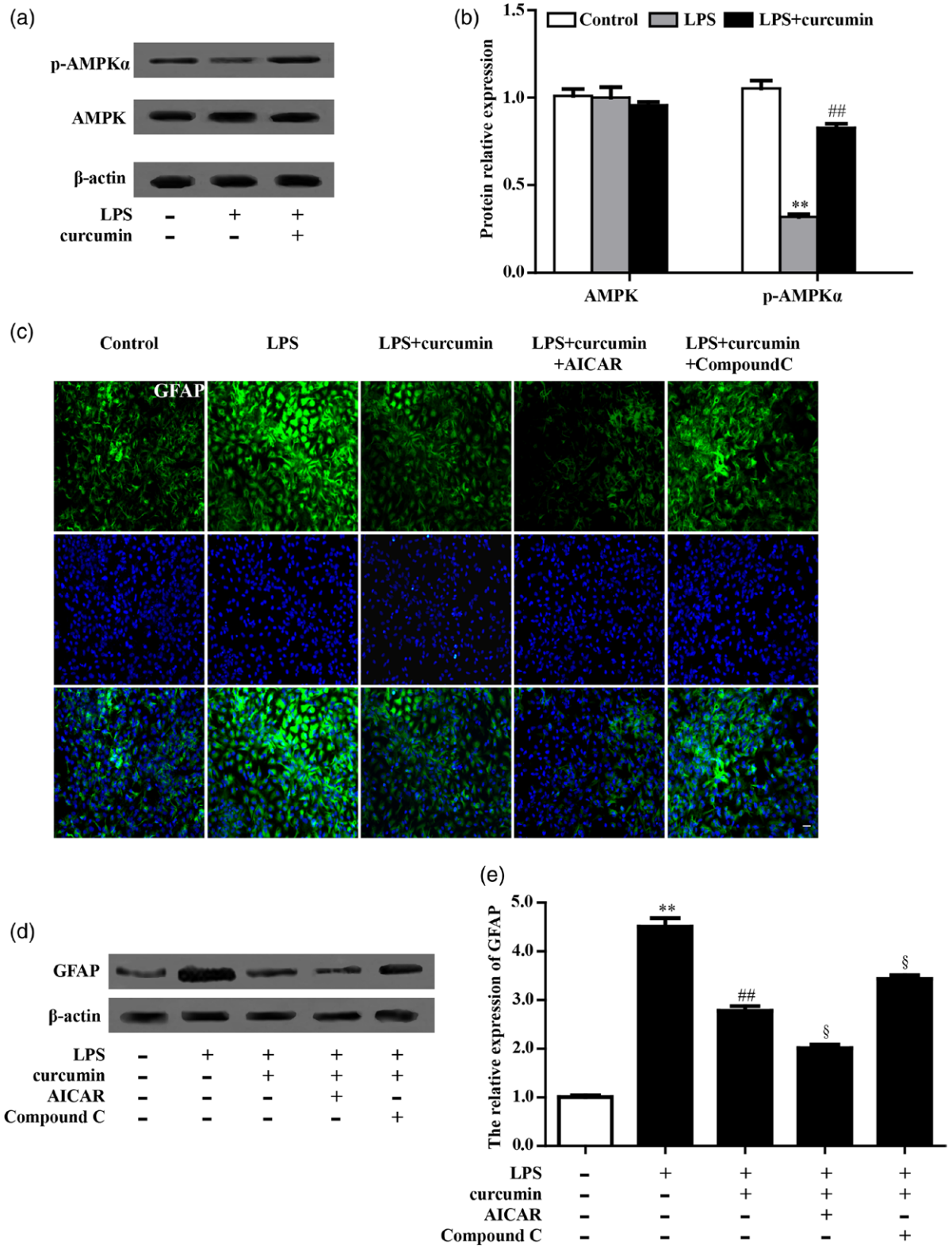
The inhibitory effects of curcumin on proliferation of LPS-activated astrocytes. (a) Analysis of the effect of curcumin on the proliferation of LPS-activated astrocytes by CCK-8 assay. The astrocytes were pretreated with curcumin for 12, 24, or 48 h and stimulation with 1 μg/ml LPS for an additional 24 h. Data (n = 3) are expressed as the mean ± SD of three independent experiments. $^{**}P < 0.001$ vs. the control group; $\#\#P < 0.001$ vs. the group treatment with LPS. (b) EdU (red) and DAPI (blue) were double stained to assess astrocytes' proliferation in LPS-activated astrocytes. Scale bar = 20 um.

effect was partially counteracted by Compound C. A similar trend was obtained when GFAP mRNA expression was examined (Fig. 2e), indicating that activation of astrocytes was accompanied with AMPK phosphorylation after LPS treatment. Together, these results suggested that AMPK signaling pathway is prominently involved in the inhibition effect of curcumin on LPS-activated astrocytes.

The pathways of AMPK were involved in curcumin-suppressed migration in LPS-activated astrocytes

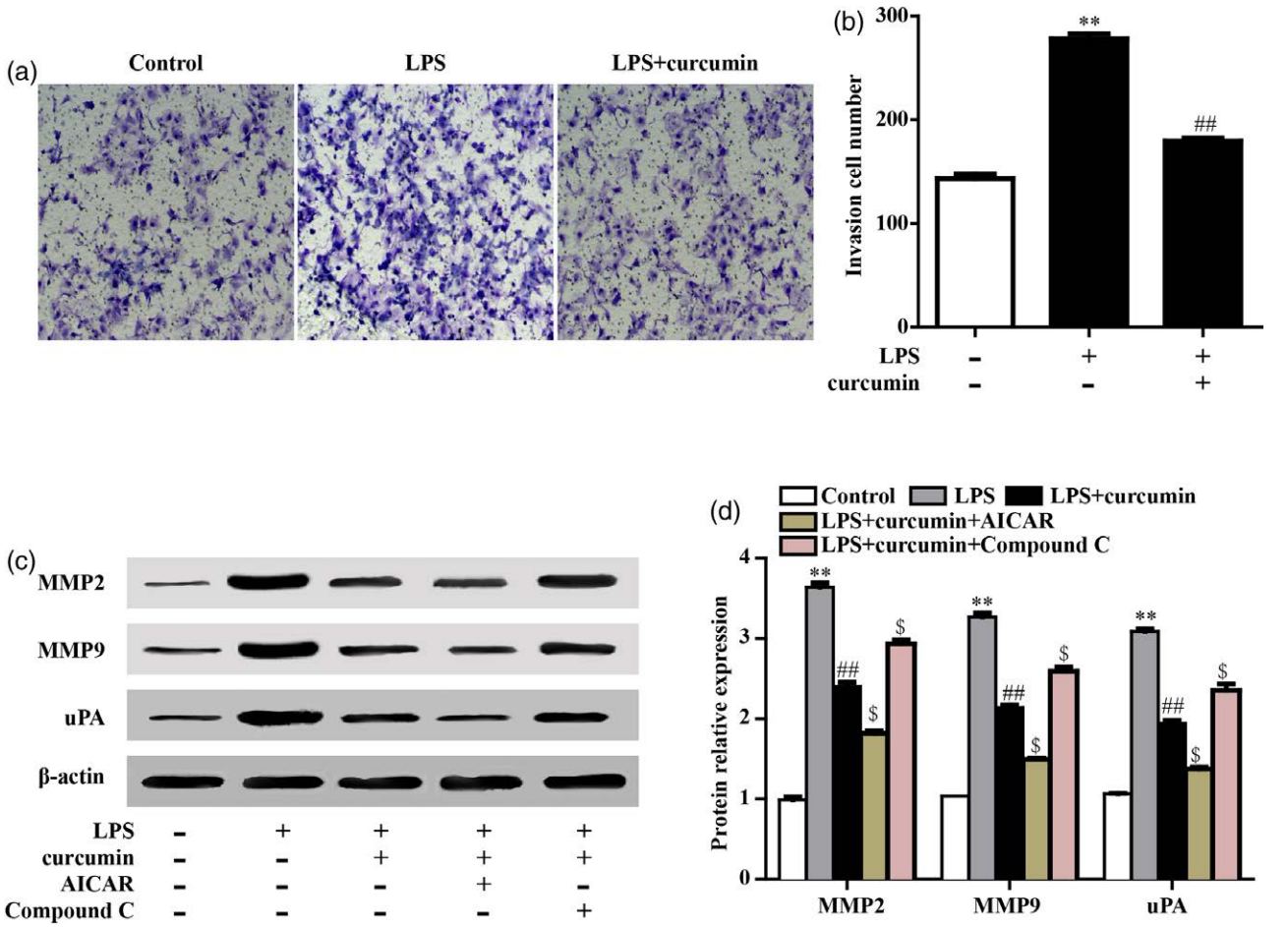
The investigation of the migration of astrocytes is of great importance during the onset and progression of nervous system diseases. Therefore, a cell migration assay was used to ascertain the effect of curcumin in activated astrocytes' migration. As shown in Fig. 3a and b, compared with the control group, the number of astrocytes migrating through the film increased significantly (283% of control) after LPS treatment. However, the increased migration could partly be inhibited by the curcumin treatment. Then, we evaluated the role of AMPK signaling pathway in curcumin-suppressed migration in LPS-activated astrocytes. We used western blotting technology to test the expressions of matrix metalloproteinases-9 (MMP9), matrix metalloproteinases-2(MMP2), and uPA, which were closely related to cell migration. Compared with the control group, the results

Fig. 2



AMPK activation was required for the inhibitory effect of curcumin on LPS-activated astrocytes. (a) Western blot bands for the protein expression of phospho-AMPKα (Thr172) and AMPKα (n = 3). (b) Densitometric analysis was performed to obtain the relative expression level of target proteins with β-actin as the internal reference. (c) Representative images of GFAP localization by immunofluorescence staining. Astrocytes were pretreated with or without Compound C (10 μM) or AICAR (1 mM) for 1 h before incubation with Curcumin or the vehicle for 24 h, and then exposed to LPS. Scale bar = 20 um. (d) Western blot analysis of GFAP expression. (e) The relative expressions of GFAP were quantified by normalizing to β-actin. $^{**}P < 0.001$ vs. the control group. $\#\#P < 0.001$ vs. the LPS group. §$P < 0.05$ vs. LPS + curcumin group.

Fig. 3



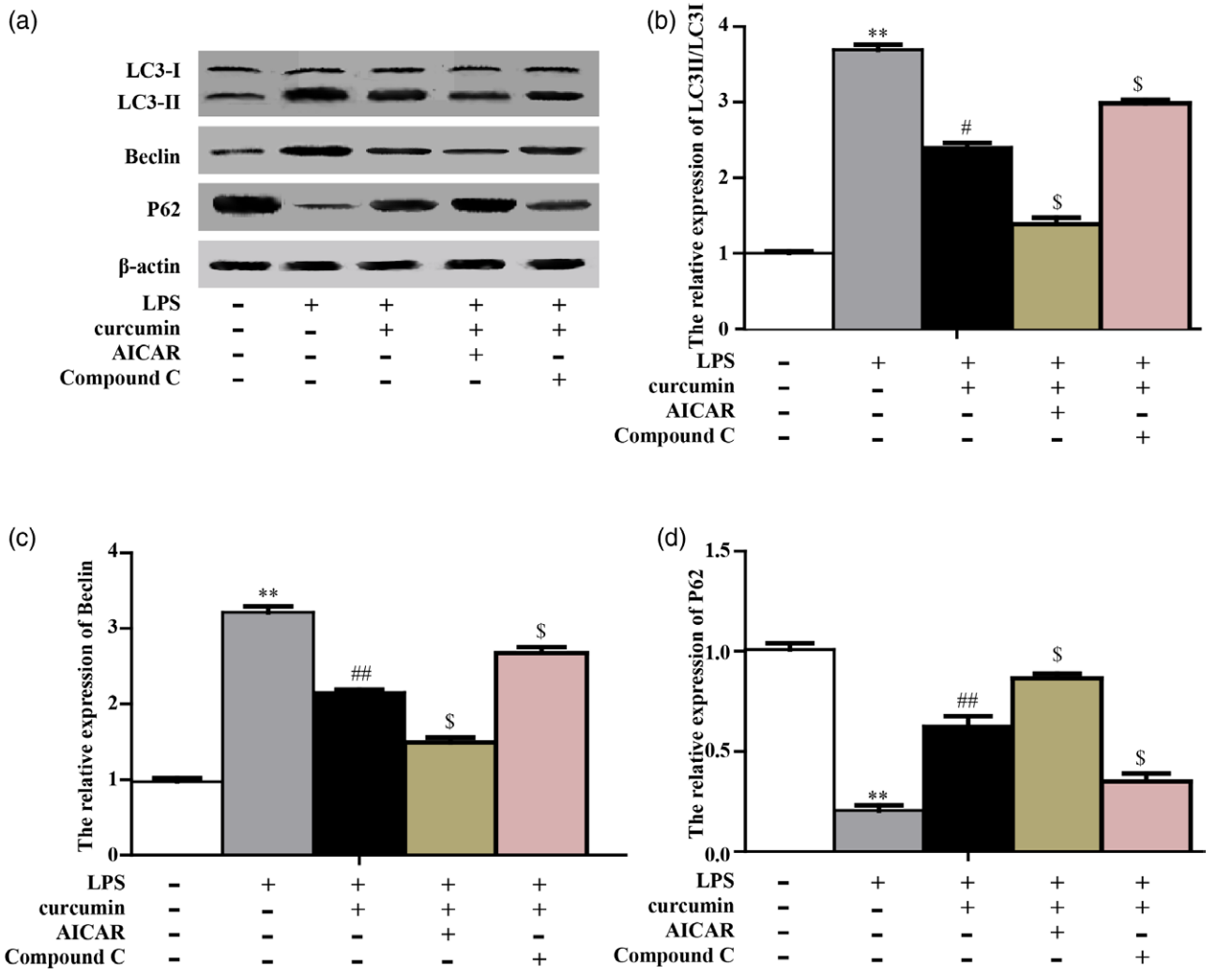
The pathways of AMPK were involved in curcumin-suppressed migration in LPS-activated astrocytes. (a) Analysis of the effect of curcumin on the migration of LPS-activated astrocytes by Transwell migration assay. (b) The data of migrated cells were counted and indicted. $^{**}P < 0.001$ vs. the control group. $^{\#\#}P < 0.001$ vs. the LPS group. Data are expressed as the mean ± SD of three independent experiments. (c) Astrocytes were pretreated with or without Compound C (10 μM) or AICAR (1 mM) for 1 h before incubation with Curcumin or the vehicle for 24 h, and then exposed to LPS. (d) Western blot analysis of MMP2, MMP9, and uPA. (d) The relative expressions of MMP2, MMP9, and uPA were quantified by normalizing to β-actin, respectively. All data are represented as mean ± SD, and obtained from three independent experiments. $^{**}P < 0.001$ vs. the control group. $^{\#\#}P < 0.001$ vs. the LPS group. §$P < 0.05$ vs. LPS + curcumin group.

in Fig. 3c and d demonstrated that the expression levels of MMP9, MMP-2, and uPA were markedly increased in LPS group, which were counteracted by curcumin. AICAR prevented the curcumin-suppressed migration in LPS-activated astrocytes, meanwhile, Compound C blocked the inhibition effect of the astrocytes' migration by curcumin. The above experimental results illuminated that curcumin activated the AMPK signaling pathway in astrocytes following LPS treatment, and AMPK pathway was involved in curcumin-suppressed migration in LPS-activated astrocytes.

Curcumin reversed the LPS-induced autophagy via AMPK pathway

Autophagy acted as one of the predominant programmed cell death pathways. To determine whether AMPK pathway contributed to curcumin-induced autophagy, western blot analysis was employed in LPS-activated astrocytes in Fig. 4. We found that the ratio of LC3-II/LC3-I was significantly upregulated, which indicated that autophagy was activated by LPS treatment, and curcumin inhibited the autophagy induced by LPS. Furthermore, the notably enhanced expression of beclin-1 and p62, two key substrates for autophagy, were also exhibited in LPS-activated astrocytes. The effect of the autophagy of LPS exposure on astrocytes was reversed by curcumin. AICAR significantly enhanced the curcumin-suppressed autophagy in LPS-activated astrocytes, meanwhile, Compound C attenuated the inhibition effect of the astrocytes' autophagy by curcumin. The results showed that curcumin could suppress the upregulation of autophagy induced by of LPS exposure via AMPK pathway.

Fig. 4



Curcumin reversed the LPS-induced autophagy via AMPK pathway. (a) The expression levels of the autophagy-related proteins including LC3-II, Beclin-1, and P62 were investigated by western blots. (b) The quantitative analysis results of LC3-II/ LC3-I were shown. (c) The relative expression of Beclin-1 was quantified by normalizing it to β-actin. (d) The relative expression of P62 was quantified by normalizing it to β-actin. All data are represented as mean ± SD, and obtained from three independent experiments. $^{**}P < 0.001$ vs. the control group. $\#\#P < 0.001$ vs. the LPS group. §$P < 0.05$ vs. LPS + curcumin group.

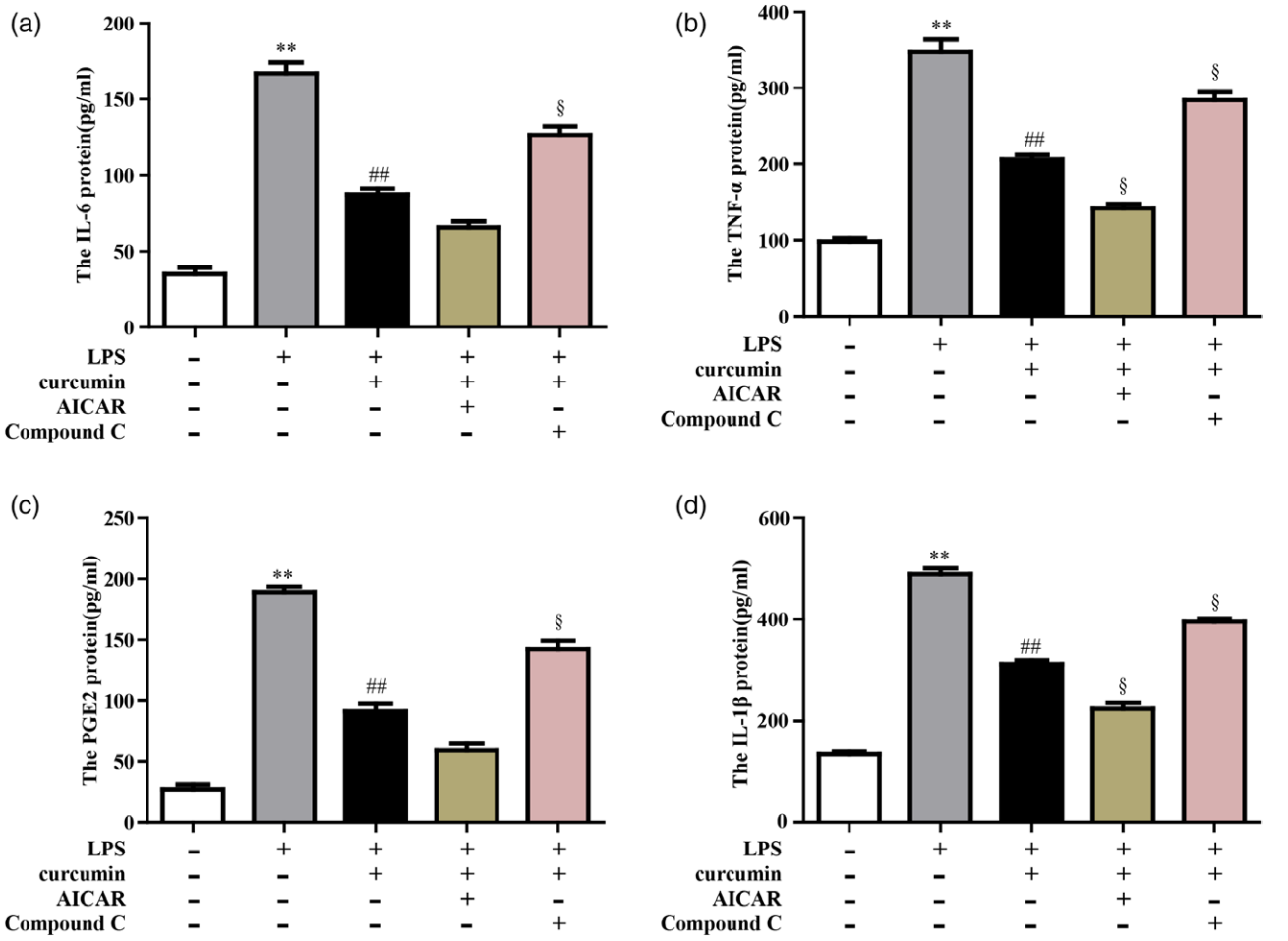
Curcumin inhibited the induced reduction of pro-inflammatory mediators by LPS exposure via AMPK pathway

Considering that astrocytes play a significant role in inflammation and immune response, the effect of AMPK pathway in curcumin-mediated inflammatory reaction was investigated. Figure 5 showed that the prominent promoting levels of TNF-α, IL-6, IL-1β, and PGE2 were observed in LPS-activated astrocytes, however, curcumin significantly suppressed the secretion of these factors and the inhibitory effects were accelerated with the AMPK activator AICAR. In contrast, the curcumin-mediated inhibitory effects on the expression levels of TNF-α, IL-6, IL-1β, and PGE2 were partially reversed by the AMPK inhibitor Compound C. These data suggested that curcumin played a significant role in LPS-activated astrocytes via AMPK signaling pathway, and activation of AMPK signaling pathway could inhibit the expression of pro-inflammatory mediators.

Curcumin inhibited the oxidative stress by inducing the activation of AMPK signaling pathway in LPS-activated astrocytes

Accumulating evidence [22] indicates the oxidative stress, such as ROS, are important regulatory mediators in many signaling processes. It is important to test the effect of curcumin on oxidative stress expression in LPS-activated astrocytes. Since we have confirmed that MPK

Fig. 5



Curcumin inhibited the induced reduction of pro-inflammatory mediators by LPS exposure via AMPK pathway. Astrocytes were pretreated with or without Compound C (10 μM) or AICAR (1 mM) for 1 h before incubation with Curcumin or the vehicle for 24 h, and then exposed to LPS. The supernatants were collected and measured for IL-6, TNF-α, and PEG2 (n = 3). $^{**}P < 0.001$ vs. the control group. $\#\#P < 0.001$ vs. the LPS group. §$P < 0.05$ vs. LPS + curcumin group.

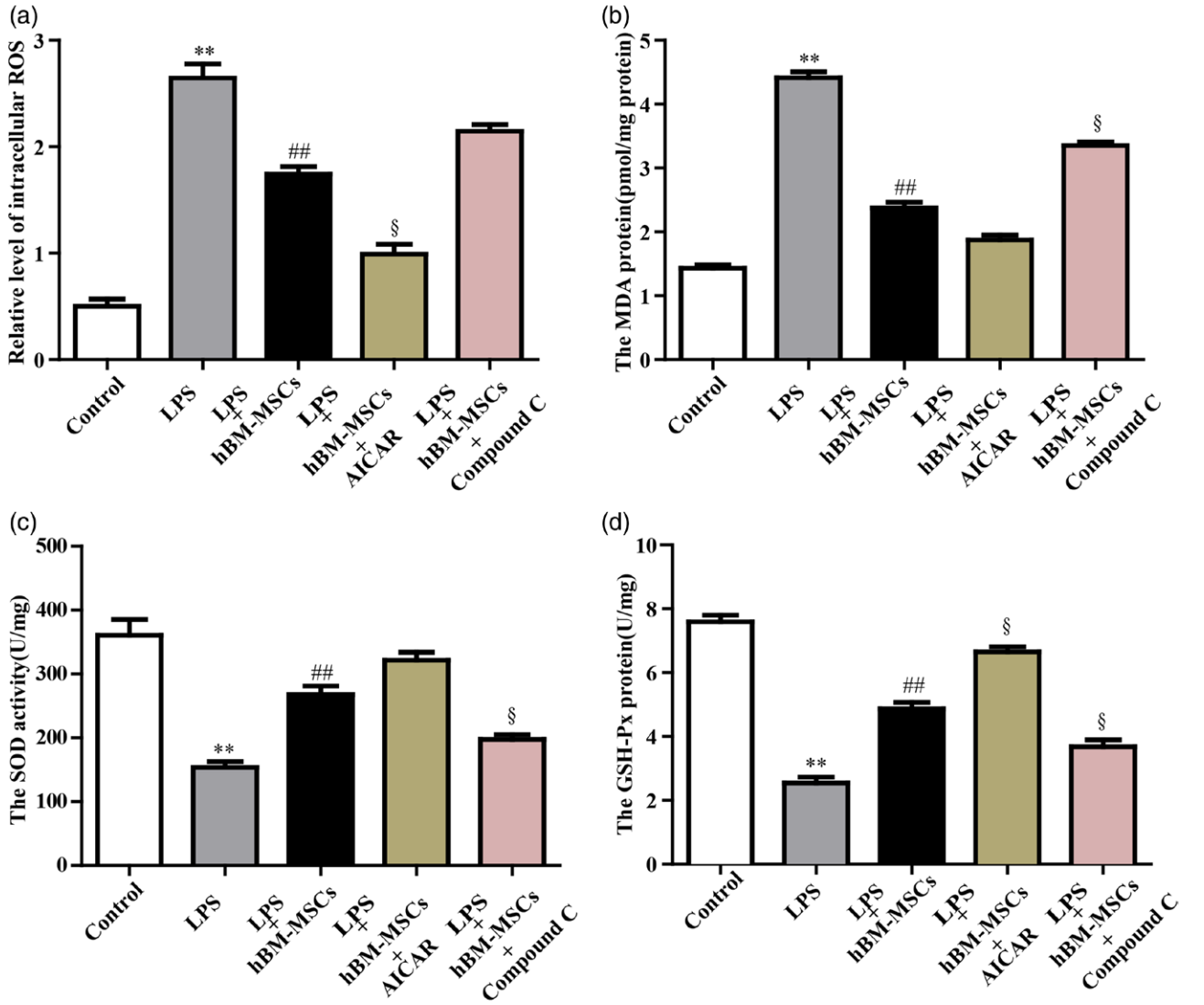
signaling pathway contributed to the inhibitory effect of curcumin on pro-inflammatory mediators in LPS-activated astrocytes. We intended to study whether the regulatory roles of curcumin in the activity of SOD and GSH-Px, the intracellular ROS production, and the level of MDA were also mediated by this pathway.

Compared to the control, LPS treatment led to a significant increase in the level of intracellular ROS and MDA (Fig. 6a and b). However, the LPS-induced the intracellular ROS and MDA expression could be inhibited in the curcumin-treated group. Our results in Fig. 6c and d also revealed that the stimulation of LPS resulted in a significant decrease in the activity of SOD and GSH-Px, whereas curcumin could reverse the SOD and GSH-Px activity. AICAR attenuated intracellular ROS production induced by curcumin, in contrast, the inhibition effects of curcumin on the intracellular ROS and MDA expression were partially counteracted by Compound. Additionally, Compared to the curcumin-treated group, the AICAR strengthened the promoting effect of the activity and GSH-Px, and Compound C attenuated the activity of SOD and GSH-Px induced by curcumin in LPS-activated astrocytes. These findings encouraged us to speculate that AMPK signaling pathway contributed to the inhibition effect of curcumin on activation of oxidative stress.

Discussion

This study established a mechanistic link between curcumin and inhibition effect on the LPS-activated astrocytes in vitro. In this work, we found that curcumin inhibited the LPS-activated proliferation of astrocytes in vitro. Furthermore, the AMPK activator AICAR could exert synergistic action on the inhibitory effect of curcumin on LPS-activated astrocytes, while Compound C, as the AMPK inhibitor, reverted the inhibitory effect of curcumin on LPS-activated astrocytes. Overall, our study focused on talking over three aspects: (1) Curcumin had the positive effect on

Fig. 6



Curcumin inhibited the oxidative stress by inducing the activation of AMPK signaling pathway in LPS-activated astrocytes. (a–d) Measurement of oxidative stress (n = 3). Astrocytes were pretreated with or without Compound C (10 μM) or AICAR (1 mM) for 1 h before incubation with Curcumin or the vehicle for 24 h, and then exposed to LPS. The amount of ROS and MDA, and the activities of SOD and GSH-Px were determined. $^{**}P < 0.001$ vs. the control group. $^{\#\#}P < 0.001$ vs. the LPS group. $^{\S}P < 0.05$ vs. LPS + curcumin group.

the phosphorylated AMPK protein in LPS-activated astrocytes; (2) Curcumin inhibited LPS-induced astrocytes migration through AMPK signaling pathway; (3) Curcumin contributed to suppress the produce of pro-inflammatory mediators and the oxidative stress by AMPK signaling pathway in LPS-activated astrocytes.

Accumulating evidence reveals astrocytes activation and subsequent neuroinflammatory response are implicated in brain injuries [23] and neurodegenerative diseases [24], such as Alzheimer's disease, stroke, and glioma. The inhibition of actrocytes activation and the neuroinflammatory response are considered to be an effective therapeutic approach to mitigate various brain diseases. Multiple signaling pathways [25] have been confirmed to be closely linked with the regulatory mechanism of on astrocytes activation, such as the mitogen-activated protein kinases (MAPKs) and the toll-like receptor 4 (TLR4)/nuclear factor-kappa B (NF-κB) signaling pathway. The AMP-activated protein kinase (AMPK), aserine–threonine kinase, which is described as a key regulator of cellular energy metabolism [26]. Recently, numerous studies [27,28] have shown the therapeutic potential of Curcumin in neurodegenerative and neuroinflammatory diseases. However, the underlying mechanisms whether the activated astrocytes are protected by curcumin via AMPK pathway are not yet well understood. In this study, firstly, we showed that Curcumin

could attenuated the expression of GFAP-reactive cells by activating AMPK pathway. Our study will shed light on the mechanism investigation of Curcumin on LPS-activated astrocytes, and providing solid foundation for using curcumin in brain diseases.

Astrocytes' migration [29] plays a pivotal role in the pathophysiology of neurodegenerative and neuroinflammatory diseases, and our results indicated that curcumin led to an inhibitory effect on migration in LPS-activated astrocyte. It has been reported that AMPK activation is involved in induction of protective autophagy [30] in astrocytes. Our results here, for the first time, proved that curcumin-induced AMPK activation suppressed migration in LPS-activated astrocytes. What is more, we used the specific AMPK inhibitor Compound C and the specific AMPK activator AICAR to test the curcumin-suppressed migration in LPS-activated astrocytes. AICAR exerted synergistic action on the curcumin-suppressed migration, and Compound C reversed the inhibition effect of the Curcumin on astrocyte migration, which suggested that the AMPK LPS-activated astrocyte and downstream responses may be involved in LPS-induced protective autophagy.

Importantly, the oxidative stress [31] and neuroinflammation [32] play central roles in a pathological feature of the CNS. Astrocytes produce pro-inflammatory cytokines, such as TNF-α, IL-1, and IL-6, which are key to the induction of brain diseases. We used the specific AMPK inhibitor Compound C and the specific AMPK activator AICAR to test pro-inflammatory mediators and the oxidative stress in LPS-activated astrocytes. Our findings encouraged us to find that the AMPK signaling pathway was involved in this process of the inhibitory effect of Curcumin on pro-inflammatory mediators and the oxidative stress in LPS-activated astrocytes.

In summary, we provided evidence showing that curcumin markedly inhibited the proliferation of in LPS-activated astrocytes. Our results demonstrated that the inhibiting effects of the curcumin on migration, autophagy, the production of pro-inflammatory mediators, and the oxidative stress were mediated at least by the AMPK signaling pathway. Therefore, curcumin, a potent anti-oxidant, could be beneficial for ameliorating the neurological diseases-related astrocytes activation. What is more, our findings may provide a new molecular mechanism for the therapeutic effects of curcumin.

Acknowledgements

This work was supported by the Natural Science Foundation of China (NSFC; 82201515) and the Natural Science Foundation of Hubei Province Youth Project (2021CFB057), received by J.C. The Natural Science Foundation of Hunan Administration of Traditional Chinese Medicine (D2022127), Natural Science Foundation of Hunan Provincial Health Commission(202204043379), and Changsha Natural Science Foundation(kq2202406), received by Y.Z. The Natural Science Foundation of China (NSFC; 82100705), received by Y.F.

Conflicts of interest

There are no conflicts of interest.

References

1 Chen Y, Sha R, Xu Li, Xia Y, Liu Y, Li X, *et al*. 2,3,7,8-Tetrachlorodibenzo-p-dioxin promotes migration ability of primary cultured rat astrocytes via aryl hydrocarbon receptor. *J Environ Sci (China)* 2019; **76**:368–376.
2 Yang B, Yin C, Zhou Y, Wang Q, Jiang Y, Bai Y, *et al*. Curcumin protects against methylmercury-induced cytotoxicity in primary rat astrocytes by activating the Nrf2/ARE pathway independently of PKCdelta. *Toxicology* 2019; **425**:152248.
3 Liu X, Tian F, Wang S, Wang F, Xiong L. Astrocyte autophagy flux protects neurons against oxygen-glucose deprivation and ischemic/reperfusion injury. *Rejuvenation Res* 2018; **21**:405–415.
4 Ouyang YB, Xu L, Yue S, Liu S, Giffard RG. Neuroprotection by astrocytes in brain ischemia: importance of microRNAs. *Neurosci Lett* 2014; **565**:53–58.
5 Hol EM, Pekny M. Glial fibrillary acidic protein (GFAP) and the astrocyte intermediate filament system in diseases of the central nervous system. *Curr Opin Cell Biol* 2015; **32**:121–130.
6 Shumakovich MA, Mencio CP, Siglin JS, Moriarty RA, Geller HM, Stroka KM. Astrocytes from the brain microenvironment alter migration and morphology of metastatic breast cancer cells. *FASEB J* 2017; **31**:5049–5067.
7 Zhan JS, Gao K, Chai RC, Jia XH, Luo DP, Ge G, *et al*. Astrocytes in migration. *Neurochem Res* 2017; **42**:272–282.
8 Palomba L, Amadori A, Cantoni O. Early release of arachidonic acid prevents an otherwise immediate formation of toxic levels of peroxynitrite in astrocytes stimulated with lipopolysaccharide/interferon-gamma. *J Neurochem* 2007; **103**:904–913.
9 Saijo K, Winner B, Carson CT, Collier JG, Boyer L, Rosenfeld MG, *et al*. A Nurr1/CoREST pathway in microglia and astrocytes protects dopaminergic neurons from inflammation-induced death. *Cell* 2009; **137**:47–59.
10 Neal M, Richardson JR. Epigenetic regulation of astrocyte function in neuroinflammation and neurodegeneration. *Biochim Biophys Acta Mol Basis Dis* 2018; **1864**:432–443.
11 Qiao C, Yin N, Gu H-Y, Zhu J-L, Ding J-H, Lu M, *et al*. Atp13a2 deficiency aggravates astrocyte-mediated neuroinflammation via NLRP3 inflammasome activation. *CNS Neurosci Ther* 2016; **22**:451–460.
12 Leng L, Zhuang K, Liu Z, Huang C, Gao Y, Chen G, *et al*. Menin deficiency leads to depressive-like behaviors in mice by modulating astrocyte-mediated neuroinflammation. *Neuron* 2018; **100**:551–563.e7.
13 Qin X, Qiao H, Wu S, Cheng J, Wan Q, Liu R. Curcumin inhibits monocyte chemoattractant protein-1 expression in TNF-alpha induced astrocytes through AMPK pathway. *Neurochem Res* 2018; **43**:775–784.
14 Babu A, Prasanth KG, Balaji B. Effect of curcumin in mice model of vincristine-induced neuropathy. *Pharm Biol* 2015; **53**:838–848.
15 Yuan J, Liu W, Zhu H, Chen Y, Zhang X, Li L, *et al*. Curcumin inhibits glial scar formation by suppressing astrocyte-induced inflammation and fibrosis in vitro and in vivo. *Brain Res* 2017; **1655**:90–103.
16 Daverey A, Agrawal SK. Curcumin protects against white matter injury through NF-κB and Nrf2 cross talk. *J Neurotrauma* 2020; **37**:1255–1265.
17 Eghbaliferiz S, Farhadi F, Barreto GE, Majeed M, Sahebkar A. Effects of curcumin on neurological diseases: focus on astrocytes. *Pharmacol Rep* 2020; **72**:769–782.
18 Mohammadi A, Hosseinzadeh Colagar A, Khorshidian A, Amini SM. The Functional roles of curcumin on astrocytes in neurodegenerative diseases. *Neuroimmunomodulation* 2022; **29**:4–14.
19 Tarassishin L, Suh HS, Lee SC. LPS and IL-1 differentially activate mouse and human astrocytes: role of CD14. *Glia* 2014; **62**:999–1013.
20 Zhai X, Qiao H, Guan W, Li Z, Cheng Y, Jia X, *et al*. Curcumin regulates peroxisome proliferator-activated receptor-gamma coactivator-1alpha expression by AMPK pathway in hepatic stellate cells in vitro. *Eur J Pharmacol* 2015; **746**:56–62.

21 Barialai L, Strecker MI, Luger A-L, Jäger M, Bruns I, Sittig ACM, *et al*. AMPK activation protects astrocytes from hypoxiainduced cell death. *Int J Mol Med* 2020; **45**:1385–1396.
22 Lehner C, Gehwolf R, Tempfer H, Krizbai I, Hennig B, Bauer H-C, *et al*. Oxidative stress and blood-brain barrier dysfunction under particular consideration of matrix metalloproteinases. *Antioxid Redox Signal* 2011; **15**:1305–1323.
23 Burda JE, Bernstein AM, Sofroniew MV. Astrocyte roles in traumatic brain injury. *Exp Neurol* 2016; **275 Pt 3**:305–315.
24 Yamanaka K, Komine O. The multi-dimensional roles of astrocytes in ALS. *Neurosci Res* 2018; **126**:31–38.
25 Colombo E, Farina C. Astrocytes: key regulators of neuroinflammation. *Trends Immunol* 2016; **37**:608–620.
26 Lu X, Wu F, Jiang M, Sun X, Tian G. Curcumin ameliorates gestational diabetes in mice partly through activating AMPK. *Pharm Biol* 2019; **57**:250–254.
27 Yu S, Wang X, He X, Wang Y, Gao S, Ren L, *et al*. Curcumin exerts anti-inflammatory and antioxidative properties in 1-methyl-4-phenylpyridinium ion (MPP(+))-stimulated mesencephalic astrocytes by interference with TLR4 and downstream signaling pathway. *Cell Stress Chaperones* 2016; **21**:697–705.
28 Lakey-Beitia J, González Y, Doens D, Stephens DE, Santamaría R, Murillo E, *et al*. Assessment of novel curcumin derivatives as potent inhibitors of inflammation and amyloid-beta aggregation in Alzheimer's disease. *J Alzheimers Dis* 2017; **60**(s1): S59–S68.
29 Zhang L, Ma P, Guan Q, Meng L, Su L, Wang L, *et al*. Protein phosphatase 2A regulates the p38 signaling pathway to affect the migration of astrocytes. *Mol Med Rep* 2018; **18**:4328–4334.
30 Zhang Y, Miao JM. Ginkgolide K promotes astrocyte proliferation and migration after oxygen-glucose deprivation via inducing protective autophagy through the AMPK/mTOR/ULK1 signaling pathway. *Eur J Pharmacol* 2018; **832**:96–103.
31 Gonzalez-Reyes RE, Nava-Mesa MO, Vargas-Sánchez K, Ariza-Salamanca D, Mora-Muñoz L. Involvement of astrocytes in alzheimer's disease from a neuroinflammatory and oxidative stress perspective. *Front Mol Neurosci* 2017; **10**:427.
32 Banks WA, Gray AM, Erickson MA, Salameh TS, Damodarasamy M, Sheibani N, *et al*. Lipopolysaccharide-induced blood-brain barrier disruption: roles of cyclooxygenase, oxidative stress, neuroinflammation, and elements of the neurovascular unit. *J Neuroinflammation* 2015; **12**:223.