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# Microglial LOX-1/MAPKs/NF-κB positive loop promotes the vicious cycle of neuroinflammation and neural injury

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ABSTRACT

Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1), a member of the scavenger receptor family, recognizes multiple ligands and participates in several inflammatory responses, but its function within the central nervous system (CNS) remains unclear. In this study, we discovered an increased LOX-1 expression in activated microglia in vivo and in vitro. Employing the specific inhibitors, we found that conditioned medium of necrotic neurons (Necrotic-CM) induced microglial LOX-1 expression through the MAPKs/NF-κB pathway. Silencing LOX-1 inhibited MAPK phosphorylation, NF-κB-p65 nuclear transportation, and pro-inflammatory factor production in microglia exposed to Necrotic-CM. Furthermore, utilizing the conditioned medium of activated microglia (MG-CM), we discovered microglial LOX-1 aggravated the neuroinflammation-induced neuronal apoptosis. Collectively, a LOX-1/MPAKs/NF-κB positive loop might promote microglia activation and drive the vicious cycle of neuroinflammation and neuronal injury.

## 1. Introduction

Accumulating studies reported that the neuroinflammatory-neuropathic malignant circulation is a common molecular mechanism for the central nervous system (CNS) diseases [1], and microglia, the resident immune cell of the CNS, acts as a pivotal driving force [2]. Under normal conditions, microglia plays a neuroprotective function by expressing anti-inflammatory mediators such as transforming growth factor-β (TGF-β), Arginase-1 (Arg-1), and Chitinase 3-like3 (Ym-1) [3]. However, under pathological conditions, microglia recognizes either the exogenous pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS), or damage-associated molecular patterns (DAMPs), the endogenous risk signals released by injured neurons, and transforms into an activated station. Persistent activated microglia release large amount of inflammatory mediators [4,5], such as tumor necrosis factor (TNF-a), Interleukin-6 (IL-6), and Interleukin-1β (IL-1β), which amplify the inflammatory response by transmitting inflammatory signals to astrocytes, and mediate chronic neuronal damage [6]. This process is considered as an important cause of neurodegenerative diseases, but the underlying molecular mechanisms are still unclear.

Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is a type II membrane protein that belongs to the C-type lectin family, and highly expressed on macrophages, vascular smooth muscle cells and endothelial cells, etc. [7]. LOX-1 plays important roles in the pathogenesis of atherosclerosis and myocardial ischemia by identifying ox-LDL [8]. LOX-1 is closely related to immune system functions, and activates macrophages by recognizing endogenous risk signals such as heat shock proteins (HSPs) [9]. After combing its ligands, LOX-1 reportedly activates several signaling pathways, such as MAPKs, PKC and NF-κB [10,11]. For example, ox-LDL induced endothelial oxidative injuries by modulating LOX-1-mediated ROS generation via the AMPK/PKC/NADPH oxidase signaling pathway [12].

The expression and function of LOX-1 in the CNS is not fully understood. Clinical studies have reported that the high serum levels of soluble LOX-1 (sLOX-1) and its ligands are associated with the risk and poor outcome of stroke [13,14]. The expression of LOX-1 was found increased in hypertension-induced brain damage model in rats and might participate in neuronal apoptosis by regulating the Bax/Bcl-2

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pathway [15]. Our previous studies confirmed that LOX-1 recognized DAMP molecules such as HSP60 released by necrotic neurons and promoted the inflammatory activation of microglia in vitro [16]. However, the molecular mechanism behind this phenomenon needs a further investigation.

In this study, utilizing a LPS-induced mice neuroinflammation model, we first determined LOX-1 expression and its correlation with neuroinflammation in vivo. In addition, we established the microglia activation model in vitro by either LPS, a typical PAMP, or the conditioned medium of necrotic neurons (Necrotic-CM), as a pool of soluble neuronal injury signals [17,18] to explore the contribution of LOX-1 to neuroinflammation, and focused on the related molecular signaling pathways. Furthermore, we analyzed the key role of LOX-1 in the vicious cycle of neuroinflammation and neural injury.

## 2. Materials and methods

### 2.1. Animal modeling

In this study, male C57BL/6 mice ($n = 63$, aged 6–8 weeks, 25–30 g, and specific pathogen free), provided by the Department of Animal Center, Nantong University, were randomly divided into seven groups including one control group and six LPS groups. The experimental mice were adapted to the environment for 7 days before the LPS administration. Briefly, the mice were injected intraperitoneally with LPS (*Escherichia coli* O111-B4; Sigma, St. Louis, MO, USA) at a dose of 9 mg/kg [19], and the time points were 6 h, 12 h, 1 day, 3 days, 5 days, and 7 days after injecting LPS [20]. The saline group served as a control. When a specific time point was reached, the mice were sacrificed, and the brain tissues were taken and stored at −80 °C for the following experiments. We tried our best to reduce the number and suffering of animals and to ensure the objectivity of experiments and data.

### 2.2. Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated from mice brain tissues. The cDNA synthesis was carried out with RevertAidTM RT Kit according to the manufacturer's protocol (Thermo Fisher Scientific), and PCR products were analyzed by agarose (1%) gel electrophoresis. Primers were purchased from Sunny Company (Shanghai, China).

DNA sequences of primers used in PCRs and expected product sizes.

| | Forward primer (5′-3′) | Reverse primer (5′-3′) | Size (bp) |
|---|---|---|---|
| GAPDH | CAAGGTCATCCATGACAACTTTG | GTCCACCACCCTGTTGCTGTAG | 496 |
| IL-6 | GCCTTCTTGGGACTGATGCT | TGGAAATTGGGGTAGGAAGGAC | 475 |
| iNOS | CAAGAGTTTGACCAGAGGACC | TGGAACCACTCGTACTTGGGA | 654 |
| IL-1β | TCATTGTGGCTGTGGAGAAG | AGGCCACAGGTATTTTGTCG | 333 |
| TNF-α | ACAGAAAGCATGATCCGCGA | TTGCTACGACGTGGGCTAC | 286 |
| Arg-1 | CTGGGGATTGGCAAGGTGAT | GCCAAGGTTAAAGCCACTGC | 290 |
| TGF-β | AGGAGACGGAATACAGGGCT | CCACGTAGTAGACGATGGGC | 482 |
| Ym-1 | TACCCTGGGTCTCGAGGAAG | GAAGCTGCTCCATGGTCCTT | 350 |

### 2.3. Cell cultures and stimulation

BV2 cells (a mouse microglial cell line) were cultured in PMI-1640 Medium and HT22 cells (a mouse hippocampal neuronal cell line) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (HyClone, Logan, UT, United States). The medium were supplemented with 10% (*v*/v) fetal bovine serum (FBS) and 100 mg/ml penicillin-streptomycin. Cells were incubated at 37 °C incubator, under 5% $CO_2$ in humidified air.

Conditioned medium of necrotic neurons (Necrotic-CM) from damaged HT22 was prepared by an improved method as described previously [18]. Briefly, when the HT22 cells grew to about 80%, we sealed and placed them in −80 °C for 30 min, then placed them in a 37 °C incubator for 2 h, and then collected the supernatants.

BV2 activation model: BV2 cells were respectively stimulated with LPS [21] (100 ng/ml LPS, *Escherichia coli* 055:B5, Sigma Aldrich, L6529) or Necrotic-CM (Damaged HT22 supernatant were diluted 1:4 by PMI-1640 medium) [16] at different time points.

Neuroinflammation-mediated neuron injury model [22]: the conditioned medium of activated microglia (MG-CM) was collected, diluted at 1:1 by DMEM medium, and then transferred to HT22 cultures to induce the neuroinflammation-mediated neuron injury model. HT22 cells were stimulated with the MG-CM for 24 h, and the cell viability and apoptosis were analyzed as described below.

### 2.4. Plasmid transfection

The LOX-1 DNA plasmid and the blank myc-vector as control were purchased from Invitrogen Life Technologies (Carlsbad, CA, USA). The sh-LOX-1 (mouse) and the negative control shRNA were purchased from Public Protein/Plasmid Library (PPL, China). Plasmid interference targets were as follows:

OLR1shRNA-1:5′-GTCAGTGACCCTTATTGTATT-3′, OLR1shRNA-2:5′-GTGGCCAGTTACTACAAATT-3′, OLR1shRNA-3:5′-GCAAGACTGGCTCTGGCATAAATT-3′.

Plasmids were transiently transfected into BV2 cells via Lipofectamine2000 Reagent according to the manufacturer's instructions.

### 2.5. Immunoblotting

After determination of the protein concentration with the Bio-Rad protein assay (BioRad, Hercules, CA, USA), protein samples were subjected to 10% SDS-polyacrylamide gel electrophoresis, and then transferred to the polyvinylidene difluoride filter (PVDF) membrane. The membrane was blocked with 5% nonfat milk, incubated with the specific primary antibodies overnight at 4 °C, followed by the secondary antibodies for 2 h at room temperature (RT). Lastly, protein bands were visualized with an enhanced chemiluminescence system (ECL, Pierce Company, Appleton, WI, USA). All results shown are representative of at least three independent experiments.

### 2.6. Immunofluorescence staining

Frozen tissue sections were blocked with a blocking solution (1% bovine serum albumin, 1% BSA) for 2 h at RT, co-incubated with primary antibodies against LOX-1 (rabbit, 1:100, Abcam, USA) and Iba-1 (goat, 1:100, Abcam, USA) overnight at 4 °C, followed by the secondary antibodies (1:400) and Hoechst (1:800) for 2 h at RT. The sections were observed under a Leica fluorescence microscope (Leica, DM 5000B, Leica CTR 5000, Germany).

BV2 cells were cultured on carrier plates and underwent the specific treatments for the indicated time. Then the cells were harvest, fixed with 4% paraformaldehyde and blocked with 1% BSA. The slides were incubated with anti-p65 (rabbit, 1:100, Cell Signaling Technology, USA) at 4 °C overnight, followed by Alexa fluor TM 488 donkey anti-rabbit IgG (H + L) (1:500, Thermo Fisher Scientific) for 2 h. Nuclei were visualized by Hoechst staining and the slides were detected under a Leica fluorescence microscope (Germany).

### 2.7. Immunohistochemistry

The frozen tissue sections were blocked with 10% donkey serum with 0.3% Triton X-100 and 1% (*w/v*) BSA for 2 h at RT, and incubated with anti-LOX-1 antibody (rabbit, 1:100) at 4 °C overnight, followed by the secondary antibodies for 30 min at RT. Afterwards, the sections were reacted with Dilute DAB dye solution, which then were dehydrated in gradient, dried, sealed with resin and examined using a Leica

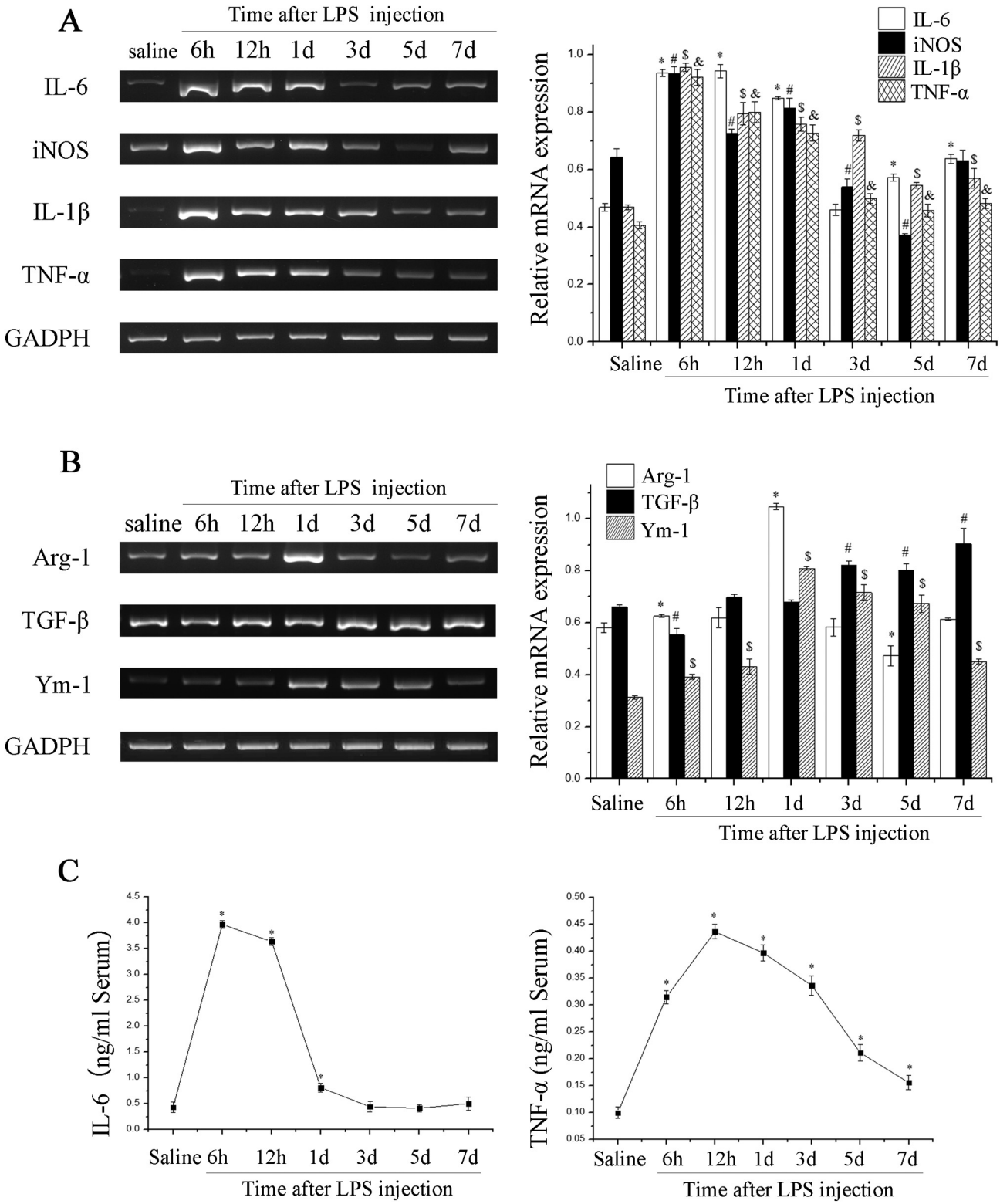


**Fig. 1.** Establishment of mice neuroinflammation model by intraperitoneal injection of LPS.
The mRNA expression of pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) and iNOS (A), as well as anti-inflammatory factors (Arg-1, TGF-β and Ym-1) (B) were detected in mice cerebral cortex by RT-PCR after intraperitoneal injection of LPS at different time points (6 h, 12 h, 1 day, 3 days, 5 days and 7 days, saline as control). The release of IL-6, TNF-α in the mice serum was detected by ELISA method (C). Data shows means ± SD in three independent experiments. (*, #, $, and &P < 0.05 compared with the saline group).

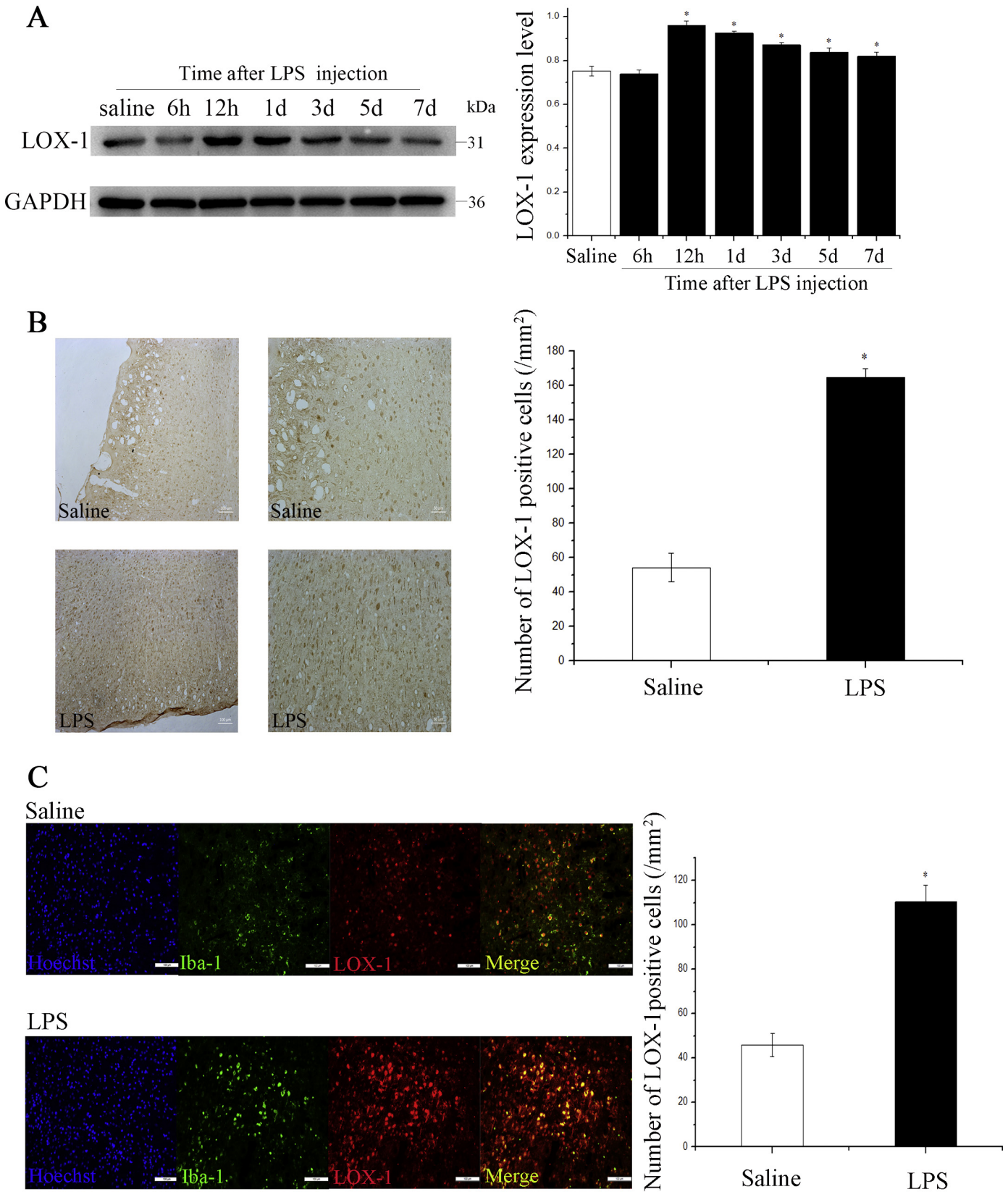


**Fig. 2.** LOX-1 protein expression increased in mice cerebral cortex after LPS injection, and localized in microglia.
The protein expression of LOX-1 in the mice cerebral cortex after LPS injection at different time points was analyzed by western blot (A). The distribution of LOX-1 in the mice cerebral cortex in saline group and 12 hour group LPS were respectively detected by Immunohistochemistry (B). Sections of mice brain cortex in saline group and 12 hour group LPS were respectively labeled with Hoechst (blue), Iba-1 (green) and LOX-1 (red), and the yellow color in the merge images represented co-localization of LOX-1 with microglia. Quantitative analysis of microglia number expressed LOX-1 in two groups (C). *P < 0.05 compared with the saline group. *Scale bars*, 100 μm or 50 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

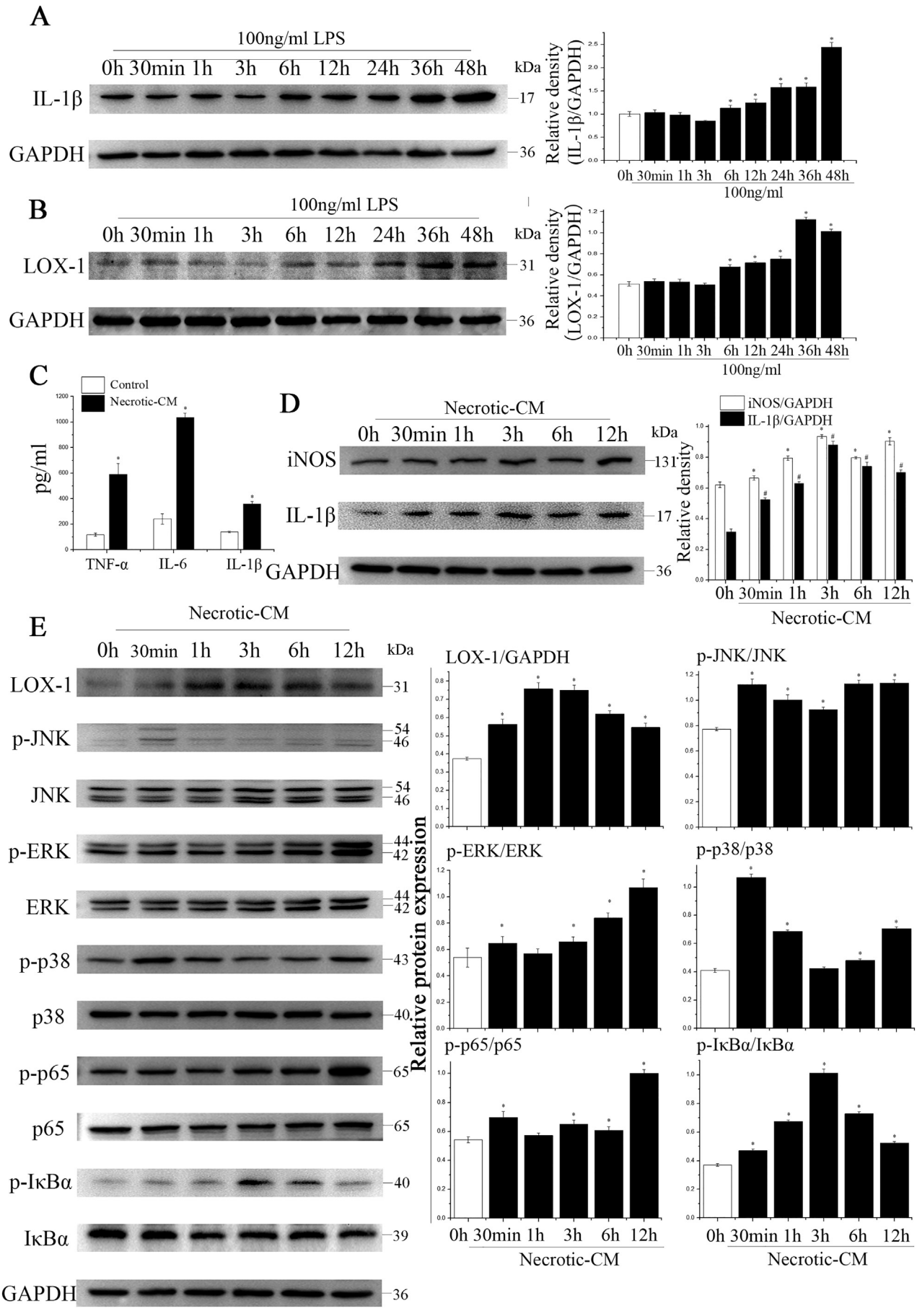


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**Fig. 3.** Increased LOX-1 expression and MAPK/NF-κB activation in microglia in response to LPS or the conditioned medium of necrotic neurons (Necrotic-CM). The protein expression of IL-1β and LOX-1 were detected at different time points (0 h, 30 min, 1 h, 3 h, 6 h, 12 h, 24 h, 36 h, and 48 h) after 100 ng/ml LPS stimulation in BV2, quantification graph indicated the relative density of IL-1β (A) or LOX-1 (B) to GAPDH. The release of pro-inflammatory factors including TNF-α, IL-6 and IL-1β were detected by ELISA in the supernatants of BV2 after Necrotic-CM stimulation for 3 h (C). Western blot detected the protein expression of iNOS and IL-1β in Necrotic-CM treated BV2 cells at different time points (D). The protein expression of LOX-1, MAPKs (JNK, ERK and p38), phosphorylated MAPKs (p-JNK, p-ERK, p-p38) and NF-κB (p-p65, p65, p-IκBα, IκBα) signaling pathways in Necrotic-CM treated BV2 cells were detected (E). The data are means ± SD ($n$ = 3). *, $^{\#}P < 0.05$ compared with the control group.

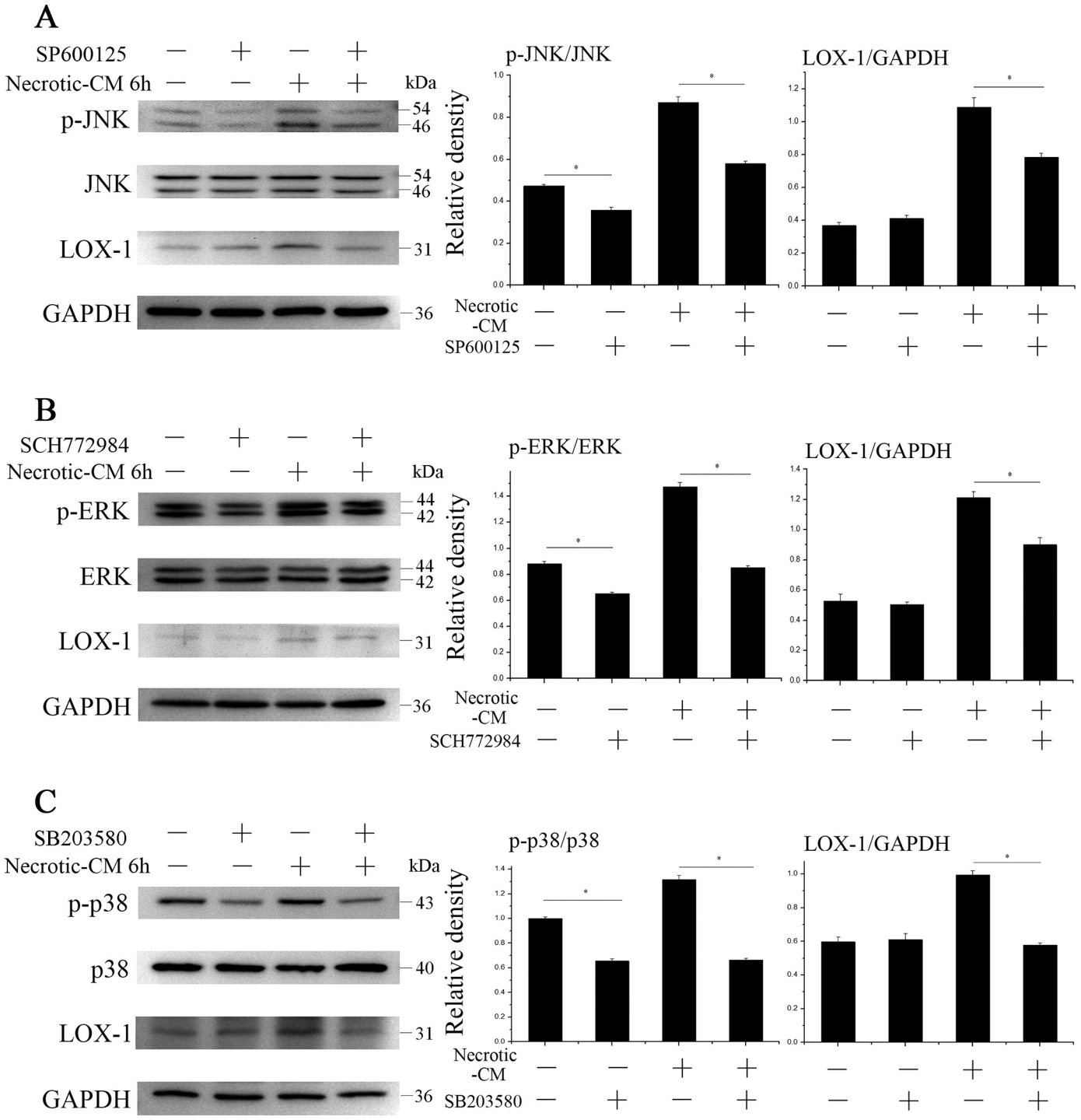


**Fig. 4.** Microglial LOX-1 expression was regulated by MAPK signaling ways in response to Necrotic-CM.
We pretreated BV2 with the JNK kinase inhibitor SP600125 (80 μM) (A), the ERK kinase inhibitor SCH772984 (80 μM) (B) or p38 kinase inhibitor SB203580 (40 μM) (C) respectively for 1 h, followed by the Necrotic-CM treatment for 6 h. Phosphorylation of JNK, ERK and p38, and the expression of LOX-1 were determined by western blot analysis. The data are means ± SD ($n$ = 3). *P < 0.05 compared with the control group.

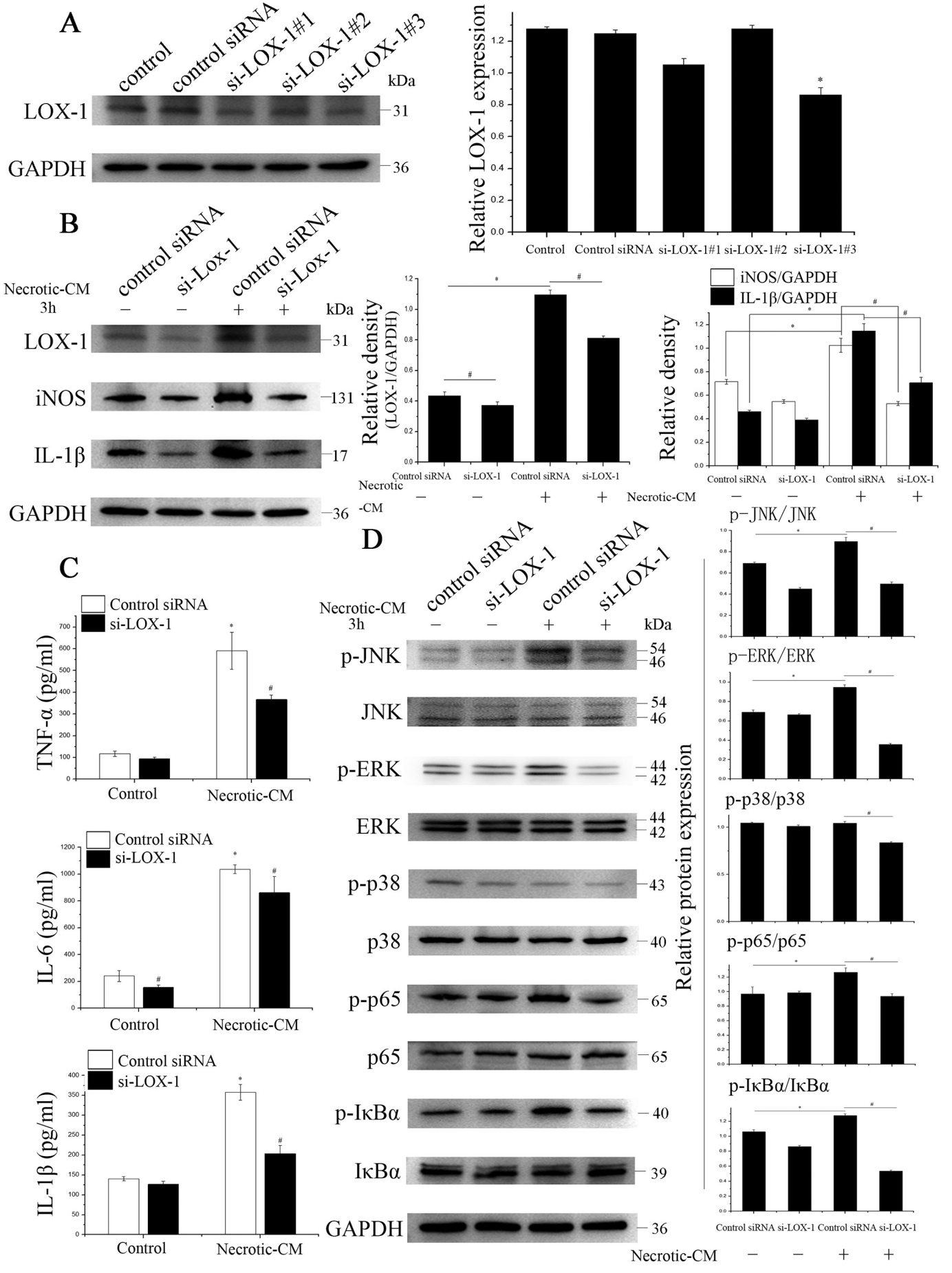


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**Fig. 5.** LOX-1 knockdown attenuated the Necrotic-CM-induced MAPK/NF-κB activation and pro-inflammatory factors expression in microglia.
We knocked down LOX-1 expression in BV2 cells by RNA interference (RNAi), and the interference efficiency of LOX-1 siRNA was detected by Western blot (A). Compared with the control siRNA group, the expression levels of LOX-1, iNOS and IL-1β in si-LOX-1 group were significantly decreased after Necrotic-CM treatment for 3 h (B). The content of pro-inflammatory cytokines IL-6, IL-1β and TNF-α were detected in BV2 after interfering LOX-1 by ELISA (C). BV2 cells were transfected with LOX-1 interfered plasmid for 48 h prior to treatment with Necrotic-CM for 3 h. Protein levels of MAPK (p-JNK, JNK, p-ERK, ERK, p-p38, p38) and NF-κB (p-p65, p65, p-IκBα, IκBα) were evaluated by western blot analysis (D). The data are means ± SD ($n$ = 3). *P < 0.05 compared with the CM-untreated group. $^{\#}$P < 0.05 compared with the control siRNA group.

microscope (Germany).

### 2.8. Nuclear and cytoplasmic extraction experiment

BV2 cells were collected after the indicated treatment, and the subcellular fractions were isolated using the NE-PER Nuclear and cytoplasmic extraction reagents (Thermo) according to the manufacturer's instruction. The cytoplasmic and nuclear protein extracts were detected for Immunoblotting.

### 2.9. Enzyme-linked immunosorbent assay (ELISA)

The serum of different treated mice and the supernatants of BV2 cells were collected for measuring TNF-α, IL-6 and IL-1β by ELISA. An instrument (BioTek, Synergy2, USA) was applied to examine optical density (OD) values in samples at a wavelength of 450 nm after measured a standard curve drawn. Each step was conducted as the manufacturers' protocols of ELISA Kit (Proteintech or Excell Biotech, Shanghai, China).

### 2.10. Detection of cell viability by cell counting kit-8 (CCK-8)

Cells were seeded into a 96-well plate at 100 μl/well. After the stimulation treatment in appropriate time, 10 μl CCK-8 (MCE, China) solution was added to each well and incubated for 4 h, The absorbance was measured at 450 nm using a plate reader.

$$\text{Cell viability} = [(\text{As} - \text{Ab}) \ / \ (\text{Ac} - \text{Ab})] \ \times \ 100\%.$$

As: absorbance of the experimental well.
Ac: absorbance of the control well.
Ab: absorbance of the blank well.

### 2.11. Antibodies

The antibodies used in this study for immunoblot were as follows:
Rabbit polyclonal anti-LOX-1 (1:1000) and Mouse monoclonal anti-iNOS (1:1000) were purchased from Abcam (USA). Mouse polyclonal anti-GAPDH (1:5000) and Mouse polyclonal anti-β-actin (1:5000) were purchased from Proteintech (USA). Rabbit monoclonal anti-NF-κB-p65 (1:1000), Rabbit monoclonal anti-Phospho-NF-κB-p65 (Ser536) (1:1000), Rabbit monoclonal anti-IκBα (1:1000), Rabbit monoclonal anti-p38-MAPK (1:1000), Rabbit monoclonal anti-JNK-MAPK (1:1000), Rabbit monoclonal anti-ERK-MAPK (1:1000), Rabbit monoclonal anti-Phospho-p38-MAPK (Thr180/Tyr182) (1:1000), Rabbit monoclonal anti-Phospho-JNK-MAPK (Thr183/Tyr185) (1:1000), Rabbit monoclonal anti-Phospho-ERK-MAPK (Thr202/Tyr204) (1:1000), Rabbit monoclonal anti-Bax (1:1000), Rabbit monoclonal anti-cleaved-PARP (1:1000), and Mouse monoclonal anti-Phospho-IκBα (Ser32/36) (1:1000) were all purchased from Cell Signaling Technology (USA). Rabbit monoclonal anti-IL-1β (1:500) was purchased from Biorbyt (California, USA) and Mouse monoclonal anti-PCNA (1:500) was from Santa Cruz Biotechnology (USA).

### 2.12. Statistical analysis

All statistical analysis was conducted by SPSS software. The differences between groups were analyzed by one-way analysis of variance (ANOVA), and followed by Tukey's post hoc multiple comparison tests. Values are presented as means ± SD, and differences are considered significant at $p < 0.05$. Each experiment consisted of at least three replicates per condition.

## 3. Results

### 3.1. The expression of LOX-1 in mice cerebral cortex was increased after LPS intraperitoneal injection

To explore the possible function of LOX-1 in neuroinflammation, we established a neuroinflammation model in mice by intraperitoneal injection of LPS. RT-PCR revealed the increased mRNA expression of pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) and iNOS in mice cerebral cortex 6 h following LPS administration (Fig. 1A). In contrast, the anti-inflammatory factors (Arg-1, TGF-β and Ym-1) [23,24], increased later than the pro-inflammatory molecules. The expression of Arg-1 and Ym-1 reached the highest level at 1 day, and TGF-β peaked at 3 days (Fig. 1B). Moreover, the elevated release of IL-6, TNF-α in the mice serum was detected by ELISA method, which further confirmed the LPS-induced inflammation (Fig. 1C). Next, we analyzed the expression of LOX-1 in the cerebral cortex of mice by western blot. The basic level of LOX-1 was low in the control group, significantly increased 12 h after LPS administration, kept a high level until 3 days, and gradually decreased thereafter (Fig. 2A). To further determine the distribution of LOX-1 after LPS injection, immunohistochemistry staining was applied. The density of LOX-1 positive cells significantly increased in the mice cerebral cortex 12 h after LPS injection (Fig. 2B). Immunofluorescence double labeling assay confirmed the elevated expression of LOX-1, and presented a clear co-localization of LOX-1 and Iba-1, a microglia marker, in LPS-treated mice brain (Fig. 2C). These results provided the in vivo evidence to support that LOX-1 is associated with microglia activation and neuroinflammation.

### 3.2. Increased LOX-1 expression and MAPK/NF-κB activation in microglia in response to LPS or the conditioned medium of necrotic neurons (Necrotic-CM)

To explore the relationship between LOX-1 and microglia activation, we stimulated BV2 microglial cells with 100 ng/ml LPS, to establish a classic in vitro microglia activation model [25]. IL-1β protein expression increased 6 h after LPS stimulation, demonstrating microglial inflammatory activation (Fig. 3A). LOX-1 expression began to increase 6 h after LPS stimulation and peaked at 36 h (Fig. 3B). Besides of exogenous PAMPs (such as LPS), the endogenous danger signals released by damaged neurons can also trigger microglia activation [18,26], and more importantly, some DAMPs (such as HSP60 and HMGB1) were identified as the ligands for LOX-1 [27]. In this study, the conditioned medium collected from the necrotic neuronal HT22 cultures (Necrotic-CM) was used as a pool of soluble DAMPs released from necrotic neurons to stimulate BV2. ELISA assay demonstrated that the Necrotic-CM promoted the release of pro-inflammatory factors including TNF-α, IL-6 and IL-1β in BV2 (Fig. 3C). Western blot detected an increased expression of iNOS and IL-1β in Necrotic-CM treated BV2 cells, further supporting the microglial activation model (Fig. 3D). The expression of LOX-1 protein increased 30 min after Necrotic-CM treatment in BV2 and reached the peak at 1 h, and kept a high level until

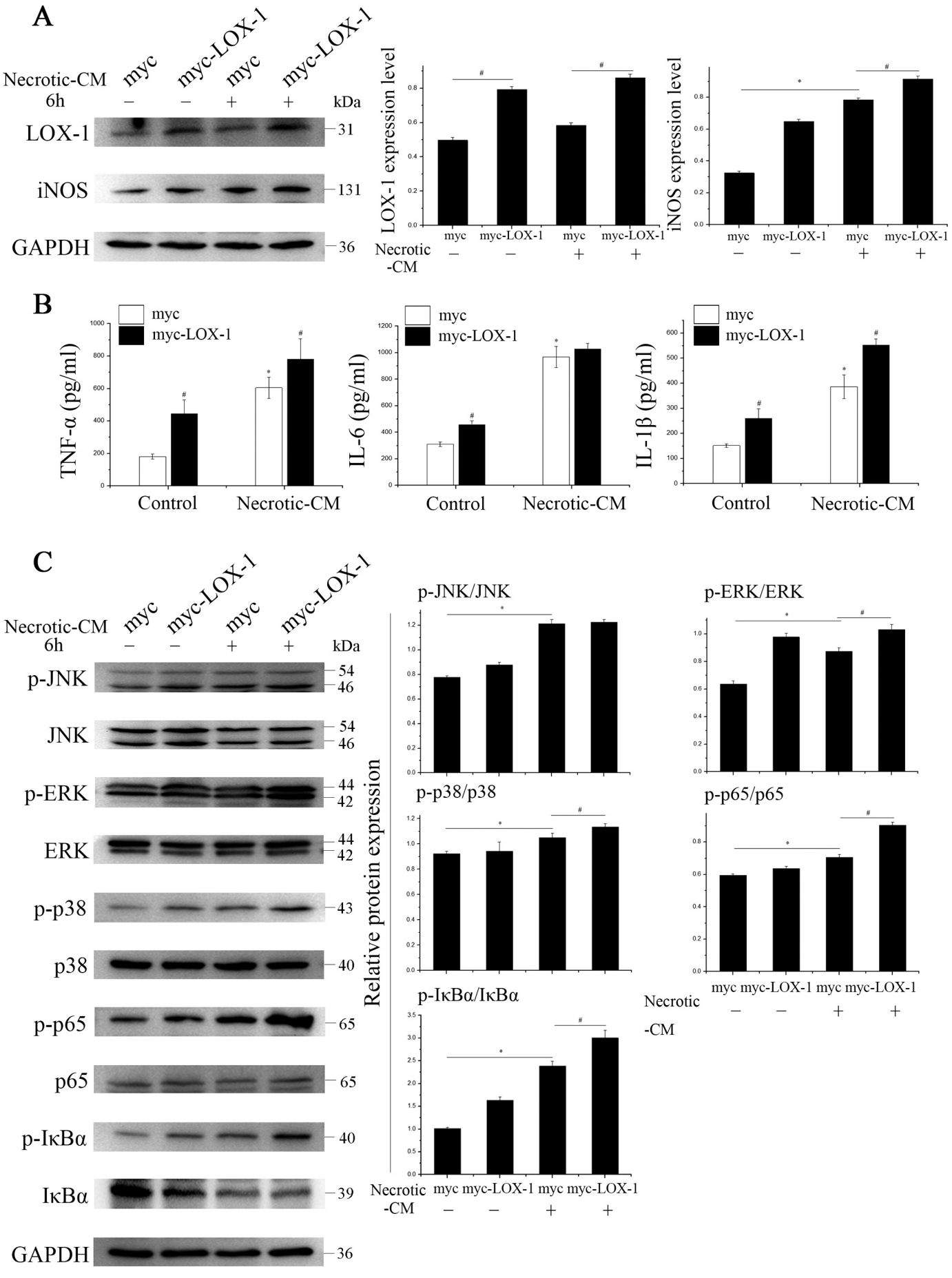


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**Fig. 6.** LOX-1 overexpression promoted the Necrotic-CM-induced microglial inflammatory activation via MAPK/NF-κB signaling pathway.
BV2 cells were transfected with the myc-LOX-1 plasmid, cultured for 48 h, and followed by the Necrotic-CM stimulation for 6 h. The protein expression of LOX-1 and iNOS were detected by western blot (A). The release of pro-inflammatory factors IL-6, IL-1β and TNF-α were detected in Necrotic-CM treated BV2 which were transfected with either myc-LOX-1 plasmid or the blank vector (myc) by ELISA (B). Representative images of western blot for the expression and phosphorylation (p-) of JNK, ERK1/2, p38, p65 and IκBα in BV2 cells (C). The data are means ± SD ($n$ = 3). *P < 0.05 compared with the CM-untreated group. #P < 0.05 compared with the blank vector (myc) group.

12 h (Fig. 3E). The MAPKs/NF-κB signaling pathway has been reported to regulate LOX-1 mediated activation of endothelial cells [28,29]. In the present study, the phosphorylation of three MAPKs (p-JNK, p-p38 and p-ERK) was induced 30 min after Necrotic-CM stimulation in BV2 (Fig. 3E). The peak of phosphorylated IκBα (p-IκBα) level appeared at 3 h following Necrotic-CM treatment, while p-p65 level began to increase at 30 min, and reached the maximum at 12 h (Fig. 3E). These data suggested collectively that necrotic neurons can stimulate MAPKs/NF-κB activation and LOX-1 expression in microglia likely by releasing the endogenous DAMPs.

### 3.3. Microglial LOX-1 expression was positively regulated by MAPK signaling in response to Necrotic-CM

Previous studies have reported a positive regulation of MAPKs on LOX-1 expression in innate immune cells when exposed to extracellular stimuli, such as LPS or TNF-α [27]. However, the regulatory mechanisms of LOX-1 expression in microglia remain unclear. In this study, we employed the specific inhibitors, SP600125, SCH772984, and SB203580, to block the activation of JNK, ERK or p38 in BV2 respectively. Western blot confirmed the inhibition efficiency (Fig. 4A–C). SP600125 significantly inhibited the Necrotic-CM-stimulated expression of LOX-1 in microglia when compared to the control group. Similar results were obtained by the other two inhibitors. These results indicated that microglial LOX-1 expression is positively regulated by MAPK signaling pathway after Necrotic-CM stimulation.

### 3.4. LOX-1 knockdown attenuated the Necrotic-CM-induced MAPK/NF-κB activation and pro-inflammatory factors expression in microglia

Once recognizing its ligands, LOX-1 promotes the release of inflammatory factors in macrophages, and exaggerates vascular inflammation [8]. To further determine the function and downstream signaling pathways of LOX-1 in microglia activation, we knocked down LOX-1 expression in BV2 by RNA interference (RNAi). The interference efficiency of LOX-1 siRNA was detected by Western blot, and si-LOX-1#3 exerted a significant inhibiting effect compared to the control siRNA (Fig. 5A). Western blot indicated that the Necrotic-CM induced expression of iNOS and IL-1β were significantly reversed by interfering LOX-1 (si-LOX-1) (Fig. 5B). Consistently, ELISA discovered that the Necrotic-CM-induced release of pro-inflammatory factors TNF-α, IL-6 and IL-1β were largely decreased after interfering LOX-1(Fig. 5C). Previous studies reported the activation of MAPK/NF-κB pathways after LOX-1 recognizing its ligands in macrophages and cardiomyocytes. Here, western blot demonstrated that silencing LOX-1 significantly attenuated the expression of p-JNK, p-ERK, p-p65 and p-IκBα in BV2 cells 3 h following Necrotic-CM stimulation (Fig. 5D), indicating a potential LOX-1/MAPKs/NF-κB axis in the necrotic neurons-activated microglia.

### 3.5. LOX-1 overexpression promoted the Necrotic-CM-induced microglial inflammatory activation via MAPK/NF-κB signaling pathway

Next, we overexpressed LOX-1 in BV2 by transiently transfection of myc-LOX-1 plasmid, and the overexpression efficiency was confirmed by Western blot. LOX-1 overexpression significantly promoted the iNOS expression (Fig. 6A), and elevated the release of pro-inflammatory factors (IL-1β and TNF-α) in Necrotic-CM treated BV2 (Fig. 6B). Although LOX-1 overexpression slightly increased IL-6 expression in BV2 without stimulation, no significant difference of the IL-6 levels was observed between the LOX-1-overexpression cells and the control group 6 h following Necrotic-CM treatment (Fig. 6B). Compared to the blank vector group, LOX-1 overexpression significantly increased the phosphorylation of ERK, p38, p65 and IκBα in BV2 cells 6 h after Necrotic-CM stimulation (Fig. 6C). These data suggested that LOX-1 promoted Necrotic-CM-induced microglial activation via the MAPK/NF-κB signaling pathway.

### 3.6. LOX-1 promotes NF-κB p65 nuclear transportation in microglia

Following extracellular stimulation, MAPKs reportedly mediate the phosphorylation and nuclear transportation of NF-κB, to promote the target gene transcription [30]. In this study, utilizing immunofluorescence assay, we detected a clear nuclear transportation of NF-κB p65 subunit in BV2 after Necrotic-CM treatment (Fig. 7A). Interestingly, LOX-1 overexpression (myc-LOX-1) significantly enhanced the entry of p65 into the nucleus in Necrotic-CM-treated BV2 when compared to the control group (Fig. 7A). Next, we separated the subcellular nuclear and cytoplasm fractions, and examined the subcellular p65 protein levels by Western blot. In consistence with the immunofluorescent result, we found Necrotic-CM increased p65 nuclear transportation in BV2 cells, which was further promoted by LOX-1 overexpression (Fig. 7B). Accordingly, inhibiting LOX-1 expression by siRNA significantly decreased the Necrotic-CM-induced p65 nuclear transportation (Fig. 7C). Altogether, we deduced LOX-1 promotes p65 nuclear transportation in microglia in response to the soluble neuronal injury signals.

### 3.7. Knocking down microglial LOX-1 attenuated the vicious cycle of neuroinflammation and neuron injury

DAMPs released from injured neurons can mediate inflammatory microglia activation [16]. Great amount of pro-inflammatory factors released by excessively activated microglia can promote neuronal apoptosis, thereby to form a vicious cycle of neuroinflammation and neuron injury [31]. To further elucidate the effects of LOX-1 on this vicious cycle, we treated BV2 (either wild type or LOX-1 knocked down group) with the Necrotic-CM for 3 h, changed to fresh medium and cultured for another 6 h, and then collected the supernatants of activated BV2 cultures (activated MG-CM) to stimulate HT22 neurons for 24 h. CCK-8 results revealed that the conditioned medium from activated BV2 significantly decreased cell viability of HT22 cells, indicating a neuroinflammation-induced neuron injury. In contrast, knocking down microglial LOX-1 significantly suppressed the viability decrease of HT22 cells following activated-MG-CM treatment (Fig. 8A). Moreover, Western blot assay demonstrated that activated-MG-CM increased the expression of cleaved-PARP and Bax, two markers for apoptosis, in HT22 cells, while which was significantly reduced when silencing LOX-1 in microglia (Fig. 8B). All these results showed that LOX-1 knockdown attenuated microglial inflammatory activation and the subsequent neuronal apoptosis.

## 4. Discussion

Neuroinflammation mediated by microglia is involved in the development of many neurological diseases. When deterioration degree of the external environment exceeds the regulation range of the microglia itself, the microglia will be over-activated, presenting a typical M1

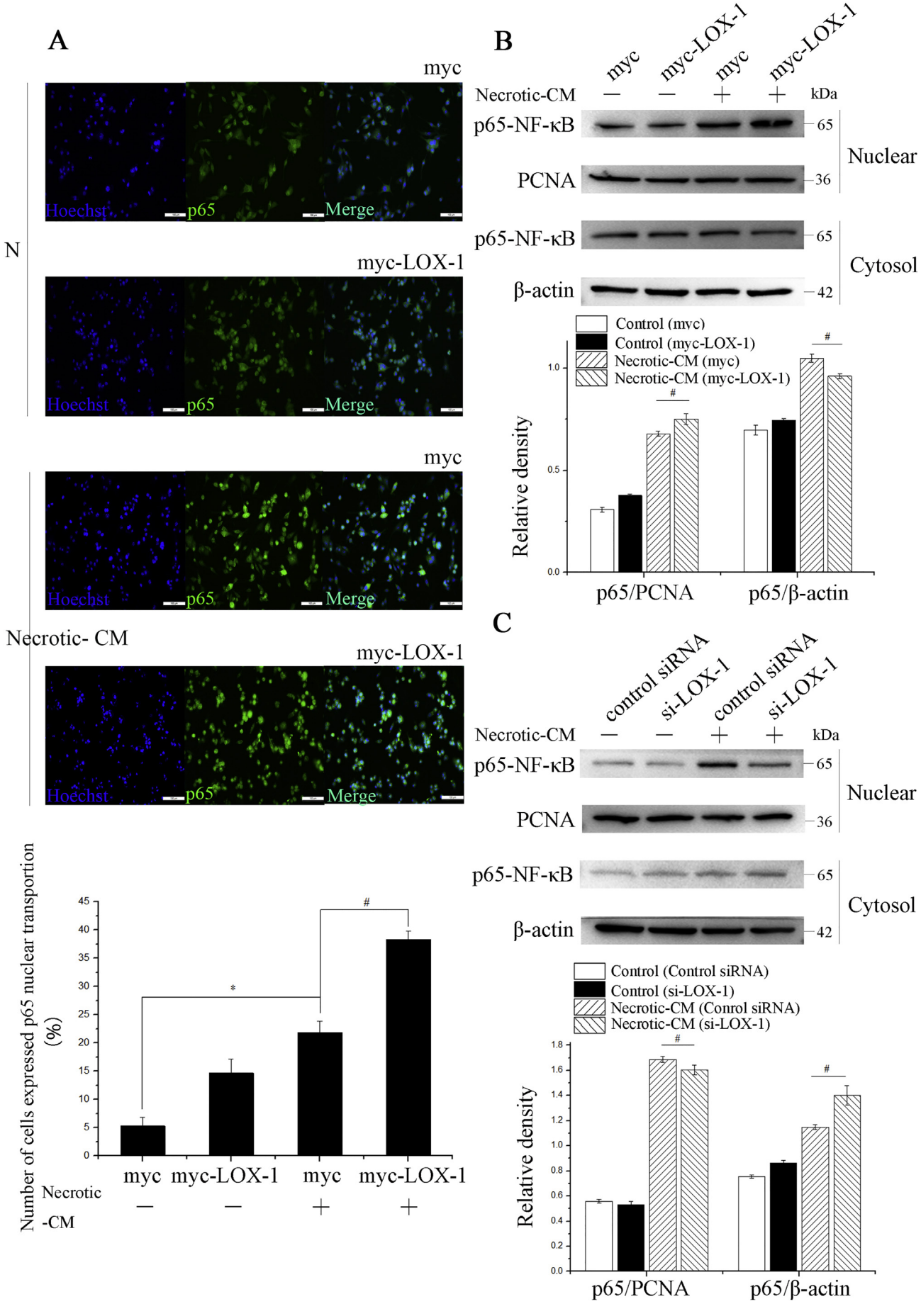


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**Fig. 7.** LOX-1 promoted NF-κB p65 nuclear transportation in microglia.
BV2 cells were transfected with myc-LOX-1 plasmid for 48 h, followed by the Necrotic-CM stimulation for 6 h. The subcellular localization of NF-κB p65 (green) was detected by immunofluorescence staining (A). The protein levels of NF-κB p65 in both cytosol and nuclear were measured by western blot (B). BV2 cells were transfected with the si-LOX-1 plasmid, cultured for 48 h, and then treated with the Necrotic-CM for 3 h. The protein levels of p65 in cytosol and nuclear was detected by western blot (C). The data are means ± SD (n = 3). *P < 0.05 compared with the CM-untreated group. #P < 0.05 compared with the control blank vector group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

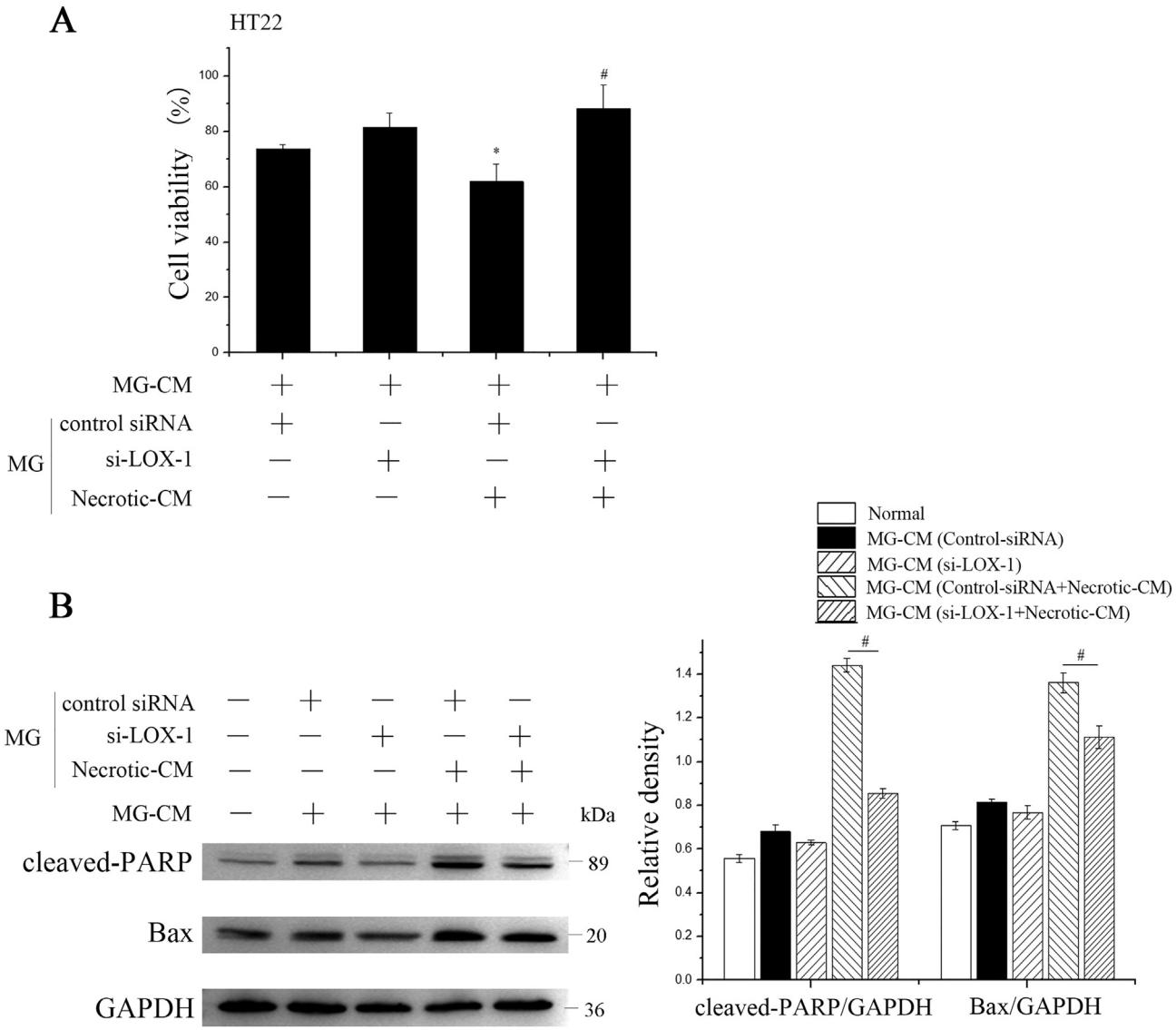


**Fig. 8.** Knocking down microglial LOX-1 attenuated the vicious cycle of neuro-inflammation and neuron injury.
BV2 cells were stimulated with or without the Necrotic-CM for 3 h, changed into fresh medium, and then cultured for another 6 h. Then the supernatants of activated BV2 cultures (MG-CM) were collected, diluted at 1:1 by DMEM medium, and then used to stimulate HT22 neurons for 24 h. CCK-8 was employed to detect the cell viability of MG-CM treated HT22 neurons (A). The protein expression of cleaved-PARP and Bax in HT22 neurons were measured by western blot (B). The data represent the means ± SD (n = 3). *P < 0.05 compared with the control group. #P < 0.05 compared with within groups.

polarization type, thereby promoting neuroinflammation [32]. There are many receptors on the surface of the microglial membrane, playing protective or damage function by binding to their ligands. For example, CD200R on the microglial membrane interacts with CD200 released by neuronal cells to maintain a steady state environment inside the brain [33]. LPS initiates the MAPKs signaling pathway through the TLR4 receptor on the microglial membrane, thereby promoting the release of inflammatory factors. In addition, these inflammatory factors can also damage surrounding neurons to amplify the inflammatory response and aggravate intracerebral damage [34]. In the present study, we first confirmed the increased expression of LOX-1 in activated microglia in the inflamed mice brain (Figs. 1 and 2), and in LPS-activated BV2 cells (Fig. 3). Considering the complexity of the brain micro-environment, we proposed LOX-1 might act as an important receptor to recognize the endogenous danger signals released by damaged neurons to stimulate microglia activation during CNS disease. According to previous study [16], we utilized the conditioned medium of necrotic neurons (Necrotic-CM) as a pool of soluble DAMPs to mimic the endogenous stimulation for microglia. Consistent with our expectation, Necrotic-CM triggered microglial inflammatory activation and significantly increased LOX-1 expression (Fig. 3).

The LOX-1 expression was stimulated by TNF-α in endothelial cells

via ERK signaling pathway. In vascular smooth muscle cells, ox-LDL mediated ROS production and promoted LOX-1 expression via JNK signaling pathway [35,36]. MAPKs signaling pathway is regarded as the major inflammatory pathway for microglia activation, however its contribution to necrotic neurons-induced microglial activation remained unclear. A recent study revealed that necrotic neurons modulated the M1/M2 polarization of macrophages and microglia through the MyD88 pathway [26]. In this study, we found that MAPKs activation in microglia appeared as early as 30 min following Necrotic-CM stimulation, which was much earlier than the maximum expression of LOX-1. Utilizing the specific inhibitors of p38, ERK, and JNK, we confirmed that Necrotic-CM stimulated LOX-1 expression and microglial inflammatory activation through the MAPK signaling pathway (Figs. 3 and 4).

The contribution of LOX-1 in immune cells has been discovered previously. For example, ox-LDL stimulated foam cell formation and macrophage apoptosis by LOX-1 [37]. However, the biological function of LOX-1 in microglial activation and neuroinflammation has not been well understood. In this study, we found that LOX-1 knockdown significantly inhibited the expression of several pro-inflammatory factors in Necrotic-CM-treated microglia (Fig. 5).

Research has shown that the LOX-1/p38 MAPK pathway contributed to the ox-LDL-induced injury in HUVECs [38]. Both JNK and p38 MAPK were involved in the LOX-1 dependent endoplasmic reticulum stress pathway, which resulted in cardiomyocyte apoptosis [39]. In this study, we inhibited LOX-1 expression by siRNA and found a significant decrease of p-JNK, p-ERK, and p-P38 expression in Necrotic-CM treated microglia. In combine with the results of LOX-1 overexpression, we confirmed that LOX-1 probably by recognizing DAMPs facilitates MAPKs signaling transduction and thus promotes microglia activation (Figs. 5 and 6).

NF-κB is an important transcription factor regulating apoptosis and inflammation [40]. The LOX-1 and NF-κB correlation has been observed before. Ox-LDL-induced inflammation in macrophages increased LOX-1 expression via NF-κB signaling pathway [41]. LOX-1 mediated NF-κB activation in endothelial cells stimulated with ox-HDL [42]. In our present results, the supernatants of necrotic neurons triggered the phosphorylation and nuclear importation of NF-κB p65 in microglia (Figs. 3 and 7). LOX-1 knockdown significantly inhibited the Necrotic-CM-induced NF-κB activation, accompanied by the decreased expression of NF-κB target genes (Fig. 7). The above evidence suggested that a LOX-1/MAPKs/NF-κB positive feedback loop participates in the necrotic neurons-induced microglial activation.

Inflammatory factors produced by activation of microglia can damage neurons and exacerbate brain damage to form a vicious cycle of neuroinflammation and neuronal injury [43]. For example, TNF-α stimulates neurons to release large amounts of HMGB1, which then promotes microglia M1 polarization featured with neurotoxic and pro-inflammatory functions [44,45]. Endogenous HSP60 is predominantly expressed in neurons and released during brain injury, which mediates microglial IL-1β production [46,47]. In line with this, we found that inhibition of LOX-1 reduced the release of inflammatory factors from Necrotic-CM-treated microglia, and reduced the subsequent apoptosis in neurons (Fig. 8).

In summary, our present data suggested both LPS, a kind of exogenous PAMPs, and necrotic neurons, probably by releasing endogenous DAMPs, can stimulate LOX-1 expression and microglial activation via the MAPK/NF-κB signaling pathway. Microglial LOX-1 elevates MAPK/NF-κB activation, aggravates microglial inflammatory activation, and amplifies the secondary neuronal apoptosis in response to the soluble danger signal(s) derived from necrotic neurons. Our results implied that a positive LOX-1/MPAKs/NF-κB feedback loop might promote the necrotic neurons induced microglia inflammation, and thus drive the vicious cycle of neuroinflammation and neuronal injury. It is important to note that although our study focused on the pro-inflammatory function of LOX-1, there must be many other important receptors and proinflammatory pathways that trigger the overall hyperinflammatory response in CNS [48]. Further studies are required to provide more direct evidence for the recognition and biological function of LOX-1/DAMPs, and to extensively explore the potential underlying molecular mechanisms of this vicious cycle. The related findings might facilitate to further elucidate the pathological mechanism of neuroinflammation and thus provide potential therapeutic targets for the related neurological diseases.

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## Conflict of interest

The authors declare that there is no conflict of interest.

## References

[1] M.T. Heneka, R.M. McManus, E. Latz, Inflammasome signalling in brain function and neurodegenerative disease, Nat. Rev. Neurosci. 19 (2018) 610–621.
[2] S. Hickman, S. Izzy, P. Sen, L. Morsett, J. El Khoury, Microglia in neurodegeneration, Nat. Neurosci. 21 (2018) 1359–1369.
[3] V. Hernandez-Rabaza, A. Cabrera-Pastor, L. Taoro-Gonzalez, A. Gonzalez-Usano, A. Agusti, T. Balzano, M. Llansola, V. Felipo, Neuroinflammation increases GABAergic tone and impairs cognitive and motor function in hyperammonemia by increasing GAT-3 membrane expression. Reversal by sulforaphane by promoting M2 polarization of microglia, J. Neuroinflammation 13 (2016) 83.
[4] M. Ohta, N. Kido, I. Nakashima, N. Kato, Adjuvant actions of linear mannan-possessing lipopolysaccharide (LPS) in LPS-resistant C3H/HeJ mice, Immunology 56 (1985) 571–573.
[5] S.M. Schindler, M.G. Frank, J.L. Annis, S.F. Maier, A. Klegeris, Pattern recognition receptors mediate pro-inflammatory effects of extracellular mitochondrial transcription factor a (TFAM), Mol. Cell. Neurosci. 89 (2018) 71–79.
[6] E.E. Beier, M. Neal, G. Alam, M. Edler, L.J. Wu, J.R. Richardson, Alternative microglial activation is associated with cessation of progressive dopamine neuron loss in mice systemically administered lipopolysaccharide, Neurobiol. Dis. 108 (2017) 115–127.
[7] J. Liu, LOX-1 and neurodegeneration, Neurosci. Lett. 580 (2014) 179–181.
[8] N.V.K. Pothineni, S.K. Karathanasis, Z. Ding, A. Arulandu, K.I. Varughese, J.L. Mehta, LOX-1 in atherosclerosis and myocardial ischemia: biology, genetics, and modulation, J. Am. Coll. Cardiol. 69 (2017) 2759–2768.
[9] J. Xie, H. Zhu, L. Guo, Y. Ruan, L. Wang, L. Sun, L. Zhou, W. Wu, X. Yun, A. Shen, J. Gu, Lectin-like oxidized low-density lipoprotein receptor-1 delivers heat shock protein 60-fused antigen into the MHC class I presentation pathway, J. Immunol. 185 (2010) 2306–2313.
[10] S. Balzan, V. Lubrano, LOX-1 receptor: a potential link in atherosclerosis and cancer, Life Sci. 198 (2018) 79–86.
[11] Y. Xu, S. Ruan, H. Xie, J. Lin, Role of LOX-1 in Ang II-induced oxidative functional damage in renal tubular epithelial cells, Int. J. Mol. Med. 26 (2010) 679–690.
[12] K.L. Tsai, L.H. Chen, S.H. Chiou, G.Y. Chiou, Y.C. Chen, H.Y. Chou, L.K. Chen, H.Y. Chen, T.H. Chiu, C.S. Tsai, H.C. Ou, C.L. Kao, Coenzyme Q10 suppresses oxLDL-induced endothelial oxidative injuries by the modulation of LOX-1-mediated ROS generation via the AMPK/PKC/NADPH oxidase signaling pathway, Mol. Nutr. Food Res. 55 (Suppl. 2) (2011) S227–S240.
[13] C. Yokota, T. Sawamura, M. Watanabe, Y. Kokubo, Y. Fujita, A. Kakino, M. Nakai, K. Toyoda, Y. Miyamoto, K. Minematsu, High levels of soluble lectin-like oxidized low-density lipoprotein Receptor-1 in acute stroke: an age- and sex-matched cross-sectional study, J. Atheroscler. Thromb. 23 (2016) 1222–1226.
[14] N. Inoue, T. Okamura, Y. Kokubo, Y. Fujita, Y. Sato, M. Nakanishi, K. Yanagida, A. Kakino, S. Iwamoto, M. Watanabe, S. Ogura, K. Otsui, H. Matsuda, K. Uchida, R. Yoshimoto, T. Sawamura, LOX index, a novel predictive biochemical marker for coronary heart disease and stroke, Clin. Chem. 56 (2010) 550–558.
[15] Y. Li, Z. Duan, D. Gao, S. Huang, H. Yuan, X. Niu, The new role of LOX-1 in hypertension induced neuronal apoptosis, Biochem. Biophys. Res. Commun. 425 (2012) 735–740.
[16] D. Zhang, L. Sun, H. Zhu, L. Wang, W. Wu, J. Xie, J. Gu, Microglial LOX-1 reacts with extracellular HSP60 to bridge neuroinflammation and neurotoxicity, Neurochem. Int. 61 (2012) 1021–1035.
[17] C. Venegas, M.T. Heneka, Danger-associated molecular patterns in Alzheimer's disease, J. Leukoc. Biol. 101 (2017) 87–98.

[18] T.F. Pais, C. Figueiredo, R. Peixoto, M.H. Braz, S. Chatterjee, Necrotic neurons enhance microglial neurotoxicity through induction of glutaminase by a MyD88-dependent pathway, J. Neuroinflammation 5 (2008) 43.
[19] A. Salazar, B.L. Gonzalez-Rivera, L. Redus, J.M. Parrott, J.C. O'Connor, Indoleamine 2,3-dioxygenase mediates anhedonia and anxiety-like behaviors caused by peripheral lipopolysaccharide immune challenge, Horm. Behav. 62 (2012) 202–209.
[20] Y. Zhang, X. Liu, H. Xue, X. Liu, A. Dai, Y. Song, K. Ke, M. Cao, Upregulation of PRDM5 is associated with astrocyte proliferation and neuronal apoptosis caused by lipopolysaccharide, J. Mol. Neurosci. 59 (2016) 146–157.
[21] H. Hara, D. Kimoto, M. Kajita, C. Takada, T. Kamiya, T. Adachi, Apomorphine prevents LPS-induced IL-23 p19 mRNA expression via inhibition of JNK and ATF4 in HAPI cells, Eur. J. Pharmacol. 795 (2017) 108–114.
[22] A.K. Verma, S. Ghosh, S. Pradhan, A. Basu, Microglial activation induces neuronal death in Chandipura virus infection, Sci. Rep. 6 (2016) 22544.
[23] R. Orihuela, C.A. McPherson, G.J. Harry, Microglial M1/M2 polarization and metabolic states, Br. J. Pharmacol. 173 (2016) 649–665.
[24] A. Islam, M.E. Choudhury, Y. Kigami, R. Utsunomiya, S. Matsumoto, H. Watanabe, Y. Kumon, T. Kunieda, H. Yano, J. Tanaka, Sustained anti-inflammatory effects of TGF-beta1 on microglia/macrophages, Biochim. Biophys. Acta Mol. basis Dis. 1864 (2018) 721–734.
[25] Y. Zheng, W. Fang, S. Fan, W. Liao, Y. Xiong, S. Liao, Y. Li, S. Xiao, J. Liu, Neurotropin inhibits neuroinflammation via suppressing NF-kappaB and MAPKs signaling pathways in lipopolysaccharide-stimulated BV2 cells, J. Pharmacol. Sci. 136 (2018) 242–248.
[26] J. Yang, Y. Zhao, L. Zhang, H. Fan, C. Qi, K. Zhang, X. Liu, L. Fei, S. Chen, M. Wang, F. Kuang, Y. Wang, S. Wu, RIPK3/MLKL-mediated neuronal necroptosis modulates the M1/M2 polarization of microglia/macrophages in the ischemic cortex, Cereb. Cortex 28 (2018) 2622–2635.
[27] S. Xu, S. Ogura, J. Chen, P.J. Little, J. Moss, P. Liu, LOX-1 in atherosclerosis: biological functions and pharmacological modifiers, Cell. Mol. Life Sci. 70 (2013) 2859–2872.
[28] Y.D. Zhou, X.Q. Cao, Z.H. Liu, Y.J. Cao, C.F. Liu, Y.L. Zhang, Y. Xie, Rapamycin inhibits oxidized low density lipoprotein uptake in human umbilical vein endothelial cells via mTOR/NF-kappaB/LOX-1 pathway, PLoS One 11 (2016) e0146777.
[29] A. Dandapat, C. Hu, L. Sun, J.L. Mehta, Small concentrations of oxLDL induce capillary tube formation from endothelial cells via LOX-1-dependent redox-sensitive pathway, Arterioscler. Thromb. Vasc. Biol. 27 (2007) 2435–2442.
[30] C.H. Zhou, X. Li, Y.Z. Zhu, H. Huang, J. Li, L. Liu, Q. Hu, T.F. Ma, Y. Shao, Y.Q. Wu, Ghrelin alleviates neuropathic pain through GHSR-1a-mediated suppression of the p38 MAPK/NF-kappaB pathway in a rat chronic constriction injury model, Reg. Anesth. Pain Med. 39 (2014) 137–148.
[31] N. Yan, Y. Liu, S. Liu, S. Cao, F. Wang, Z. Wang, S. Xi, Fluoride-induced neuron apoptosis and expressions of inflammatory factors by activating microglia in rat brain, Mol. Neurobiol. 53 (2016) 4449–4460.
[32] Y. Tang, Editorial: microglial polarization in the pathogenesis and therapeutics of neurodegenerative diseases, Front. Aging Neurosci. 10 (2018) 154.
[33] M. Hernangomez, L. Mestre, F.G. Correa, F. Loria, M. Mecha, P.M. Inigo, F. Docagne, R.O. Williams, J. Borrell, C. Guaza, CD200-CD200R1 interaction contributes to neuroprotective effects of anandamide on experimentally induced inflammation, Glia 60 (2012) 1437–1450.
[34] B. Xiang, C. Xiao, T. Shen, X. Li, Anti-inflammatory effects of anisalcohol on lipopolysaccharide-stimulated BV2 microglia via selective modulation of microglia polarization and down-regulation of NF-kappaB p65 and JNK activation, Mol. Immunol. 95 (2018) 39–46.
[35] X. Ran, W. Zhao, W. Li, J. Shi, X. Chen, Cryptotanshinone inhibits TNF-alpha-induced LOX-1 expression by suppressing reactive oxygen species (ROS) formation in endothelial cells, Korean J Physiol Pharmacol 20 (2016) 347–355.
[36] Y. Sun, X. Chen, Ox-LDL-induced LOX-1 expression in vascular smooth muscle cells: role of reactive oxygen species, Fundam Clin Pharmacol 25 (2011) 572–579.
[37] H. Zhang, Q. Liu, J.L. Lin, Y. Wang, R.X. Zhang, J.B. Hou, B. Yu, Recombinant human Thioredoxin-1 protects macrophages from oxidized low-density lipoprotein-induced foam cell formation and cell apoptosis, Biomol Ther (Seoul) 26 (2018) 121–129.
[38] L. Zhang, Y.H. Jia, X.S. Zhao, F.H. Zhou, Y.Y. Pan, Q. Wan, X.B. Cui, X.G. Sun, Y.Y. Chen, Y. Zhang, S.B. Cheng, Trichosanatine alleviates oxidized low-density lipoprotein induced endothelial cells injury via inhibiting the LOX-1/p38 MAPK pathway, Am. J. Transl. Res. 8 (2016) 5455–5464.
[39] T. Liu, Y. Zhou, Y.C. Liu, J.Y. Wang, Q. Su, Z.L. Tang, L. Li, Coronary microembolization induces cardiomyocyte apoptosis through the LOX-1-dependent endoplasmic reticulum stress pathway involving JNK/P38 MAPK, Can J Cardiol 31 (2015) 1272–1281.
[40] H. Yang, Y. Chen, L. Yu, Y. Xu, Esculentoside a exerts anti-inflammatory activity in microglial cells, Int. Immunopharmacol. 51 (2017) 148–157.
[41] N. Sun, H. Wang, L. Wang, Ghrelin inhibits oxLDL-induced inflammation in RAW264.7 mouse macrophages through down-regulation of LOX-1 expression via NF-kappaB signaling pathway, Cell Mol Biol (Noisy-le-grand) 62 (2016) 57–61.
[42] T. Matsunaga, S. Hokari, I. Koyama, T. Harada, T. Komoda, NF-kappa B activation in endothelial cells treated with oxidized high-density lipoprotein, Biochem. Biophys. Res. Commun. 303 (2003) 313–319.
[43] I.E. Papageorgiou, A. Lewen, L.V. Galow, T. Cesetti, J. Scheffel, T. Regen, U.K. Hanisch, O. Kann, TLR4-activated microglia require IFN-gamma to induce severe neuronal dysfunction and death in situ, Proc. Natl. Acad. Sci. U. S. A. 113 (2016) 212–217.
[44] K.A. Kigerl, W. Lai, L.M. Wallace, H. Yang, P.G. Popovich, High mobility group box-1 (HMGB1) is increased in injured mouse spinal cord and can elicit neurotoxic inflammation, Brain Behav. Immun. 72 (2018) 22–33.
[45] Y. Shi, L. Zhang, J. Teng, W. Miao, HMGB1 mediates microglia activation via the TLR4/NF-kappaB pathway in coriaria lactone induced epilepsy, Mol. Med. Rep. 17 (2018) 5125–5131.
[46] K. Rosenberger, P. Dembny, K. Derkow, O. Engel, C. Kruger, S.A. Wolf, H. Kettenmann, E. Schott, A. Meisel, S. Lehnardt, Intrathecal heat shock protein 60 mediates neurodegeneration and demyelination in the CNS through a TLR4- and MyD88-dependent pathway, Mol. Neurodegener. 10 (2015) 5.
[47] S. Swaroop, A. Mahadevan, S.K. Shankar, Y.K. Adlakha, A. Basu, HSP60 critically regulates endogenous IL-1beta production in activated microglia by stimulating NLRP3 inflammasome pathway, J. Neuroinflammation 15 (2018) 177.
[48] E. Gulke, M. Gelderblom, T. Magnus, Danger signals in stroke and their role on microglia activation after ischemia, Ther. Adv. Neurol. Disord. 11 (2018) 1756286418774254.