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Original Article

Baicalin inhibits LPS-induced inflammation in RAW264.7 cells through miR-181b/HMGB1/TRL4/NF-κB pathway

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Abstract: Purpose: Inflammation out of control may induce many diseases. Baicalin has certain anti-inflammatory effects, but its mechanism of action is not clear. Therefore, this study was designed to explore a potential mechanism of anti-inflammation. Methods: In this study, RAW264.7 cells were induced by 1.0 g/mL lipopolysaccharide (LPS) and then exposed to baicalin at various concentrations (0.1-1.0 μmol/L). Then, we investigated the effect of baicalin in RAW264.7 inflammation models. Results: In this study, 0.1-1.0 μmol/L baicalin, especially baicalin at 1.0 μmol/L, effectively inhibited the expression of inflammatory factors (TNF-α, IL-1β, IL-6, Cox, and iNOS), decreased the activity of High Mobility Group Box 1 (HMGB1)/Toll-like Receptor 4 (TLR4)/nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway, and stimulated miR-181b expression. HMGB1 was proved to be negatively regulated by miR-181b. Here, up-regulation of miR-181b or down-regulation of HMGB1 exerted similar effects as baicalin and down-regulated miR-181b reversed the anti-inflammatory effect of baicalin in RAW264.7 inflammation models. Conclusion: Baicalin can inhibit LPS-induced inflammation in RAW264.7 cells via the miR-181b/HMGB1/TRL4/NF-κB pathway.

Keywords: Murine macrophage RAW264.7, inflammation, baicalin, miR-181b/HMGB1/TRL4/NF-κB pathway

Introduction

Inflammation refers to physiological responses to external stimuli such as infection, injury, or chemical stimulus [1]. It is classified into acute inflammation and chronic inflammation according to its duration. Chronic inflammation lasts longer than acute inflammation and involves immune cells, leading to angiogenesis, tissue fibrosis, and necrosis [2]. The inflammatory response induced by lipopolysaccharide (LPS) is chronic and is closely related to the Toll-like receptor (TLR) family. For example, LPS induces macrophages to secrete factors from the TLRs, thereby activating the expression or abnormal activity of mediators including tumor necrosis factor-α (TNF-α), and ultimately initiating inflammatory responses in endothelial cells [3]. Many studies [4-8] have revealed that macrophages are crucial during inflammation, so regulation of macrophages may relieve inflammation. High-mobility group box 1 (HMGB1) is a protein encoded by the HMGB1 gene, containing the HMG-box domain, which is secreted by immune cells and can bind to TLR4 protein to affect the inflammatory response [9-11].

MiR-181b is a microRNA located on human chromosome 2, which can regulate gene expression via its target binding to mRNA at the post-transcriptional level. A previous study [12] suggests that the up-regulation of miR-181b is related to the endotoxin resistance of RAW264.7 and can suppress excessive immune response induced by LPS. Therefore, miR-181b is essential in the inflammatory response network.

Baicalin is a flavonoid compound extracted from the roots of Scutellaria baicalensis [13]. A growing number of studies have confirmed the anti-inflammatory role of baicalin. The research by Wu et al. [14] found that baicalin alleviated

Table 1. Primer sequences

Gene	Upstream primer (5'-3')	Downstream primer (5'-3')
miR-181b	CCAGCTGGGCTCACTGAACAATGA	CAACTGGTGTCGTGGAGTCGGC
HMGB1	TCAAAGGAGAACATCCTGGCCTGT	CTGCTTGTCATCTGCAGCAGTGTT
U6	CTCGCTTCGGCAGCACA	TGGTGTCGTGGAGTCG
GAPDH	TCAACGACCACTTTGTCAAGCTCA	GCTGGTGGTCCAGGGGTCTTACT

cellular inflammation caused by mycoplasma infection through TLR2/NF-κB (Nuclear factor kappa light chain enhancer of activated B cells). In the study by Ishfaq et al. [15], they also noted that baicalin inhibited inflammation and apoptosis caused by mycoplasma infection through regulating energy metabolism. Ishfaq et al. [16] suggested that baicalin regulated oxidative stress and apoptosis caused by mycoplasma infection through NF-κB-mediated signaling pathways. Another study [17] suggested that baicalin might relieve LPS-induced inflammation in macrophages via the TLR4/NF-κB pathway. But the mechanism underlying the regulation of baicalin on macrophage inflammation is not clear. MiRNAs are important cell regulators, and baicalin may modulate miRNAs to regulate inflammation [18].

Here, we established RAW264.7 cell models (murine macrophages) of inflammation induced by 1.0 g/ml lipopolysaccharide (LPS) and exposed cells to baicalin at various concentrations (0.1-1.0 μmol/L). We examined the effects of different doses of baicalin on inflammation and explored the mechanism of baicalin in RAW264.7 inflammation models, aiming to identify the potential value of baicalin in inflammation mitigation and treatment.

Methods

RAW264.7 cell models of inflammation induced by LPS

We purchased murine macrophages (RAW264.7) from the American Type Culture Collection (ATCC) cell bank and cultured them with RPMI1640 (Cell Biotechnology Saba Arna, Fars, Iran) basal medium supplemented with 10% fetal bovine serum (FBS; Invitrogen, Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA) and antibiotics (1%) until they grew well. Then, we seeded RAW264.7 cells into the 6-well plate and added 1 g/ml LPS (Sigma-Aldrich, Inc., St. Louis, MO, USA) into each well. After 24 hours, we examined whether the RAW264.7 inflammation models were successfully constructed. MiR-181b mimic (5'-AACAUUCAUUGCUGUCGGUGGGU-3')/inhibitor (5'-ACCCACCGACAGCAAUGAAUGUU-3') was used to up/down-regulate the expression of miR-181b, and HMGB1 siRNA to downregulate the expression of HMGB1. The miR-181b mimic/inhibitor and HMGB1 siRNA (5'-GGUUCUGUGUCCUAGGAUAAC-3') were purchased from Shanghai Sangon Biotech.

Exposure of RAW264.7 inflammation models to baicalin

Successful RAW264.7 models of inflammation induced by LPS were exposed to baicalin (Sigma, St. Louis, Missouri, USA) at different concentrations (0.1-1.0 μmol/L). After 24 hours, we tested the inflammatory responses, cell activity, and HMGB1/TRL4/NF-κB pathway activity in RAW264.7 cells.

qPCR assay

We prepared cell suspension, isolated the total RNA with the Trizol reagent (Invitrogen, Carlsbad, CA, USA), and quantified its purity. Then, miR-181b and HMGB1 mRNAs were subjected to reverse transcription and amplification. The primer sequences were designed and synthesized by Sangon Biotech (Shanghai, China). Reverse transcription and qPCR amplification kits were purchased from Solarbio (Beijing, China). All procedures were carried out following the kit manual. After determining the Ct value of samples, we calculated the expression levels with the $2^{-\Delta\Delta t}$ method. The sequences of primers are shown in **Table 1**.

Western blot assay

We prepared cell suspension and lysed cells with the RIPA lysis buffer (Cell Signaling Technology, Inc., Danvers, MA, USA), followed by centrifugation for 20 minutes. We discarded the precipitate and collected the supernatant for testing the protein concentration using the BCA kit (Thermo Fisher, Waltham, MA, USA). The protein was subjected to electrophoresis separation with the sodium dodecyl sulfate-

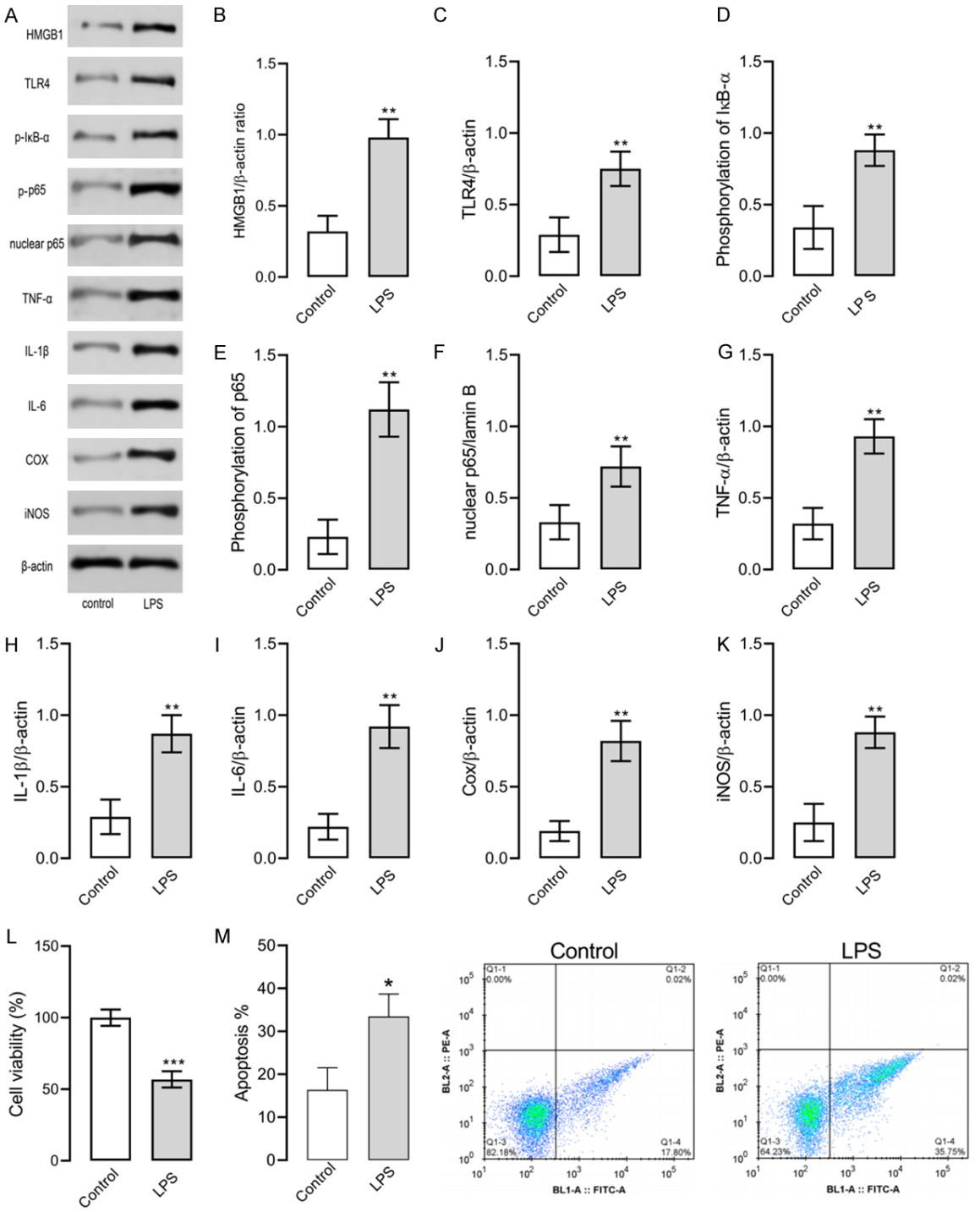
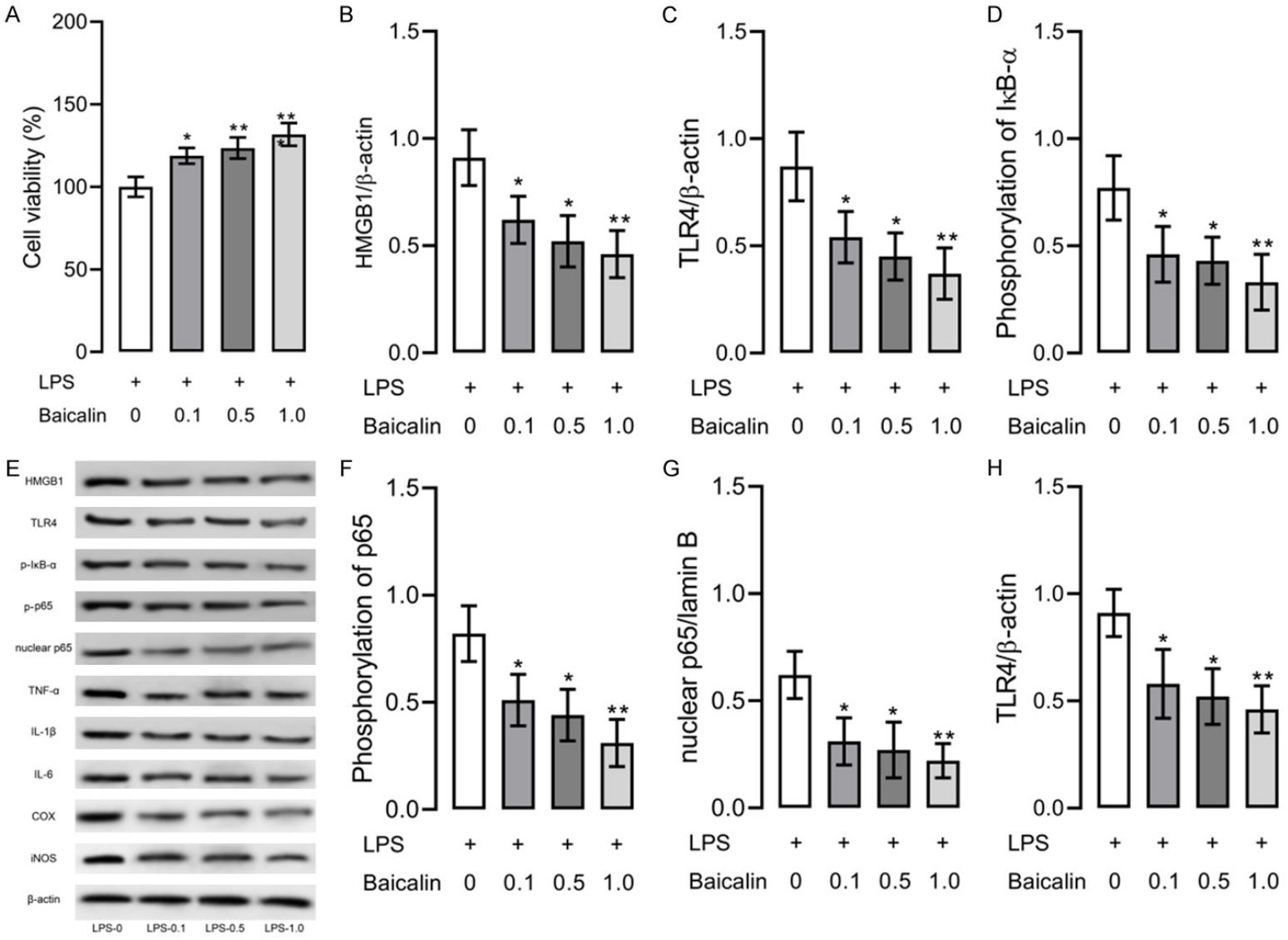


Figure 1. RAW264.7 cell models of inflammation induced by LPS. A. Western blot results, and P-IκB means phosphorylation of IκB and P-p65 means phosphorylation of p65. B. HMGB1 protein level. C. TLR4 protein level. D. IκB-α phosphorylation level. E. NF-κB p65 phosphorylation level. F. Nuclear NF-κB p65 level. G. TNF-α level. H. IL-1β level. I. IL-6 level. J. Cox level. K. iNOS level. L. Cell viability. M. Apoptosis of each group. ** indicates P<0.01 and *** indicates P<0.001 when compared with the control group. The experiment was repeated 3 times (n=3).

polyacrylamide gel electrophoresis (SDS-PAGE) (Solarbio, Beijing, China) and then transferred to a polyvinylidene fluoride membrane (Solarbio, Beijing, China). We mixed the protein to be tested with the primary antibodies and kept them still over 12 h at 4°C. After being washed


A
Cell viability (%)
200
150
100
50
0
LPS + + + +
Baicalin 0 0.1 0.5 1.0
B
HMGB1/β-actin
1.5
1.0
0.5
0.0
C
TLR4/β-actin
D
Phosphorylation of IκB-α
E
HMGB1
TLR4
p-IκB-α
p-p65
nuclear p65
TNF-α
IL-1β
IL-6
COX
iNOS
β-actin
LPS-0 LPS-0.1 LPS-0.5 LPS-1.0
F
Phosphorylation of p65
G
nuclear p65/lamin B
H
TLR4/β-actin

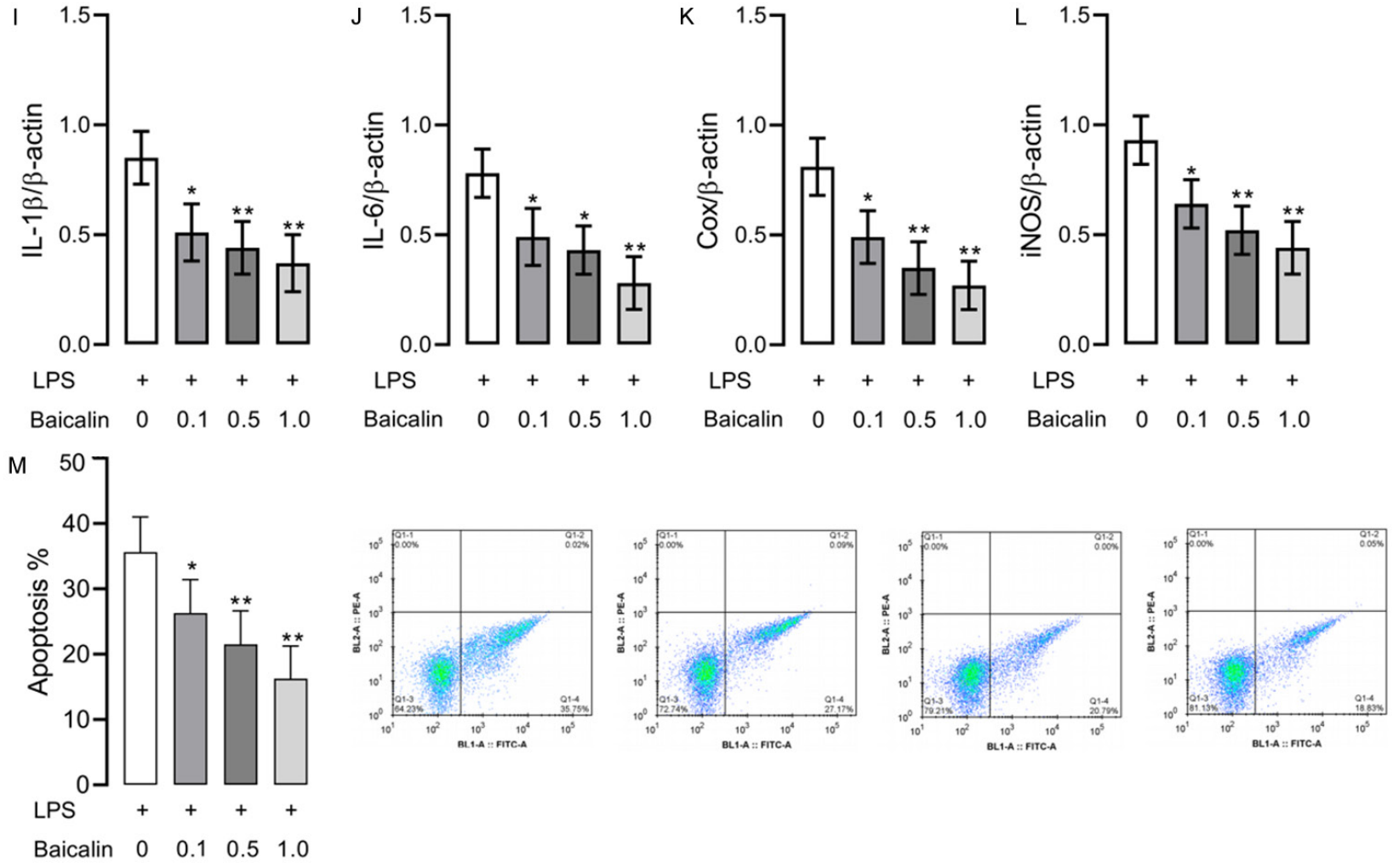


Figure 2. RAW264.7 cell models of LPS-induced inflammation exposed to various baicalin concentrations (0.1-1.0 μmol/L). A. Cell viability. B. HMGB1 protein level. C. TLR4 protein level. D. IκB-α phosphorylation level. E. Western blot results, and P-IκB means phosphorylation of IκB and P-p65 means phosphorylation of p65. F. NF-κB p65 phosphorylation level. G. Nuclear NF-κB p65 level. H. TNF-α level. I. IL-1β level. J. IL-6 level. K. Cox level. L. iNOS level. M. Apoptosis of each group. * indicates P<0.05, ** indicates P<0.01 and *** indicates P<0.001 when compared with the LPS group. The experiment was repeated 3 times (n=3).

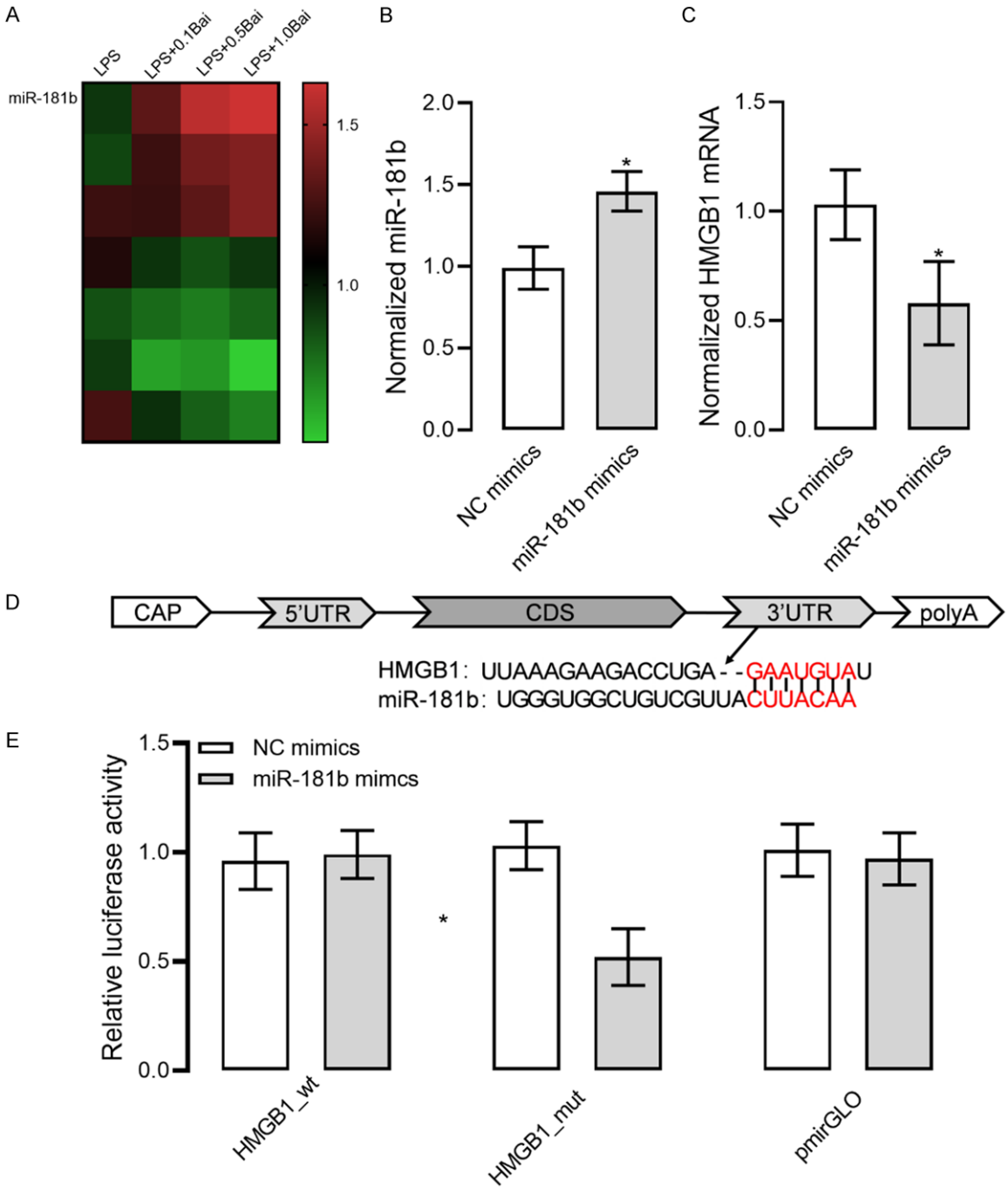


Figure 3. HMGB1 as a target gene of miR-181b. A. Increased miR-181b expression in RAW264.7 cell models of LPS-induced inflammation exposed to baicalin. B. Up-regulated miR-181b expression in RAW264.7 cells. C. Decreased HMGB1 mRNA expression in RAW264.7 cells with up-regulated miR-181b. D. Targetscan7.2 prediction of the targeting relationship between miR-181b and HMGB1 mRNA. E. Results of the dual-luciferase reporter assay. * indicates $P<0.05$ when compared with the NC mimics group. The experiment was repeated 3 times (n=3).

3 times with PBS buffer, the membrane was incubated with goat anti-rabbit secondary antibody for 1 hour. Finally, after PBS washing, the membrane was mixed with the ECL kit. β-actin and LaminB were chosen as internal reference protein controls. The proteins to be tested were acquired from Abcam (Cambridge, MA, USA). The proteins to be tested include HMGB1 (1:500), TLR4 (1:1000), phosphorylation of IκB (1:1000), phosphorylation of p65 (1:1000),

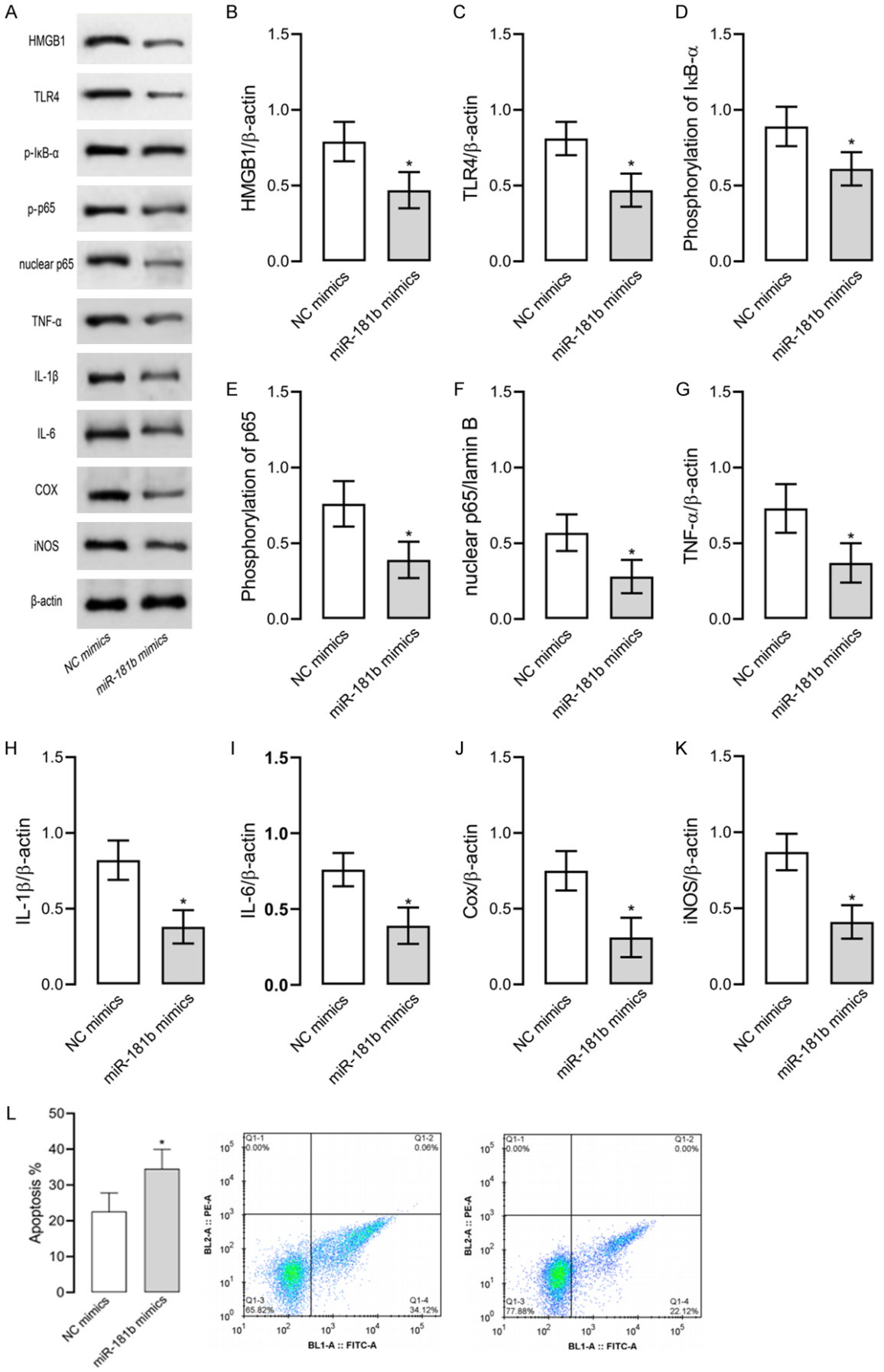

A
HMGB1
TLR4
p-IκB-α
p-p65
nuclear p65
TNF-α
IL-1β
IL-6
COX
iNOS
β-actin
NC mimics
miR-181b mimics
B
HMGB1/β-actin
C
TLR4/β-actin
D
Phosphorylation of IκB-α
E
Phosphorylation of p65
F
nuclear p65/lamin B
G
TNF-α/β-actin
H
IL-1β/β-actin
I
IL-6/β-actin
J
Cox/β-actin
K
iNOS/β-actin
L
Apoptosis %
Q1-1 0.00%
Q1-2 0.06%
Q1-3 65.82%
Q1-4 34.12%
Q1-1 0.00%
Q1-2 0.00%
Q1-3 77.88%
Q1-4 22.12%
BL2-A :: PE-A
BL1-A :: FITC-A

Figure 4. Up-regulated miR-181b in RAW264.7 models of inflammation. Up-regulation of miR-181b leads to decreased levels of HMGB1, TLR4, nuclear NF-κB p65, TNF-α, IL-1β, IL-6, Cox, and iNOS, as well as lowered NF-κB p65 and IκB-α phosphorylation levels. A. Western blot results. P-IκB means phosphorylation of IκB and P-p65 means phosphorylation of p65. B. HMGB1 protein level. C. TLR4 protein level. D. IκB-α phosphorylation level. E. NF-κB p65 phosphorylation level. F. Nuclear NF-κB p65 level. G. TNF-α level. H. IL-1β level. I. IL-6 level. J. Cox level. K. iNOS level. L. Apoptosis of each group. * indicates P<0.05 when compared with the NC mimics group. The experiment was repeated 3 times (n=3).

nuclear p65 (1:1000), TNF-α (1:1000), IL-1β (1:1000), IL-6 (1:1000), COX (1:500) and iNOS (1:1000). All antibodies were purchased from Abcam Company (USA).

MTT assay

We seeded RAW264.7 cells with inflammation into 12 wells of a 96-well plate and equally divided the 12 wells into 4 groups: LPS group, LPS+0.1 μmol/L baicalin group, LPS+0.5 μmol/L baicalin group, and LPS+1.0 μmol/L baicalin group. After 24 hours, we added 10 μL MTT solutions (5 mg/ml) into each well and cultured at 37°C for 4 hours (5% CO_2). Then we removed the medium, added 100 μL dimethyl sulfoxide (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) into each well and shook it well. Finally, we measured the OD value of each well at 570 nm with a microplate reader (Thermo Fisher Scientific).

Dual-luciferase reporter assay

The sequences of miR-181b and HMGB1 mRNAs were analyzed using Targetscan7.2. We constructed wild-type HMGB1 (with miR-181b binding site) and mutant-type HMGB1 (without HMGB1 binding site) using the pmirGLO vector. Then we transfected the vectors separately into cells and tested the luciferase intensity using the dual-luciferase reporter assay system (Promega, Madison, WI, USA). The 293T cell lines were used for dual-luciferase reporter assay. The mutant sequence of HMGB1 (5'-3'): UUAAAGAAGACCUGA-CGGUCUAU.

Apoptosis

The apoptosis of bladder cancer cells was detected with an Annexin V-FITC/PI fluorescence double staining kit (Procell, Wuhan, China) and flow cytometer (BD Biosciences, USA). First, cell suspension was prepared, followed by 5 min centrifugation, and then the supernatant was discarded. Subsequently, 500 μL 1× Annexin V binding buffer, 5 μL Annexin V-FITC and 5 μL propidium iodide were successively added into the solution, followed by 20 min incubation at room temperature in the dark. Finally, the cell apoptosis was analyzed with a flow cytometer and CellQuest software (BD Biosciences).

Statistical analysis

All tests were repeated 3 times. SPSS20.0 software (IBM, Armonk, NY, USA) was used for statistical analysis. The results were expressed as mean ± standard deviation (mean ± SD). The independent sample t-test was used for the comparison of data between any two groups, and one-way ANOVA followed with LSD t-test for the comparison among multiple groups. The confidence interval in this study was 95%. P< 0.05 was regarded as statistically significant.

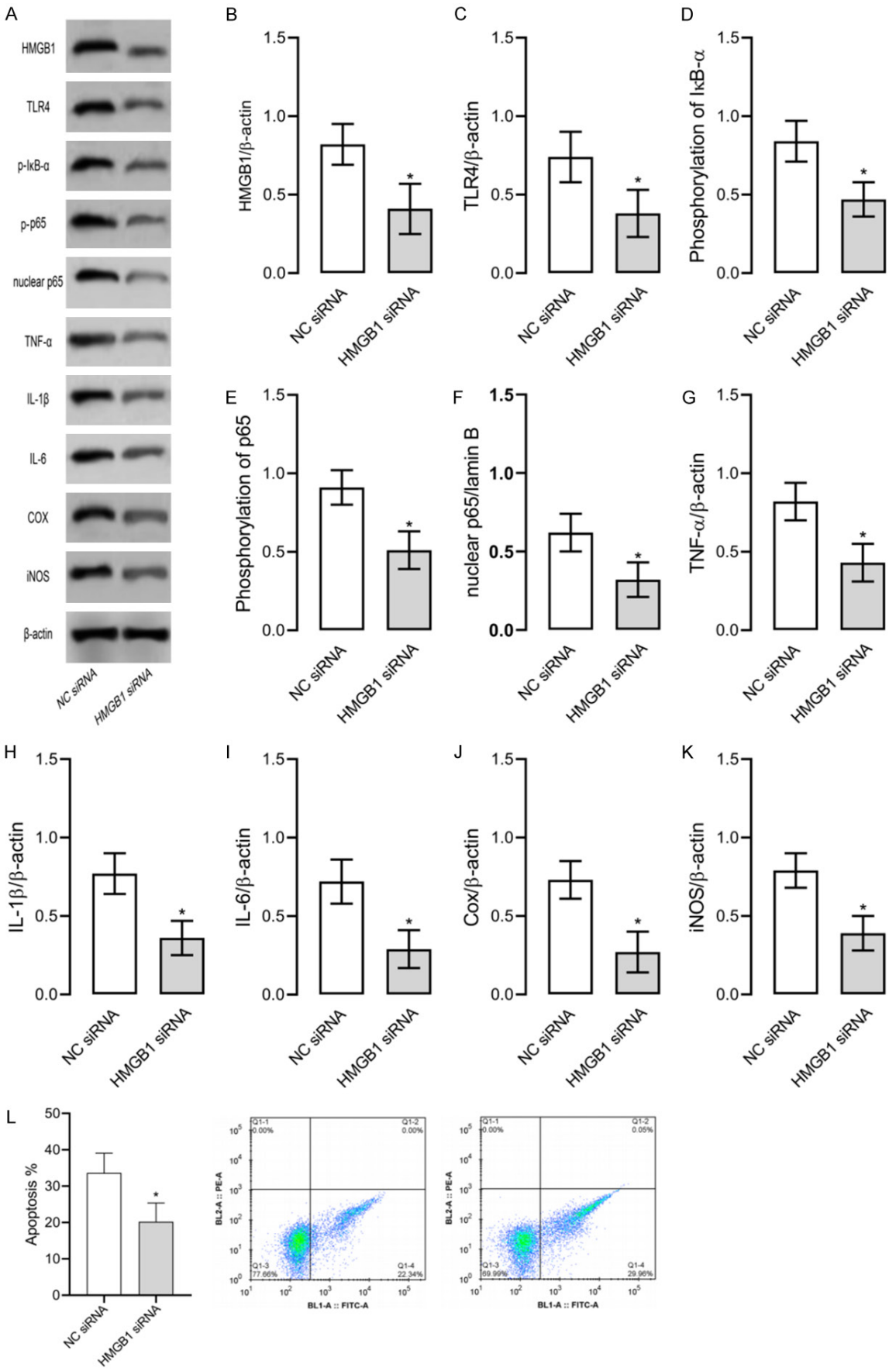
Results

LPS induced inflammation and oxidative stress in RAW264.7 cells

Here we established RAW264.7 cell models of inflammation induced by 1 g/ml LPS. Then we tested cell viability with the MTT assay, and the levels of TNF-α, IL-1β, IL-6, Cox, iNOS and proteins involved in the HMGB1/TLR4/NF-κB pathway were determined with the Western blot assay. **Figure 1** illustrates that LPS treatment decreased cell viability, induced apoptosis, increased concentration of proteins involved in the HMGB1/TLR4/NF-κB pathway, and up-regulated the expression of TNF-α, IL-1β, IL-6, Cox, and iNOS in RAW264.7 cells. Such results indicate that the RAW264.7 cell model of inflammation was successfully constructed.

Baicalin inhibited LPS-induced inflammation and oxidative stress in RAW264.7 cells

Successful RAW264.7 models of inflammation induced by LPS were exposed to baicalin at different concentrations (0.1-1.0 μmol/L). **Figure 2** illustrates that RAW264.7 cells exposed to baicalin garnered higher cell activity, lower concentrations of proteins involved in the HMGB1/


A
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p-p65
nuclear p65
TNF-α
IL-1β
IL-6
COX
iNOS
β-actin
NC siRNA
HMGB1 siRNA
B
HMGB1/β-actin
C
TLR4/β-actin
D
Phosphorylation of IκB-α
E
Phosphorylation of p65
F
nuclear p65/lamin B
G
TNF-α/β-actin
H
IL-1β/β-actin
I
IL-6/β-actin
J
Cox/β-actin
K
iNOS/β-actin
L
Apoptosis %
BL2-A :: PE-A
BL1-A :: FITC-A
Q1-1 0.00%
Q1-2 0.00%
Q1-3 77.66%
Q1-4 22.34%
Q1-1 0.00%
Q1-2 0.05%
Q1-3 69.99%
Q1-4 29.96%

Figure 5. Down-regulated HMGB1 expression in RAW264.7 models of inflammation. Down-regulation of HMGB1 leads to decreased levels of HMGB1, TLR4, nuclear NF-κB p65, TNF-α, IL-1β, IL-6, Cox, and iNOS, as well as lowered NF-κB p65 and IκB-α phosphorylation levels. A. Western blot results. P-IκB means phosphorylation of IκB and P-p65 means phosphorylation of p65. B. HMGB1 protein level. C. TLR4 protein level. D. IκB-α phosphorylation level. E. NF-κB p65 phosphorylation level. F. Nuclear NF-κB p65 level. G. TNF-α level. H. IL-1β level. I. IL-6 level. J. Cox level. K. iNOS level. L. Apoptosis of each group. * indicates $P<0.05$ when compared with the NC mimics group. The experiment was repeated 3 times (n=3).

TLR4/NF-κB pathway, and lower expression of TNF-α, IL-1β, IL-6, Cox, and iNOS, as compared with cells not exposed to baicalin, with baicalin at 1.0 μmol/L showing the strongest inhibition of inflammation and apoptosis in cells. Such results indicate that baicalin can effectively control LPS-induced inflammation in RAW264.7 cells and suppress the activity of the HMGB1/TLR4/NF-κB pathway.

HMGB1 is a target gene of miR-181b

Figure 3A shows that different doses of baicalin can increase miR-181b expression in RAW264.7 cells. **Figure 3B** and **3C** show that up-regulated miR-181b caused down-regulation of HMGB1 mRNA. **Figure 3D** indicates that there are binding sites of miR-181b in the 3' non-transcribed region of HMGB1. **Figure 3E** illustrates that the co-transfection of HMGB1_wt and miR-181b mimics resulted in the decrease in relative luciferase activity, which imply that HMGB1 could bind to miR-181b via predictive site. The above results suggest that HMGB1 is a target gene of miR-181b.

Upregulated miR-181b inhibited inflammation in RAW264.7 cells

Here we discovered that baicalin increased miR-181b expression in cells, so we up-regulated miR-181b expression to determine its effect on LPS-induced RAW264.7 cells and HMGB1/TLR4/NF-κB pathway. As shown in **Figure 4**, up-regulation of miR-181b led to decreased levels of HMGB1, TLR4, nuclear NF-κB p65, TNF-α, IL-1β, IL-6, Cox, and iNOS, as well as lowered levels of phosphorylated NF-κB p65 and IκB-α. Such results indicate that up-regulated miR-181b can inhibit LPS-induced inflammation and apoptosis in RAW264.7 cells and suppress the activity of the HMGB1/TLR4/NF-κB pathway.

Downregulated HMGB1 inhibited inflammation in RAW264.7 cells

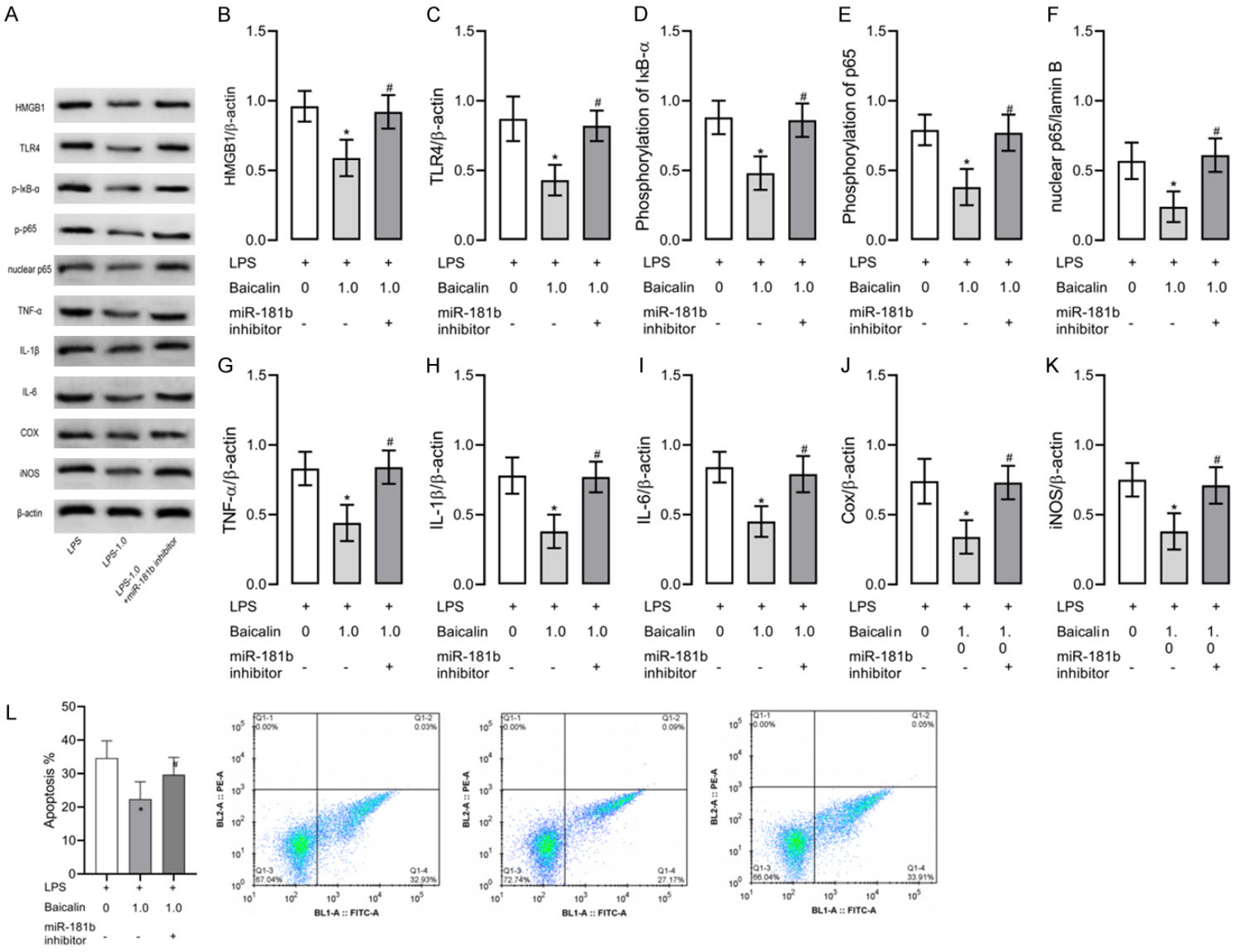
Here we discovered that baicalin decreased HMGB1 expression in cells, so we down-regulated HMGB1 expression to determine its effect on LPS-induced RAW264.7 cells and HMGB1/TLR4/NF-κB pathway. As shown in **Figure 5**, down-regulation of HMGB1 led to decreased levels of HMGB1, TLR4, nuclear NF-κB p65, TNF-α, IL-1β, IL-6, Cox, and iNOS, as well as phosphorylated NF-κB p65 and IκB-α. Such results indicate that down-regulation of HMGB1 can inhibit LPS-induced inflammation and apoptosis in RAW264.7 cells and suppress the activity of the HMGB1/TLR4/NF-κB pathway.

Baicalin inhibited LPS-induced inflammation in RAW264.7 cells via miR-181b/HMGB1/TLR4/NF-κB pathway

Here we exposed LPS-induced RAW264.7 cells to 1.0 μmol/L baicalin and down-regulated miR-181b expression in cells (**Figure 6**). Down-regulation of miR-181b reversed the inhibitory effect of 1.0 μmol/L baicalin on cell inflammation, apoptosis and HMGB1/TLR4/NF-κB pathway. These results indicate that baicalin can inhibit LPS-induced inflammation in RAW264.7 cells through the miR-181b/HMGB1/TLR4/NF-κB pathway (**Figures 7**, **8**).

Discussion

Inflammation may induce tumor growth [19], pain [20], endothelial dysfunction [21], metabolic disorders [22], and other subsequent diseases, endangering the health of the patients. A variety of diseases or external damages will cause inflammation in organisms, along with abnormally expressed genes or proteins. LncRNA NEAT1 can regulate sepsis-induced inflammation through miR-495, miR-211, and miR-125a-2p [23, 24]. MiR-155 is abnormally up-regulated in patients with inflammation caused by kidney injury, and inhibiting miR-155 expression can relieve macrophage infiltration and reduce inflammatory toxicity [25]. Inflammation, if not controlled in time, may lead to life-threatening outcomes. Baicalin has potential anti-inflammatory effects. Here we explored the mechanism of baicalin in inhibit-


A
HMGB1
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p-p65
nuclear p65
TNF-α
IL-1β
IL-6
COX
iNOS
β-actin
LPS
LPS-1.0
LPS-1.0 +miR-181b inhibitor
B
HMGB1/β-actin
C
TLR4/β-actin
D
Phosphorylation of IκB-α
E
Phosphorylation of p65
F
nuclear p65/lamin B
G
TNF-α/β-actin
H
IL-1β/β-actin
I
IL-6/β-actin
J
Cox/β-actin
K
iNOS/β-actin
LPS
Baicalin
miR-181b inhibitor
L
Apoptosis %
BL2-A :: PE-A
BL1-A :: FITC-A

Figure 6. RAW264.7 cell models of inflammation with down-regulated miR-181b expression and exposed to 1.0 μmol/L baicalin. Down-regulation of miR-181b reverses the inhibition on cell inflammation and HMGB1/TLR4/NF-κB pathway by 1.0 μmol/L baicalin. A. Western blot results. P-IκB means phosphorylation of IκB and P-p65 means phosphorylation of p65. B. HMGB1 protein level. C. TLR4 protein level. D. IκB-α phosphorylation level. E. NF-κB p65 phosphorylation level. F. Nuclear NF-κB p65 level. G. TNF-α level. H. IL-1β level. I. IL-6 level. J. Cox level. K. iNOS level. L. Apoptosis of each group. * indicates P<0.05 when compared with the LPS group. # indicates P<0.05 when compared with the LPS+1.0 μmol/L baicalin group. The experiment was repeated 3 times (n=3).

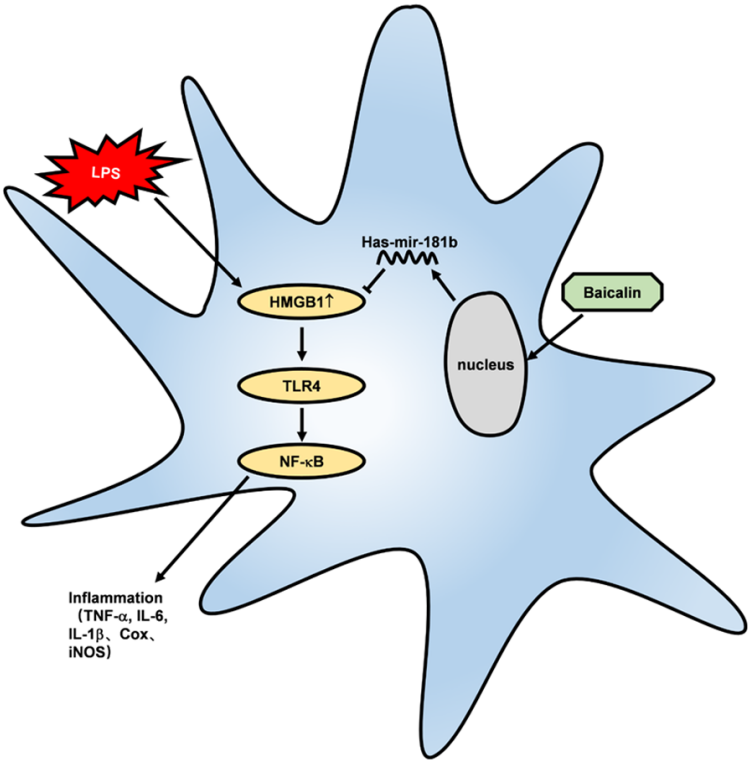

Figure 7. LPS activates the HMGB1/TLR4/NF-κB pathway in RAW264.7 cells and induces the abnormal secretion of inflammatory factors (TNF-α, IL-6, IL-1β, Cox, and iNOS), triggering Inflammation. Baicalin inhibits the expression of HMGB1 by promoting miR-181b, thus inhibiting the HMGB1/TLR4/NF-κB pathway mediated by HMGB1 and relieving inflammation.

ing inflammation, aiming to provide an accurate reference for its application.

In this study, we established RAW264.7 cell models of inflammation induced by LPS and exposed them to various baicalin concentrations (0.1-1.0 μmol/L). During the baicalin exposure, the activity of the HMGB1/TLR4/NF-κB pathway in RAW264.7 cells was inhibited, the levels of downstream factors including TNF-α, IL-6, IL-1β, Cox, and iNOS were decreased, and cell viability was increased. The exposure to baicalin also promoted miR-181b expression in cells. MiR-181b is involved in the occurrence and development of diseases such as prostate cancer bone metastasis [26], liver fibrosis [27], and breast cancer [28]. Hence, miR-181b, which is affected by baicalin, may also play a significant role in inflammation. In this study, HMGB1 was proved to be negatively regulated by miR-181b. Many studies [9-11, 29-31] revealed that HMGB1 was closely related to inflammatory response. When miR-181b expression is affected by baicalin, HMGB1 may be negatively regulated by miR-181b, thereby altering the downstream inflammation pathways. These results suggest that the up-regulated miR-181b may be the effective target of baicalin to inhibit inflammation in RAW264.7 cells.

The results of this study showed that up-regulation of miR-181b or down-regulation of HMGB1 could inhibit LPS-induced inflammation in RAW264.7 cells. In this study, the exposure to baicalin along with the down-regulation of miR-181b at the same time enhanced the activity of HMGB1/HMGB1/TLR4/NF-κB pathway and increased the levels of TNF-α, IL-6, IL-1β, Cox, and iNOS in RAW264.7 cells.

We speculate that LPS activates the HMGB1/TLR4/NF-κB pathway in RAW264.7 cells and induces the abnormal secretion of inflammatory factors (TNF-α, IL-6, IL-1β, Cox, and iNOS), finally triggering inflammation. In this study, baicalin inhibited the expression of HMGB1 by promoting miR-181b, thus inhibiting the HMGB1/TLR4/NF-κB pathway mediated by HMGB1 and relieving inflammation.

This study selected RAW264.7 cells for construction of inflammation model and discovered the possible mechanism of baicalin in the

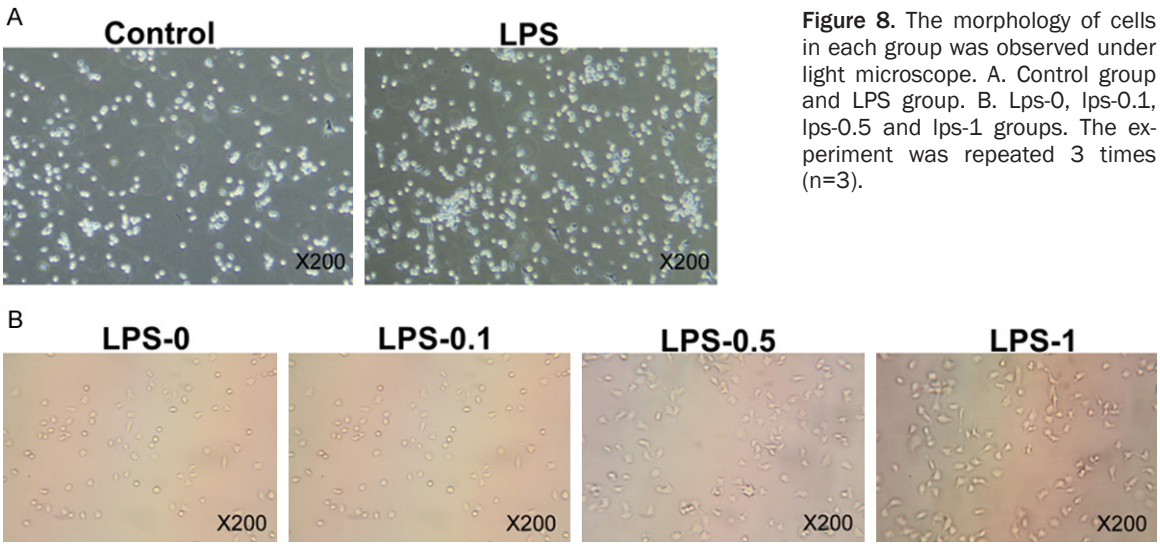

Figure 8. The morphology of cells in each group was observed under light microscope. A. Control group and LPS group. B. Lps-0, lps-0.1, lps-0.5 and lps-1 groups. The experiment was repeated 3 times (n=3).

treatment of inflammation. But there are still some limitations, for example, this study only investigated cell models of inflammation but did not explore the application of baicalin in animal models of inflammation. Besides, this study confirmed that baicalin at 1.0 μmol/L had a good anti-inflammation effect, but it did not identify the optimal concentration of baicalin in treating inflammation. Therefore, we should conduct high-throughput screening in the future to determine the optimal concentration of baicalin. Here, we note that baicalin regulates inflammation through the miR-181b/HMGB1/TLR4/NF-κB pathway, but whether baicalin can inhibit inflammation through other pathways needs further exploration.

Conclusion

In summary, baicalin inhibits LPS-induced inflammation in RAW264.7 cells through the miR-181b/HMGB1/TLR4/NF-κB pathway and its inhibition on inflammation is fairly effective at the concentration of 1.0 μmol/L. Therefore, the effect of baicalin is worthy of further study.

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Disclosure of conflict of interest

None.

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