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ORIGINAL ARTICLE

Baicalin relieves inflammation stimulated by lipopolysaccharide via upregulating TUG1 in liver cells

Yanqiu Huang[1] · Mengyan Sun[2] · Xuefang Yang[2] · Aiyu Ma[1] · Yujie Ma[1] · Aiying Zhao[1]

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Abstract

Hepatitis has become a major social, health, and economic problem worldwide. Herein, we tested the beneficial influence of baicalin, a flavonoid extracted from the roots of *Scutellaria baicalensis*, on human normal liver L-02 and THLE2 cell apoptosis and inflammatory reaction stimulated by lipopolysaccharide (LPS) and possible molecular mechanisms. L-02 and THLE2 cell viability and apoptosis after LPS and/or baicalin treatment were tested using CCK-8 assay and Annexin V-FITC/PI apoptosis kit, respectively. qRT-PCR was used to measure the MCP-1, IL-6, TNF-α, and lncRNA taurine upregulated gene 1 (TUG1) expressions in L-02 and THLE2 cells. sh-TUG1 was transfected to knockdown TUG1. SB203580 was used as inhibitor of p38MAPK pathway, while SP600125 was used as inhibitor of JNK pathway. We discovered that LPS stimulation caused L-02 and THLE2 cell apoptosis and inflammatory reaction. Baicalin relieved the L-02 and THLE2 cell apoptosis and inflammatory reaction stimulated by LPS. Moreover, LPS lowered the TUG1 expression in L-02 cells, while baicalin promoted the TUG1 expression in L-02 and L-02 and THLE2 cells, as well as inactivated p38MAPK and JNK pathways in LPS-stimulated L-02 cells. Besides, knockdown of TUG1 activated p38MAPK and JNK pathways and promoted inflammatory cytokine expression in L-02 cells. In conclusion, this study further affirmed the beneficial influences of baicalin on LPS-stimulated human normal liver cell apoptosis and inflammatory reaction. Baicalin relived liver cell inflammation stimulated by LPS might be via upregulating TUG1 and then inactivating p38MAPK and JNK pathways.

Keywords Hepatitis · Baicalin · Lipopolysaccharide · LncRNA TUG1 · p38MAPK pathway · JNK pathway

Introduction

Hepatitis is collectively referred as inflammation of liver, which is caused by viruses, bacteria or parasite infection, chemical poisons, drug or alcohol ingestion, and influence of autoimmune factors [1, 26, 29]. The main clinical features of hepatitis are loss of appetite, bloating, tired of greasy food, nausea and vomiting, which are accompanied with abnormal expression levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholinesterase (CHE), alkaline phosphatase (ALP), and γ-glutamyl transpeptidase (GGTP) in serum [3, 7, 22]. Although many advances have been made in the treatment of hepatitis in recent years, there are still lots of patients who present insufficient response to therapy [8, 28]. It is crucial to search for other compounds that can alleviate inflammation of liver cells.

Baicalin (CAS number: 21967-41-9, Fig. 1) is a flavonoid found and isolated from the roots of *Scutellaria baicalensis* Georgi, which accounts for approximately 8.1–15.6% of the dry weight of *Scutellaria baicalensis* roots [19, 30]. During the past few years, pharmacological studies have proved that baicalin possesses a satisfying anti-inflammatory activity [9, 38]. In terms of liver cells, He et al. reported that baicalin could relieve liver damage caused by chronic plus binge

Highlights
1. Baicalin relieves L-02 cell apoptosis and inflammatory reaction stimulated by LPS.
2. Baicalin promotes TUG1 expression in L-02 cells.
3. Baicalin mitigates LPS-stimulated activation of p38MAPK and JNK pathways.
4. TUG1 knockdown activates p38MAPK and JNK pathways.
5. Baicalin also relieves LPS-caused THLE2 cell apoptosis and inflammatory reaction.

✉ Aiying Zhao
zhaoaiying0022@sina.com

[1] Department of Infectious Diseases, Heze Municipal Hospital, No2888 Caozhou Road, Heze 274000, Shandong, China

[2] Department of Clinical Laboratory, Heze Municipal Hospital, Heze 274000, China

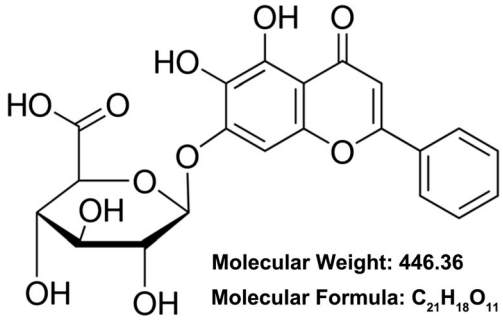

Fig. 1 Chemical skeleton structure of baicalin

ethanol feeding in mice [13]. Zhong and Liu indicated that baicalin could mitigate diet-stimulated nonalcoholic steatohepatitis through suppressing inflammatory reaction [39]. Moreover, Cheng et al. demonstrated that baicalin could ameliorate liver inflammation stimulated by lipopolysaccharide (LPS) in chicken via inhibiting toll-like receptor 4 (TLR-4)-mediated nuclear factor kappa B (NF-κB) activation [6]. More studies are still needed to further explore the beneficial influences of baicalin on hepatitis.

Long non-coding RNAs (lncRNAs) are closely related to the regulation of multiple cell functions [20]. Taurine upregulated gene 1 (TUG1) is a newly discovered lncRNA that exerts critical regulatory functions on cell inflammation reaction [31, 37]. Su et al. indicated that overexpression of TUG1 could protect mouse livers from cold-induced damage via suppressing inflammatory reaction [31]. Liang et al. reported that TUG1 participated in the anti-inflammatory activity of emodin, an anthraquinone derivate isolated from the rhizomes of *Rheum palmatum* L. [16].

In the current research, human normal liver L-02 and THLE2 cells were exposure to LPS stimulation to cause inflammatory damage. Then, we further investigated the anti-inflammatory activity of baicalin on LPS-stimulated L-02 and THLE2 cell apoptosis and inflammatory cytokine expressions, as well as tested the molecular mechanism related to TUG1. The findings of our research will provide experimental evidence for further comprehending the beneficial influences of baicalin on hepatitis.

Materials and methods

Cell culture and treatment

L-02 cells were received from China Center for Type Culture Collection (CCTCC, Wuhan, China) and cultivated in 5% CO_2 at 37 °C in RPMI-1640 medium (R2405, Sigma-Aldrich, CA, USA) containing 10% (v/v) fetal bovine serum (FBS, 3011-8611, Hangzhou Sijiqing Biological Engineering Materials Co, Ltd., Hangzhou, China), 1% (v/v) penicillin-streptomycin-glutamine solution (Gibco, CA, USA), and 2.5 mg/ml sodium bicarbonate ($NaHCO_3$, Sigma-Aldrich). THLE2 cells were supplied by American Type Culture Collection (ATCC, VA, USA) and cultivated in 5% CO_2 at 37 °C in Dulbecco's modified Eagle's medium (DMEM, D0819, Sigma-Aldrich) containing 10% (v/v) FBS and 1% (v/v) penicillin-streptomycin solution.

LPS and baicalin (purity > 95%) were both purchased from Sigma-Aldrich (catalog nos. L2630 and 572667). Ultrapure water was used to dissolve LPS to 5 mg/ml. Dimethyl sulfoxide (DMSO, Sigma-Aldrich) was used to dissolve baicalin to 20 mM. Cells were pre-treated with baicalin for 12 h before LPS stimulation.

p38MAPK specific inhibitor SB203580 and JNK specific inhibitor SP600125 were also obtained from Sigma-Aldrich (catalog nos. S8307 and S5567).

Detection of cell viability

Cell counting kit-8 (CCK-8) assay (HY-K0301, MedChem Express, NJ, USA) was carried out to test the viabilities of L-02 and THLE2 cells. In brief, 1×10^4 L-02 or THLE2 cells were seeded into 96-well plate overnight and treated by different dosages of LPS and/or baicalin for 12 or 24 h. Then, 10 μl kit solution was added into culture medium. Followed by placing at 37 °C for 1 h, the absorbance of each well was recorded using Micro-plate Reader (Molecular Device, CA, USA) at 450 nm. Cell viability (%) was represented as the percentage of control.

Assessment of cell apoptosis

Annexin V-FITC/PE apoptosis kit (C1065, Beyotime Biotechnology, Shanghai, China) and flow cytometer analysis were carried out to assess apoptosis of L-02 and THLE2 cells. In brief, 3×10^4 L-02 or THLE2 cells were seeded into 24-well plate overnight and treated by different dosages of LPS and/or baicalin for 12 or 24 h. Then, cells were harvested in line with experimental group and washed with kit buffer. Subsequently, cells were stained using kit solution at 37 °C for 15 min in the dark. The rates of apoptotic L-02 and THLE2 cells were measured and analyzed using flow cytometer (Attune Nxt, Thermo Fisher Scientific, MA, USA).

shRNA transfection

Short-hairpin RNA directed against TUG1 was constructed into U6/GFP/Neo plasmid (Invitrogen, CA, USA) to form sh-TUR1. Empty U6/GFP/Neo plasmid was used as negative control (sh-NC). sh-TUG1 and sh-NC were transfected into L-02 cells using Lipofectamine 3000 reagent (L3000-008, Invitrogen), respectively. Quantitative reverse transcription PCR (qRT-PCR) was carried out to test transfection efficiency.

qRT-PCR

Total RNAs were isolated from L-02 or THLE2 cells using RNAiso Plus kit (9109, Takara Biomedical Technology, Beijing, China). Then, cDNA was synthesized by PrimeScript cDNA Synthesis kit (6210, Takara Biomedical Technology). The monocyte chemoattractant protein-1 (MCP-1), interleukin-6 (IL-6), tumor necrosis factor α (TNF-α), and TUG1 expression levels were evaluated using TB Green Fast qPCR Mix kit (RR430a, Takara Biomedical Technology) and normalized to β-actin. Data were calculated using $2^{-\Delta\Delta Ct}$ method [14].

Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) was conducted to test the concentrations of MCP-1, IL-6, and TNF-α in culture supernatants of L-02 and THLE2 cells. In brief, 3×10^4 L-02 or THLE2 cells were seeded into 24-well plate overnight and treated by 8 μg/ml LPS and/or 25 or 50 μM baicalin for 12 or 24 h. Then, culture supernatant was collected in line with experimental group. Human MCP-1 ELISA kit (#88-7399-22, Invitrogen), Human IL-6 ELISA kit (#KHC0061, Invitrogen), and Human TNF-α ELISA kit (#KHC3011, Invitrogen) were carried out respectively in line with the instructions from supplier.

Western blotting assay

Total proteins were isolated from L-02 or THLE2 cells using Cell Lysis Buffers (No. 635656, Clontech, CA, USA) containing with Protease Inhibitor Cocktail-ProteoGuard (No. 635673, Clontech) and quantified using TaKaRa BCA Protein Assay kit (T9300A, Takara Biomedical Technology). Western blotting assay was performed as previously described [15]. The following antibodies were used: anti-caspase 3 antibody (sc-166589), anti-Bcl-2 antibody (sc-509), anti-Bax antibody (sc-70408), anti-MCP-1 antibody (sc-130328), anti-IL-6 antibody (sc-130326), anti-TNF-α antibody (sc-130349), anti-t-p38MAPK antibody (sc-136210), anti-p-p38MAPK antibody (sc-7973), anti-t-JNK antibody (sc-7345), anti-p-JNK antibody (sc-293136), anti-t-c-Jun antibody (sc-166540), anti-p-c-Jun antibody (sc-53182), anti-β-actin antibody (sc-130065), Goat anti-Rabbit IgG-HRP second antibody (sc-2004), and Goat anti-Mouse IgG-HRP second antibody (sc-2005, Santa Cruz Biotechnology, CA, USA). The signal of proteins and intensities of bands were measured and analyzed using ChemiDoc™ XRS system (Bio-Rad Laboratories, CA, USA) and Image Lab™ software (Bio-Rad Laboratories), respectively.

Statistical analysis

All experiments were repeated three times. Statistical analysis was carried out with the help of Prism 6.0 software (Graphpad Software, CA, USA). Results were expressed as mean ± standard deviation (SD). *P* values were calculated using ANOVA with Tukey's post hoc analysis. Statistical significance was set at $P < 0.05$.

Results

LPS stimulated L-02 cell apoptosis and inflammatory reaction

Firstly, we examined the influence of LPS on L-02 cell viability, apoptosis, and inflammatory cytokine expressions. The different concentrations of LPS (0, 2, 4, 8, and 10 μg/ml) were used to stimulate L-02 cells. After 4, 8, or 10 μg/ml LPS stimulation for 12 h, L-02 cell viability was significantly decreased (Fig. 2a, $P < 0.05$ for 4 μg/ml, $P < 0.01$ for 8 μg/ml, and $P < 0.001$ for 10 μg/ml). Then, 4 and 8 μg/ml LPS stimulation were selected for use in the following experiments. The results of Fig. 2b exhibited that 4 or 8 μg/ml LPS stimulation dramatically caused L-02 cell apoptosis ($P < 0.05$ for 4 μg/ml and $P < 0.01$ for 8 μg/ml). Moreover, the cleaved-caspase 3 and Bax protein levels in L-02 cells were increased, while the Bcl-2 protein level was decreased after 4 or 8 μg/ml LPS stimulation (Fig. 2c). Besides, Fig. 2 d displays that 8 μg/ml LPS stimulation remarkably elevated the MCP-1, IL-6, and TNF-α mRNA levels in L-02 cells ($P < 0.01$ for MCP-1 and IL-6, $P < 0.001$ for TNF-α). The MCP-1, IL-6, and TNF-α protein levels in L-02 cells were also hoisted after 8 μg/ml LPS stimulation (Fig. 2e). Furthermore, after 8 μg/ml LPS stimulation, the MCP-1, IL-6, and TNF-α concentrations in the culture supernatant of L-02 cells were also notably increased (Fig. 2f, $P < 0.01$ for MCP-1 and IL-6, $P < 0.001$ for TNF-α). These above outcomes evidenced that LPS stimulation could cause L-02 cell apoptosis and inflammatory reaction.

Baicalin relieved LPS-stimulated L-02 cell apoptosis and inflammatory reaction

Then, we tested the impacts of baicalin on LPS-stimulated L-02 cell apoptosis and inflammatory cytokine expressions. L-02 cells were exposure to 25, 50, 75, or 100 μM baicalin to choose optimum treatment concentration of baicalin. The results of Fig. 3a showed that 25 or 50 μM baicalin treatment had no obvious impact on L-02 cell viability, while 75 or 100 μM baicalin treatment lowered the L-02 cell viability ($P < 0.05$). Considering that we want to probe the protective effect of baicalin on LPS-caused L-02 cell apoptosis and inflammatory

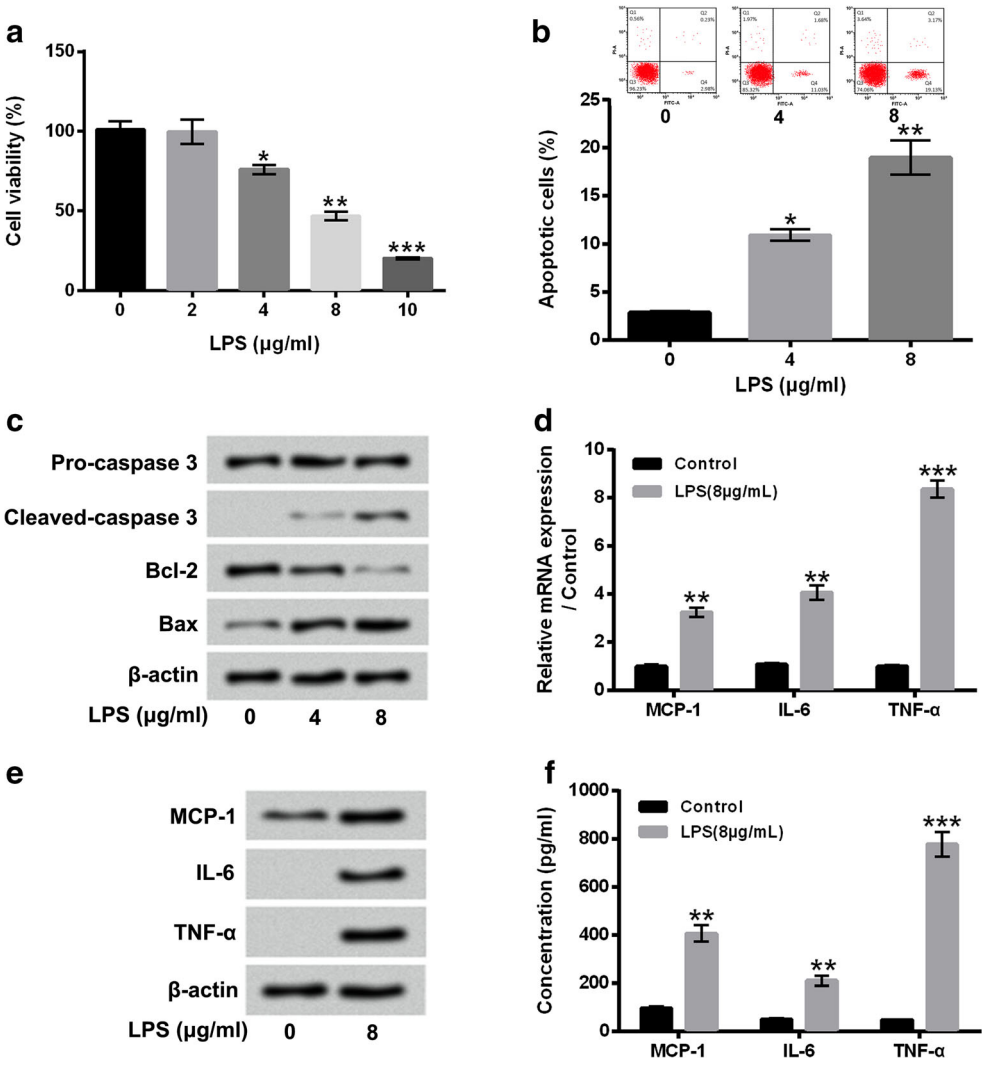

Fig. 2 LPS stimulated L-02 cell apoptosis and inflammatory reaction. **a** L-02 cell viability after 2, 4, 8, or 10 μg/ml LPS stimulation for 12 h was tested using CCK-8 assay. **b** The rate of apoptotic L-02 cells after 4 or 8 μg/ml LPS stimulation for 12 h was assessed using Annexin V-FITC/PI apoptosis kit. **c** The pro-caspase 3, cleaved-caspase 3, Bcl-2, and Bax protein levels in L-02 cells after 4 or 8 μg/ml LPS stimulation for 12 h were examined using western blotting analysis. **d**, **e** The mRNA and protein levels of MCP-1, IL-6, and TNF-α in L-02 cells after 8 μg/ml LPS stimulation for 12 h were detected using qRT-PCR and western blotting analysis, respectively. **f** The MCP-1, IL-6, and TNF-α concentrations in the culture supernatant of L-02 cells after 8 μg/ml LPS stimulation for 12 h were tested using ELISA. $N = 3$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

reaction, 25 and 50 μM baicalin treatment was chosen for the following experiments. Figure 3 b exhibits that 50 μM baicalin treatment notably relieved the 8 μg/ml LPS stimulation-caused L-02 cell viability reduction ($P < 0.05$). Additionally, 8 μg/ml LPS stimulation-caused L-02 cell apoptosis was also softened by 25 or 50 μM baicalin treatment (Fig. 3c, $P < 0.05$ for 25 μM and $P < 0.01$ for 50 μM). Relative to 8 μg/ml LPS stimulation group, the cleaved-caspase 3 and Bax protein levels in L-02 cells were reduced, while the Bcl-2 protein level was enhanced in 8 μg/ml LPS stimulation + 25 or 50 μM baicalin treatment group (Fig. 3d). Besides, 50 μM baicalin treatment extraordinary mitigated the 8 μg/ml LPS stimulation-caused enhancement of MCP-1, IL-6, and TNF-α mRNA levels in L-02 cells (Fig. 3e, $P < 0.05$). Analogous results were found in MCP-1, IL-6, and TNF-α protein levels in L-02 cells and MCP-1, IL-6, and TNF-α concentrations in culture supernatant of L-02 cells (Fig. 3f, g; $P < 0.05$ for concentrations in culture supernatant). These above outcomes proposed that baicalin could relief LPS stimulation-caused L-02 cell apoptosis and inflammatory reaction.

Baicalin promotes TUG1 expression in L-02 cells

In order to explore whether TUG1 was associated with the impact of baicalin on L-02 cells stimulated by LPS, the TUG1 expression in L-02 cells was tested after LPS stimulation or baicalin incubation. As presented in Fig. 4a, 4 or 8 μg/ml LPS stimulation dramatically lowered the TUG1 expression in L-02 cells ($P < 0.05$ for 4 μg/ml and $P < 0.01$ for 8 μg/ml). Moreover, data in Fig. 4b showed that 25 or 50 μM baicalin treatment noticeably upregulated the TUG1 expression in L-02 cells ($P < 0.05$ for 25 μM and $P < 0.01$ for 50 μM), which hinted that baicalin relieved LPS-caused L-02 cell apoptosis and inflammatory reaction might be via elevating TUG1 expression.

Baicalin mitigated LPS-stimulated activation of p38MAPK and JNK pathways in L-02 cells

The influence of LPS stimulation and/or baicalin treatment on p38MAPK and JNK pathways in L-02 cells

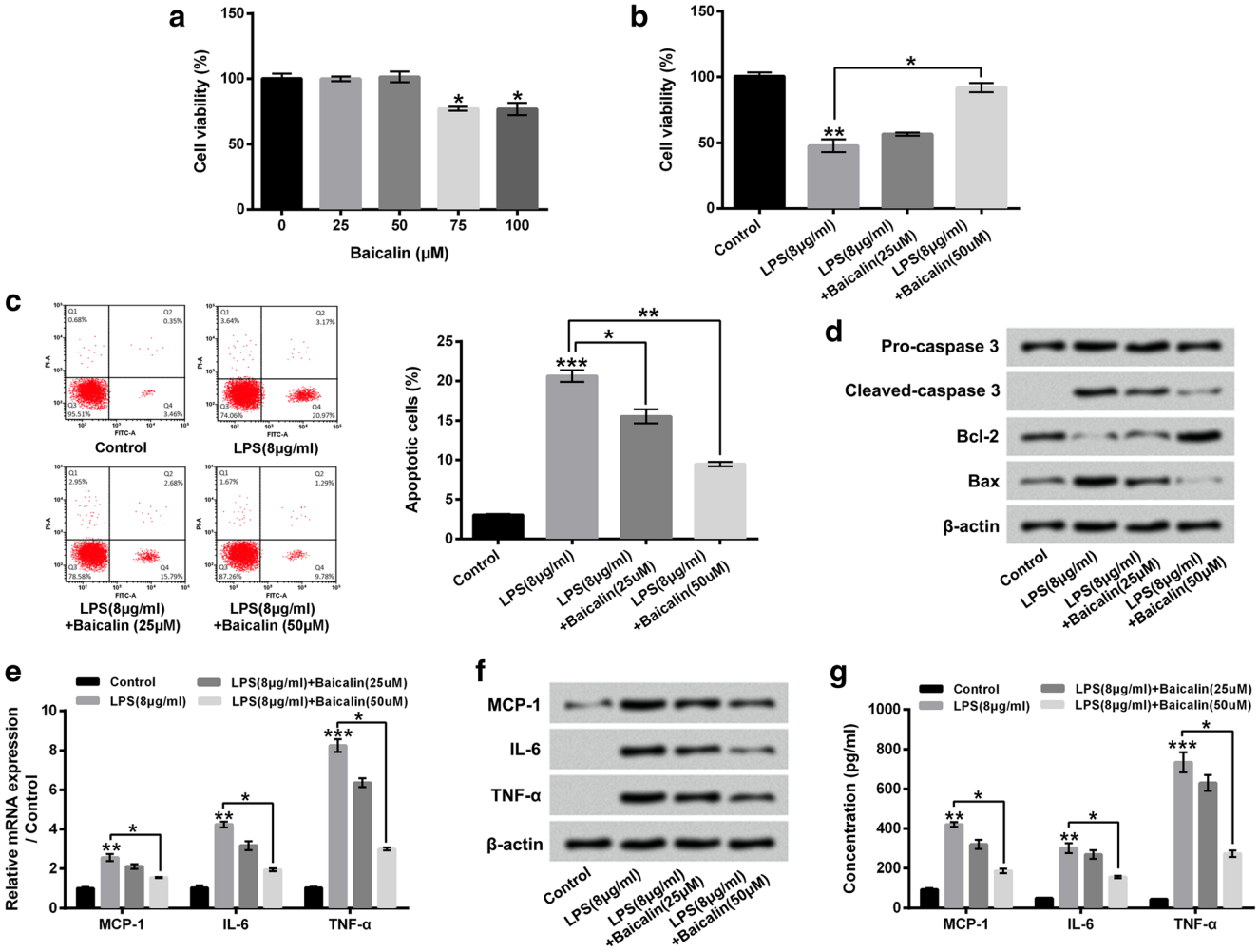

Fig. 3 Baicalin relieved L-02 cell apoptosis and inflammatory reaction stimulated by LPS. **a** L-02 cell viability after 25, 50, 75, or 100 μM baicalin treatment for 24 h was tested using CCK-8 assay. After 8 μg/ml LPS stimulation and/or 25 or 50 μM baicalin treatment for 24 h, **b** L-02 cell viability was tested using CCK-8 assay; **c** the rate of apoptotic L-02 cells was assessed using Annexin V-FITC/PI apoptosis kit; **d** the pro-caspase 3, cleaved-caspase 3, Bcl-2, and Bax protein levels in L-02 cells were examined using western blotting analysis; **e**, **f** the mRNA and protein levels of MCP-1, IL-6, and TNF-α in L-02 cells were detected using qRT-PCR and western blotting analysis, respectively; **g** the MCP-1, IL-6, and TNF-α concentrations in the culture supernatant of L-02 cells were tested using ELISA. $N = 3$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

were examined using western blotting analysis. Results illustrated that 8 μg/ml LPS stimulation drastically activated p38MAPK and JNK pathway in L-02 cells via elevating p/t-p38MAPK, p/t-JNK, and p/t-c-Jun expression rates (Fig. 5, $P < 0.05$ for p/t-JNK, $P < 0.01$ for p/t-p38MAPK and p/t-c-Jun). Besides, 25 or 50 μM baicalin treatment significantly softened the activation of p38MAPK and JNK pathway in L-02 cells stimulated by LPS through reducing p/t-p38MAPK, p/t-JNK, and p/t-c-Jun expression rates ($P < 0.05$). These outcomes indicated that baicalin could mitigate activation of p38MAPK and JNK pathways stimulated by LPS in L-02 cells.

Knockdown of TUG1 activated p38MAPK and JNK pathways and promoted inflammatory cytokine expression in L-02 cells

sh-TUG1 was transfected into L-02 cells to knockdown TUG1. The effects of TUG1 knockdown on inflammatory cytokine expressions in L-02 cells treated by LPS and/or baicalin were analyzed. Figure 6 a presents that sh-TUG1 transfection notably lowered the TUG1 expression in L-02 cells ($P < 0.01$). SB203580 (10 μM) was used as p38MAPK specific inhibitor and SP600125 (10 μM) was used as JNK specific inhibitor to added in this research. The results in Fig. 6b exhibited that 10 μM SB203580 incubation obviously

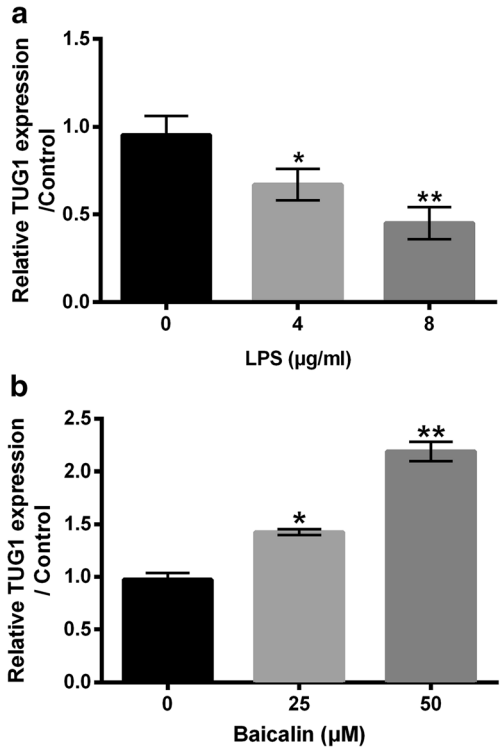

Fig. 4 Baicalin upregulated TUG1 expression in L-02 cells. **a** The TUG1 expression in L-02 cells after 4 or 8 μg/ml LPS stimulation for 24 h was tested using qRT-PCR. **b** The TUG1 expression in L-02 cells after 25 or 50 μM baicalin treatment for 24 h was tested using qRT-PCR. $N = 3$. $^{*}P < 0.05$, $^{**}P < 0.01$

inactivated p38MAPK pathway in L-02 cells stimulated by 8 μg/ml LPS. Ten-micromolar SP600125 incubation remarkably inactivated JNK pathway in L-02 cells stimulated by 8 μg/ml LPS. However, Fig. 6 c displays that sh-TUG1 transfection notably activated p38MAPK and JNK pathway in L-02 cells via elevating p/t-p38MAPK, p/t-JNK, and p/t-c-Jun expression rates ($P < 0.05$). Data in Fig. 7a showed that TUG1 knockdown reversed the impacts of 50 μM baicalin on MCP-1, IL-6, and TNF-α protein levels in LPS-stimulated L-02 cells. Ten-micromolar SB203580 or SP600125 incubation relieved the influence of TUG1 knockdown on MCP-1, IL-6, and TNF-α protein levels in LPS + baicalin-treated L-02 cells. Similar results were found in MCP-1, IL-6, and TNF-α concentrations in the culture supernatant of L-02 cells (Fig. 7b, d; $P < 0.05$ or $P < 0.01$ as marked). These above outcomes implied that knockdown of TUG1 could activate p38MAPK and JNK pathways and promote inflammatory reaction in L-02 cells.

Baicalin also relieved LPS-stimulated THLE2 cell apoptosis and inflammatory reaction by elevating TUG1 expression

Finally, we further tested the influences of baicalin on LPS-stimulated THLE2 cell apoptosis and inflammatory cytokine expressions, as well as the expression of TUG1 in THLE2 cells after baicalin treatment. Figure 8 a shows that 25 or 50 μM baicalin treatment also had no significant influence on THLE2 cell viability, while 75 or 100 μM baicalin treatment reduced the viability of THLE2 cells ($P < 0.05$). Data in Fig. 8b presented that 8 μg/ml LPS stimulation declined the THLE2 cell viability, while 50 μM baicalin treatment remarkably mitigated the 8 μg/ml LPS stimulation-caused THLE2 cell viability loss ($P < 0.05$). Moreover, 8 μg/ml LPS stimulation dramatically induced THLE2 cell apoptosis, while 25 or 50 μM baicalin treatment both weakened the 8 μg/ml LPS stimulation-caused THLE2 cell apoptosis (Fig. 8c, $P < 0.05$ for 25 μM and $P < 0.01$ for 50 μM), which was accompanied with the decreased protein levels of cleaved-caspase 3 and Bax, along with the increased protein level of Bcl-2 in the 8 μg/ml LPS stimulation + 25 or 50 μM baicalin treatment group (Fig. 8d). In addition, Fig. 8e, f and g point out that 8 μg/ml LPS stimulation enhanced the mRNA and protein levels of MCP-1, IL-6, and TNF-α in THLE2 cells, as well

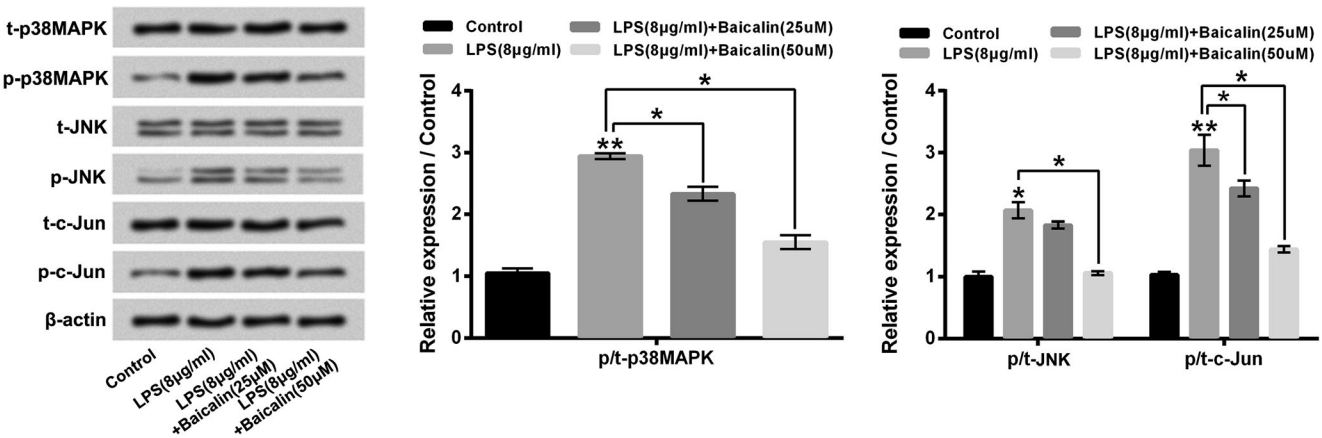

Fig. 5 Baicalin mitigated LPS-stimulated activation of p38MAPK and JNK pathways in L-02 cells. The t-p38MAPK, p-p38MAPK, t-JNK, p-JNK, t-c-Jun, and p-c-Jun expression levels in L-02 cells after 8 μg/ml LPS stimulation and/or 25 or 50 μM baicalin treatment for 24 h were examined using western blotting analysis. $N = 3$. $^{*}P < 0.05$, $^{**}P < 0.01$

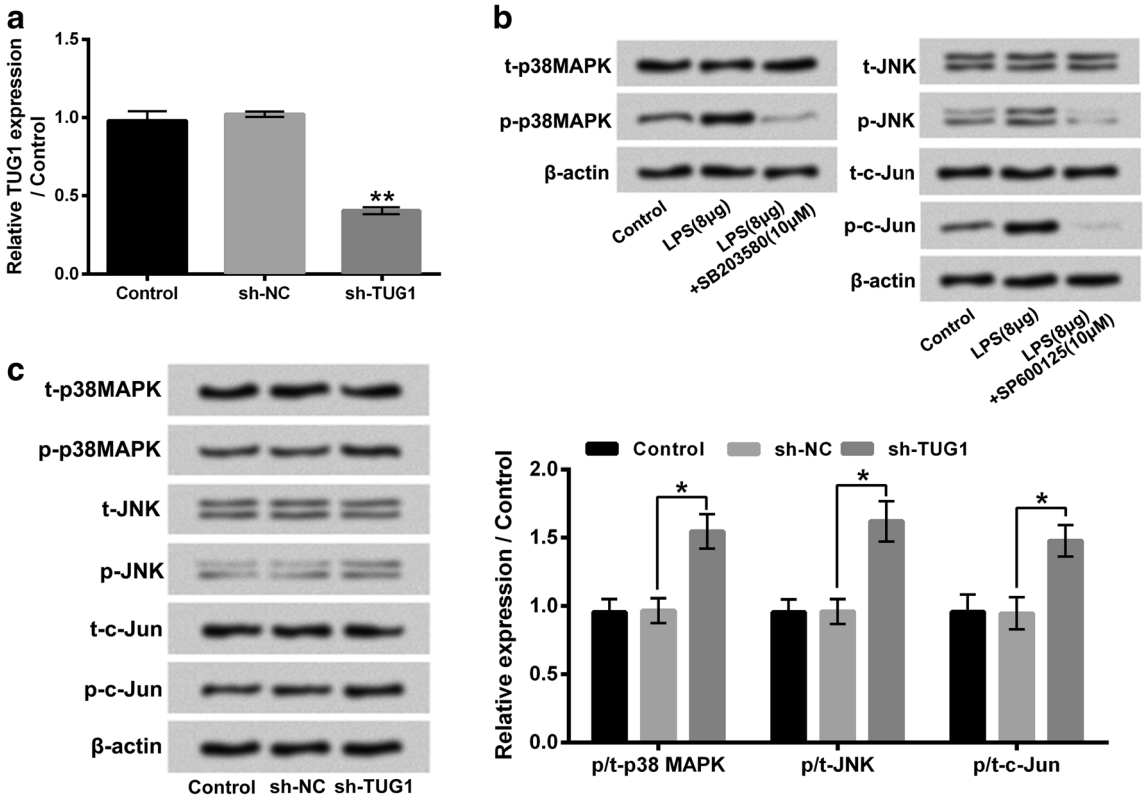

Fig. 6 Knockdown of TUG1 activated p38MAPK and JNK pathways in L-02 cells. **a** The TUG1 expression in L-02 cells after sh-NC or sh-TUG1 transfection was examined using qRT-PCR. **b** The t-p38MAPK, p-p38MAPK, t-JNK, p-JNK, t-c-Jun, and p-c-Jun expression levels in L-02 cells after 8 μg/ml LPS stimulation and/or SB203580 (10 μM) or SP600125 (10 μM) incubation for 24 h were tested using western blotting analysis. **c** The t-p38MAPK, p-p38MAPK, t-JNK, p-JNK, t-c-Jun, and p-c-Jun expression levels in L-02 cells after sh-NC or sh-TUG1 transfection were tested using western blotting analysis. $N = 3$. $^{*}P < 0.05$, $^{**}P < 0.01$

as MCP-1, IL-6, and TNF-α concentrations in the culture supernatant of THLE2 cells ($P < 0.01$ or $P < 0.001$ as marked), while 25 or 50 μM baicalin treatment relieved the 8 μg/ml LPS stimulation-caused enhancement of mRNA and protein levels of MCP-1, IL-6, and TNF-α in THLE2 cells, as well as MCP-1, IL-6, and TNF-α concentrations in the culture supernatant of THLE2 cells ($P < 0.05$ or $P < 0.01$ as marked). Besides, Fig. 8 h displays that 25 or 50 baicalin treatment also notably elevated the TUG1 expression in THLE2 cells ($P < 0.05$ for 25 μM and $P < 0.01$ for 50 μM). These above outcomes evidenced that baicalin also relieved LPS-stimulated THLE2 cell apoptosis and inflammatory reaction might be through elevating TUG1 expression.

Discussion

Hepatitis has become a major social, health, and economic problem worldwide [3, 18]. Herein, we further verified the beneficial influence of baicalin on human normal liver L-02 and THLE2 cell apoptosis and inflammatory reaction caused by LPS stimulation. More importantly, we found that LPS downregulated the TUG1 expression in L-02 cells, while baicalin promoted the TUG1 expression in L-02 and THLE2 cells. Besides, we discovered that baicalin suppressed LPS-caused activation of p38MAPK and JNK pathways in L-02 cells. Knockdown of TUG1 activated p38MAPK and JNK pathways and promoted inflammatory reaction in L-02 cells.

LPS is one of the most important compositions of the outer membrane of Gram-negative bacteria [33]. The pro-inflammatory roles of LPS have been substantiated by a series of in vitro and in vivo experimental research [25, 34]. LPS-caused hepatitis models have been widely used to testing the potential therapeutic medicines for hepatitis [10, 35]. In the present research, LPS stimulation caused human normal liver L-02 and THLE2 cell viability loss and apoptosis. The concentrations of MCP-1, IL-6, and TNF-α, which are closely related to the liver cell inflammatory reaction in hepatitis [11, 21], were all increased in both inside and outside of L-02 and THLE2 cells after LPS stimulation. These outcomes evidenced that LPS-stimulated L-02 and THLE2 cells could be used to test the beneficial influences of baicalin on hepatitis.

Flavonoids are polyphenolic compounds widely disturbed in plants with satisfactory anti-inflammatory activity [24]. Some flavonoids have been tested as the effective medicines

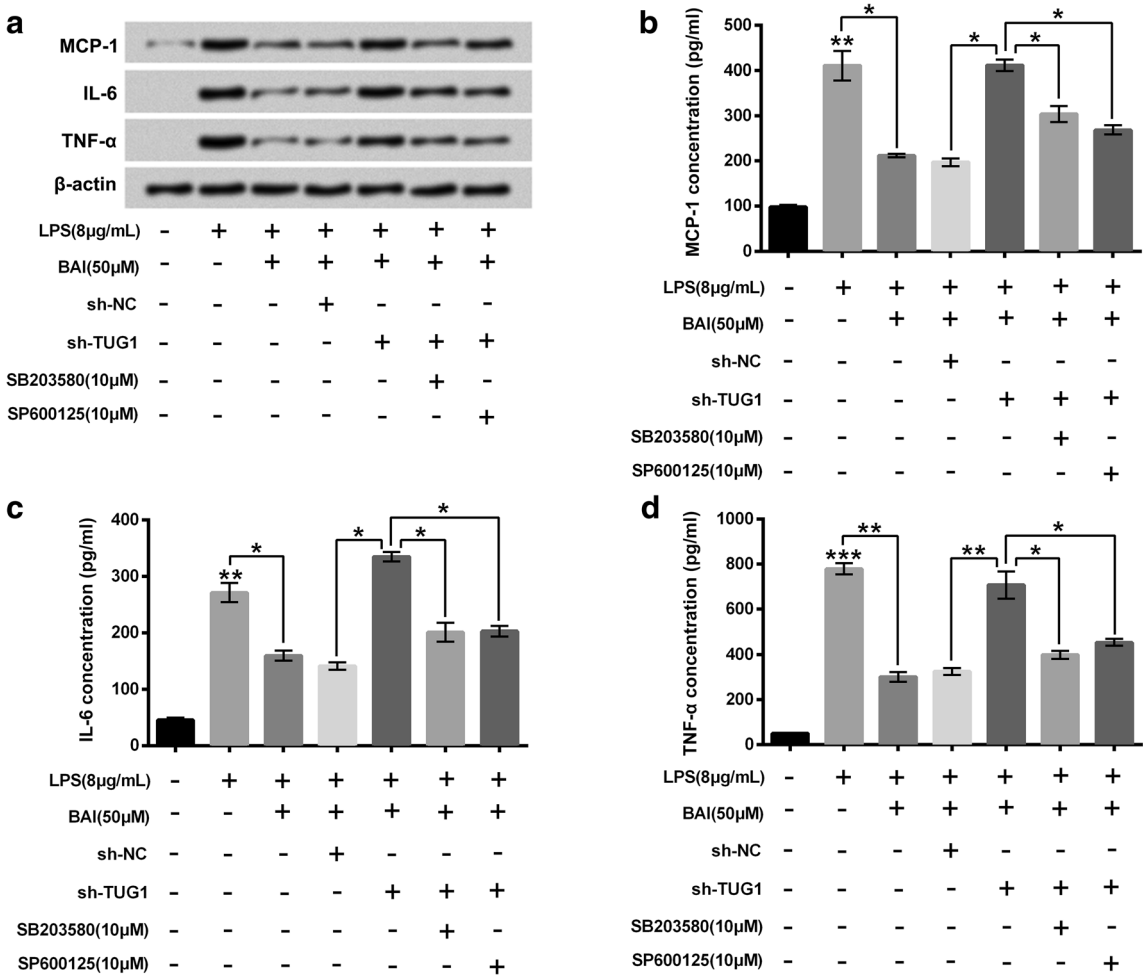

Fig. 7 Knockdown of TUG1 promoted the inflammatory cytokines expressions in L-02 cells. **a**–**d** The MCP-1, IL-6, and TNF-α protein levels in L-02 cells, as well as the MCP-1, IL-6, and TNF-α concentrations in the culture supernatant of L-02 cells after 8 μg/ml LPS stimulation and/or 50 μM baicalin treatment or sh-TUG1 transfection or SB203580 (10 μM) or SP600125 (10 μM) incubation for 24 h were examined using western blotting analysis and ELISA, respectively. $N = 3$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

for human inflammatory diseases, including hepatitis [5, 27]. Baicalin is the most vital flavonoid extracted from the roots of *Scutellaria baicalensis* with satisfying anti-inflammatory activity [19]. More and more pharmacological researches in recent years demonstrated that baicalin might have effective protective and therapeutic effects on inflammatory damage of liver cells [6, 13, 39]. In consistent with the previous studies, we discovered that baicalin relieved the L-02 and THLE2 cell apoptosis and inflammatory cytokine expressions stimulated by LPS, which further provided a strong experimental rationale for comprehending the beneficial activity of baicalin on hepatitis.

Followed by the advances of high-throughput sequencing techniques, more and more lncRNAs has been found to act as critical regulatory factors of multiple cell functions [17]. TUG1 has been reported to be associated with the regulation of cell inflammation reaction [37]. Previous experiments confirmed that TUG1 could exert anti-inflammatory effects on cold-stimulated liver injury in mouse [31]. Herein, we pointed out that LPS declined the TUG1 expression in L-02 cells, while baicalin elevated the TUG1 expression in L-02 and THLE2 cells, which implied that upregulation of TUG1 induced by baicalin might play key roles in the anti-inflammatory activity of baicalin on L-02 and THLE2 cell apoptosis and inflammatory reaction stimulated by LPS.

p38MAPK and JNK pathways are considered to be associated with the regulation of liver cell inflammatory reaction [4, 23]. The unusual activation of p38MAPK and JNK pathways and its roles in the development of hepatitis have been affirmed in previous literatures [2, 12]. Moreover, Zhong et al. and Sun et al. reported that baicalin participated in the regulation of p38MAPK and JNK pathways in cells [32, 39]. Zhang et al. indicated that TUG1 could modulate MAPK pathway in cells [36]. Therefore, in this study, we further analyzed the influences of baicalin on p38MAPK and JNK pathways in LPS-stimulated L-02 cells. We discovered that LPS activated

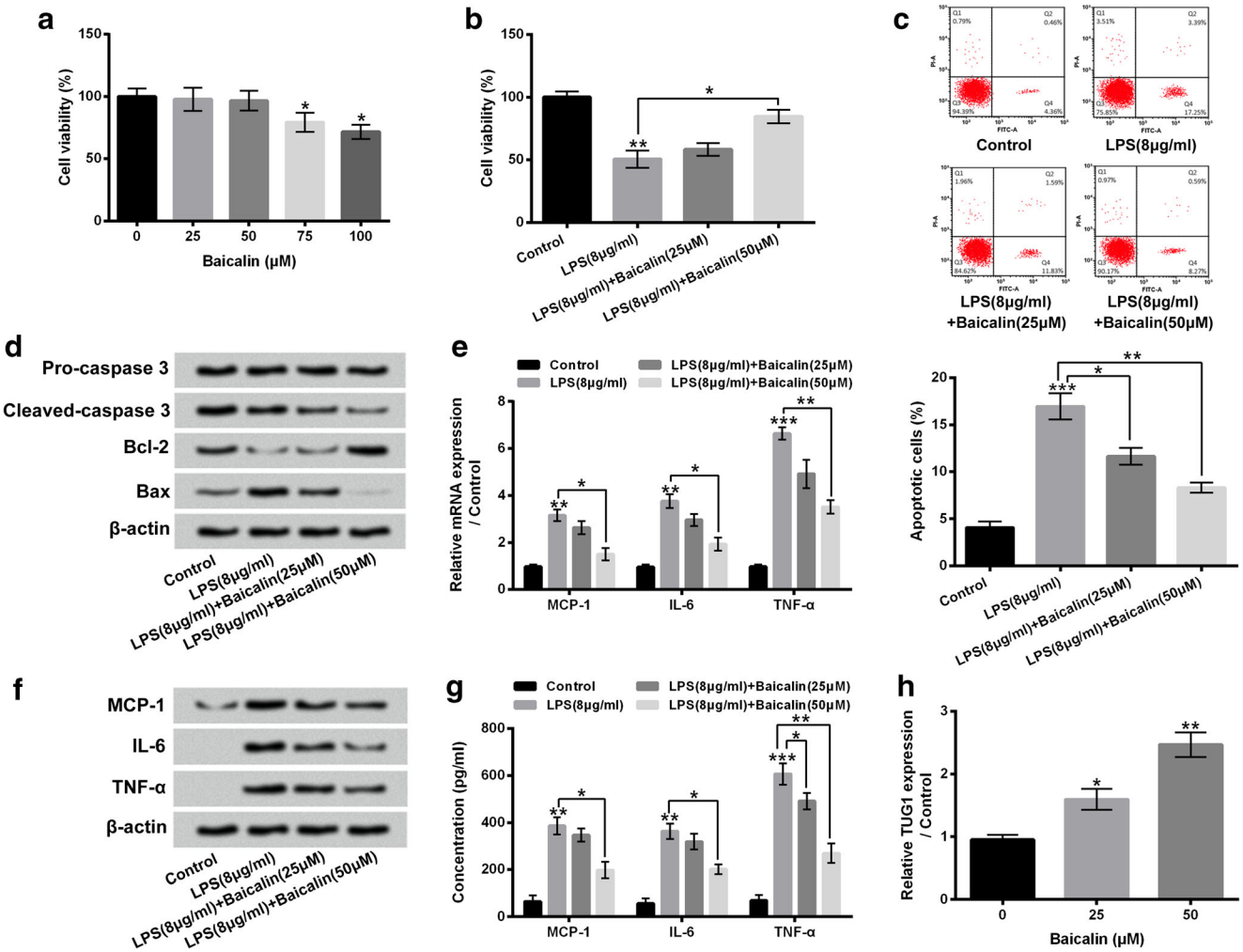

Fig. 8 Baicalin also relieved LPS-stimulated THLE2 cell apoptosis and inflammatory reaction by elevating TUG1 expression. **a** THLE2 ell viability after 25, 50, 75, or 100 μM baicalin treatment for 24 h was tested using CCK-8 assay. After 8 μg/ml LPS stimulation and/or 25 or 50 μM baicalin treatment for 24 h, **b** THLE2 cell viability was tested using CCK-8 assay; **c** the rate of apoptotic THLE2 cells was assessed using Annexin V-FITC/PI apoptosis kit; **d** the pro-caspase 3, cleaved-caspase 3, Bcl-2, and Bax protein levels in THLE2 cells were examined using western blotting analysis; **e**, **f** the mRNA and protein levels of MCP-1, IL-6, and TNF-α in L-02 cells were detected using qRT-PCR and western blotting analysis, respectively; **g** the MCP-1, IL-6, and TNF-α concentrations in the culture supernatant of L-02 cells were tested using ELISA. **h** The TUG1 expression in THLE2 cells after 25 or 50 μM baicalin treatment for 24 h was tested using qRT-PCR. $N = 3$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

p38MAPK and JNK pathways in L-02 cells, while baicalin mitigated the LPS-caused activation of p38MAPK and JNK pathways in L-02 cells. More importantly, knockdown of TUG1 activated the p38MAPK and JNK pathways in L-02 cells and reversed the impact of baicalin on inflammatory cytokines MCP-1, IL-6, and TNF-α expression in LPS-stimulated L-02 cells. Inhibitors of p38MAPK pathway (SB203580) and JNK pathway (SP600125) attenuated the influence of TUG1 knockdown on inflammatory cytokine expression in LPS + baicalin-treated L-02 cells. These outcomes implied that baicalin relieved LPS-caused L-02 cell inflammatory cytokine expression might be achieved through elevating TUG1 expression and then inactivating p38MAPK and JNK pathways.

Taken together, this study further affirmed the anti-inflammatory activity of baicalin on LPS-stimulated human normal liver cell apoptosis and inflammatory reaction. Baicalin relived inflammation stimulated by LPS in human normal liver L-02 cells via upregulating TUG1 and then inactivating p38MAPK and JNK pathways. We propose that baicalin might be a potential preventive and therapeutic medicine for hepatitis, despite further animal and clinical experiments are still needed.

Author contributions Yanqiu Huang, Aiying Zhao: conceived and designed the experiments.

Yanqiu Huang, Mengyan Sun, Xuefang Yang, Aiyu Ma, Yujie Ma: data collection.

Yanqiu Huang, Aiying Zhao: manuscript writing.

Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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