Experimental and Molecular Pathology 111 (2019) 104317

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

journal homepage: www.elsevier.com/locate/yexmp

Tetramethylpyrazine alleviates lipopolysaccharide-induced damage in ATDC5 cells via down-regulating MyD88

Qiang Li[a,1], Wanying Xing[b,1], Xiong Xu[a], Sulong Wang[a], Yisha He[a], Yueshu Wang[a], Hongbin Sun[a,*]

[a] Department of Hand Surgery, China-Japan Union Hospital of Jilin University, Changchun 130033, Jilin, China
[b] Department of Breast Surgery, China-Japan Union Hospital of Jilin University, Changchun 130033, Jilin, China

ARTICLE INFO

Keywords:
Osteoarthritis
Tetramethylpyrazine
Lipopolysaccharide
Myeloid differentiation factor 88
NF-κB
p38MAPK

ABSTRACT

Background: Tetramethylpyrazine (TMP) has been reported to play a significant role in the cardiovascular and neuronal diseases. But, the functions of TMP in osteoarthritis (OA) remain unclear. In this investigation, we intended to probe the protective effectiveness of TMP in lipopolysaccharide (LPS)-caused damage in ATDC5 cells.

Methods: ATDC5 cells were managed with LPS (5 μg/mL) for 12 h, and then the effects of TMP on these cells were evaluated. Cell viability and apoptosis of these treated cells were detected by CCK-8 and flow cytometry methods. The secretions of IL-1β, IL-6 and TNF-α were examined via applying ELISA kits. qRT-PCR was utilized to measure cell inflammatory factors and MyD88 expression. After transfection with pc-MyD88, the above-involved cell processes were reassessed, and NF-κB and p38MAPK pathways were examined by western blot assay.

Results: LPS treatment induced a series of inflammatory destructions, which reduced viability, accelerated apoptosis and cell inflammatory factors release in ATDC5 cells. However, TMP precondition clearly mitigated LPS-triggered ATDC5 cell injury. Additionally, TMP down-regulated MyD88 expression in LPS-treated ATDC5 cells, as well as overexpression of MyD88 overturned the impacts of TMP on LPS-induced cell injury in ATDC5 cells. Beyond that, TMP restrained LPS-triggered the activations of NF-κB and p38MAPK via repression of MyD88.

Conclusion: The above consequences exhibited that TMP exhibited a protective effect to lighten LPS-caused cell damage via mediating MyD88/NF-κB/p38MAPK pathways. These findings suggested that TMP perhaps an effective agent for the clinical treatment of OA.

1. Introduction

Osteoarthritis (OA) is a bone and synovial joint disease, which usually arises in elderly people (Losina et al., 2013). The main pathological features of OA contain articular cartilage degenerative and destruction, synovial membrane inflammation and osteophyte formation (Mobasheri et al., 2017). Chondrocytes are the only cells in joint articular cartilage, which play a significant role in the cartilage formation. In the process of OA, the apoptotic rate of articular chondrocytes and the expression of inflammatory factors are observably increased, which is one of the key causes of OA cartilage degeneration (Antonelli and Starz, 2012). Recently, various strategies such as surgical, pharmacologic treatment and NSAIDs have been applied to treat OA, which can improve joint function and reduce painful during OA process (Lohmander and Roos, 2007; Zhang et al., 2010). However, these strategies present a few of drawbacks in cartilage destruction and can cause considerable cardiovascular and gastrointestinal side effects. Therefore, searching novel drugs for the treatment of cartilage inflammatory injury are great significance for remedying OA clinically.

Tetramethylpyrazine (TMP) is one of the effective ingredients derived from *Ligusticum chuanxiong hort* (Cao et al., 2015). TMP has a favorable clinical potency in cerebrovascular and cardiovascular diseases, alcoholic liver injury and angina pectoris (Cao et al., 2016; Gao et al., 2015; Shao et al., 2015). Moreover, recent study also disclosed that TMP emerged an important role in the mediation of apoptosis and inflammation (Koushki et al., 2015). In an animal research, TMP has

* Corresponding authors at: Department of Hand Surgery, China-Japan Union Hospital of Jilin University, No.126 Xiantai Street, Changchun 130033, Jilin, China.
E-mail addresses: wang16yq@sina.com (Y. Wang), sun27ho@sina.com (H. Sun).
[1] Equal first authors.

https://doi.org/10.1016/j.yexmp.2019.104317
Received 7 May 2019; Received in revised form 27 August 2019; Accepted 7 October 2019
Available online 23 October 2019
0014-4800/

been testified to prohibit the articular cartilage explants and chondrocytes apoptosis through suppressing reactive oxygen species (ROS) production and down-regulating caspase-3 activity (Ju et al., 2010). Simultaneously, other study reported that intraarticular injection of TMP could relieve inflammatory bruise and improve joint abrasion in the treatment of OA (Zhang et al., 2018). Therefore, an in-depth study for exploring the role and mechanism of TMP is important for the treatment of OA.

The differentiation ability of ATDC5 cell is highly consistent with chondrocyte. Under in vitro culture conditions, ATDC5 cells maintain an undifferentiated state and has strong proliferative capacity, so it is regarded as a reliable cell model to simulate cartilage formation (Yao and Wang, 2013). In this research, we explored the protective functions of TMP in LPS-injured ATDC5 cells in vitro. In addition, we also detected myeloid differentiation factor 88 (MyD88), nuclear factor kappa B (NF-κB), and p38 mitogen activated protein kinase (p38MAPK) pathways to further disclose the possible regulatory mechanisms of TMP protecting ATDC5 cells against LPS-triggered injury. This study might provide a potential strategy for OA clinical treatment.

2. Materials and methods

2.1. Cell culture and operation

ATDC5 cells were acquired from RIKEN (Tsukuba, Japan). The resuscitative ATDC5 cells were cultured in Dulbecco's modified Eagle's medium/nutrient mixture F12 (DMEM/F12) (1:1) (Life Technologies, California, USA) medium including 10% fetal bovine serum (FBS, Thermo Fisher Scientific, Massachusetts, USA). Then cells were cultivated in a constant temperature (37 °C) incubator (Thermo Fisher Scientific) within 70%–80% saturated humidity and 5% CO_2. ATDC5 cells were managed with LPS (5 μg/mL or 2.5 μg/mL) (Sigma-Aldrich, Missouri, USA) for 12 h. TMP (Sigma-Aldrich) was formulated for six concentration gradients (0, 10, 50, 100, 150 and 200 μM) with DMSO (Sigma-Aldrich). The control group (no TMP) was utilized the same amount of DMSO to treat ATDC5 cells. ATDC5 cells were pretreated with TMP at different concentrations for 24 h before LPS management.

2.2. Cell viability

After TMP and/or LPS treatment, Cell Counting Kit-8 (CCK-8) acquired from Beyotime Biotechnology (Shanghai, China) was exploited for the detection of cells viability. ATDC5 cells were inoculated in a 96-well plate with 200 μL medium, and then 10 μL CCK-8 solution was mixed into the culture medium cultivating for 3 h at 37 °C. The absorbing was measured via using a Microplate Reader (Thermo Fisher Scientific) at 450 nm and the experiment was repeated for three times.

2.3. Cell apoptosis

Flow cytometry was executed to evaluate the apoptotic cells via adopting Annexin V-EGFP/PI kit (Invitrogen, California, USA). After TMP and/or LPS stimulation, ATDC5 cells were digested by trypsin containing 0.25% EDTA. Then, cells were re-suspended in binding buffer, and then stained with Annexin V-EGFP (5 μL) and PI (10 μL). The strained cells were cultured at room temperature for 15 min in the dark. Finally, flow cytometry (Guava Technologies, California, USA) was utilized to detect the number of apoptotic cells.

2.4. Enzyme-linked immunosorbent assay (ELISA)

Cells suspension was collected from different treatment group, and was sowned in a 24-well plate. After 24 h cultivation, the concentrations of interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α) were respectively evaluated by utilizing mouse IL-1β, IL-6 and TNF-α ELISA kits (R&D Company, Abingdon, UK).

2.5. Transfection

The pcDNA3.1 and pc-MyD88 plasmids were achieved from Genechem Corporation (Shanghai, China). ATDC5 cells in good growth state were digested by 2.5 g/L trypsin and diluted 1×10^5 mL^{-1}, and then cultivated in an incubator at 37 °C with 5% CO_2. Cell transfection was then conducted via applying Lipofectamine 2000 reagent purchased from Invitrogen.

2.6. ROS assay

The ROS level was determined by staining with 2,7-dichlorofluorescein diacetate (DCFH-DA; Nanjing Jiancheng, Nanjing, China). After treatment with TMP and/or LPS, ATDC5 cells were washed twice with PBS, and then cultivated in DMEM comprising 10 μM DCFH-DA for 20 min at 37 °C in the dark. Subsequently, the above-treated cells were washed with PBS again, and the samples were collected by exploiting 0.25% trypsin-EDTA. After centrifugation, these cells were re-suspended in 500 μL PBS, and then were subjected into a flow cytometer (488 nm excitation, 521 nm emission) for the determination of fluorescent intensity.

2.7. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was derived from above treated cells via adopting TRIzont (Invitrogen). Reversed transcription synthesis cDNA was executed via applying PrimeScript™ RT reagent Kit (Invitrogen). The qRT-PCR reaction was operated by utilizing the Fast SYBR™ Green Master (ROX) Kit (Applied Biosystems, California, USA) and ABI 7500 Fluorescence PCR System Operating Guide (Thermo Fisher Scientific). The housekeeping gene of β-actin was exploited for standardization of IL-1β, IL-6, TNF-α and MyD88 expression. The $2^{-\Delta\Delta Ct}$ method was applied for measuring the relative mRNA expression levels of genes (Ish-Shalom and Lichter, 2010).

2.8. Western blot

RIPA lysis buffer purchased from Beyotime Biotechnology (Shanghai, China) was employed for extracting total protein from ATDC5 cells after management with TMP and/or LPS. The BCA Protein Assay Kit (Beyotime Biotechnology) was used to quantify proteins content. The proteins were then loaded into SDS-PAGE, and were transferred into nitrocellulose (NC) membranes (Merck Millipore, Darmstadt, Germany). The NC membrane was blocked for 1 h by utilizing 50 g/L bovine serum albumin (BSA) (Beyotime Biotechnology) at room temperature, and then was incubated with the primary antibodies at 4 °C overnight. The above primary antibodies contained Pro-Caspase-3 (ab32499), Cleaved Caspase-3 (ab214430), IL-1β (ab9722), IL-6 (ab208113), TNF-α (ab6671), p-IκBα (ab133462), t-IκBα (ab32518), p-p65 (ab86299), t-p65 (ab32536), p-p38MAPK (ab47363), t-p38MAPK (ab27986), β-actin (ab16039, Abcam, Cambridge, UK) and Caspase-9 (C9) Mouse mAb (#9508)(Cell Signaling Technology, Boston, USA). The NC membranes were rinsed three times by Tris-Buffered Saline and Tween (TBST) (Beyotime Biotechnology) and then co-cultivated with secondary antibody (ab97051, Abcam) for 1 h at room temperature. The ChemiDocTM XRS system was utilized to capture proteins signals (Bio-Rad, Hercules, CA, USA). The Image Lab™ software (Bio-Rad) was exploited to analyze the gray value of the exposed protein bands.

2.9. Statistical analysis

All tests were replicated at least three times. The experiments results were disclosed as the mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) and Student *t*-test were utilized to

calculate P-values via GraphPad Prism 6.0 software (Graphpad, California, USA). P < .05 was considered to be a significant difference result.

3. Results

3.1. TMP attenuated LPS-stimulated apoptosis in ATDC5 cells

TMP molecular structure was shown in Fig. 1A. At first, we discovered that TMP administration did not influence ATDC5 cells viability at different concentrations (0, 10, 50, 100, 150, 200 μM, Fig. 1B), indicating that TMP had no toxicity effect on ATDC5 cells. Under the treatment of LPS (5 μg/mL), cell viability was obviously decreased as comparison to control group (P < .001, Fig. 1C). However, pretreatment with TMP (100, 150 and 200 μM) obviously accelerated cell viability as relative to LPS treatment group, especially in 150 μM TMP treatment (P < .001, Fig. 1C). Simultaneously, flow cytometry results showed that LPS-induced cell apoptosis was obviously prohibited by TMP pretreatment (P < .01) in ATDC5 cells (Fig. 1D). Additionally, we used 2.5 μg/mL LPS to stimulate ATDC5 cells, and then the influence of TMP in cell apoptosis was determined again. The results showed that TMP completely reversed the effect of low dose LPS on cell apoptosis (P < .001, Supplementary Fig. 1). Outside of this, Fig. 1E and F revealed that LPS treatment significantly enhanced the Cleaved-Caspase-3 and Cleaved-Caspase-9 expression as compared to control group (P < .001). Compared to LPS alone treated cells, we observed that TMP pretreatment clearly decreased Cleaved-Caspase-3 and Cleaved-Caspase-9 expression (P < .001). This data disclosed that TMP attenuated LPS-induced cell apoptosis in ATDC5 cells.

3.2. TMP attenuated LPS-induced cellular inflammatory factor productions and ROS level

To explore whether TMP could reduce LPS-induced accumulation of cellular inflammatory factors, ATDC5 cells received LPS and TMP stimulation. As shown in Fig. 2A–C, the results revealed that the mRNA, secretions and protein expression of IL-1β, IL-6 and TNF-α were significantly elevated under LPS treatment group compared to under control group (P < .001). However, this consequence was markedly suppressed under TMP intervention compared with LPS alone treatment group (P < .01 or P < .001, Fig. 2A–C). Likewise, we observed that LPS significantly elevated ROS generation in ATDC5 cells (P < .001). But, TMP significantly prohibited the level of ROS in LPS-treated ATDC5 cells (P < .01, Fig. 2D). These consequences proposed that TMP reduced LPS-stimulated accumulation of cellular inflammatory factors and elevation of ROS level in ATDC5 cells.

3.3. TMP hindered LPS-stimulated NF-κB and p38MAPK signaling activations

Inflammation factors are triggered by multiple intracellular signaling pathways, such as NF-κB and p38MAPK signaling pathways. Fig. 3A–D conferred that LPS treatment obviously increased the protein expression of p-lκBα, p-p65 and p-p38MAPK (P < .001). However, TMP pretreatment clearly prohibited LPS-triggered the enhancements of the protein expression of p-lκBα, p-p65 (P < .001) and p-p38MAPK (P < .01) as compared with LPS treatment group in ATDC5 cells (Fig. 3A–D). These outcomes indicated that NF-κB and p38MAPK signaling pathways were effectively blocked by TMP in LPS-stimulated ATDC5 cells.

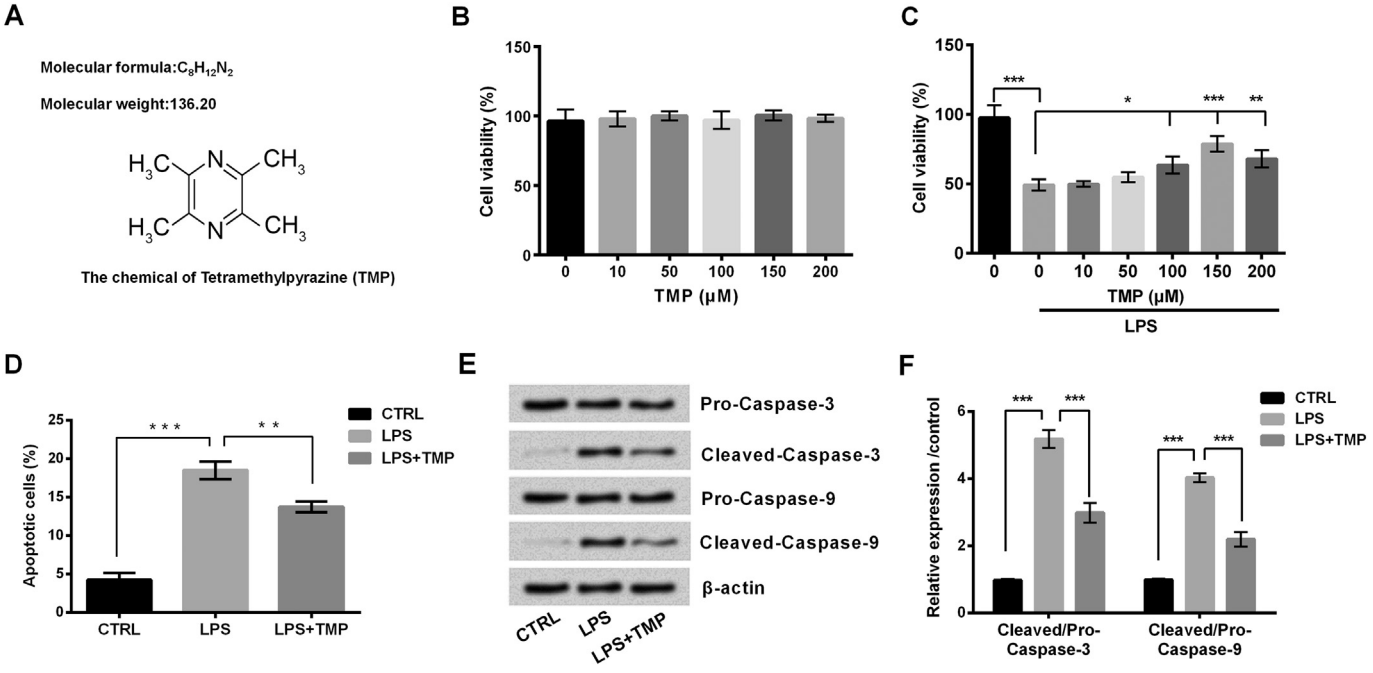

Fig. 1. Effects of TMP on cell apoptosis.
(A) The molecular structure of TMP.
(B) ATDC5 cells were treated with the different concentrations (0, 10, 50, 100, 150, 200 μM) of TMP for 24 h, the viability of ATDC5 cells was detected by CCK-8.
(C) ATDC5 cells were pre-treated with the different concentrations (0, 10, 50, 100, 150, 200 μM) of TMP for 24 h, and then were managed with LPS (5 μg/mL) for 12 h, cell viability was reassessed by CCK-8.
(D) The percentage of ATDC5 apoptotic cells under TMP pretreatment (24 h) and LPS treatment (12 h) was measured by flow cytometry assay.
(E and F) The protein levels of Pro/Cleaved-Caspase-3 and Pro/Cleaved-Caspase-9 under TMP pretreatment (24 h) and LPS treatment (12 h) was measured by western blot.
*, P < .05; **, P < .01; ***, P < .001.

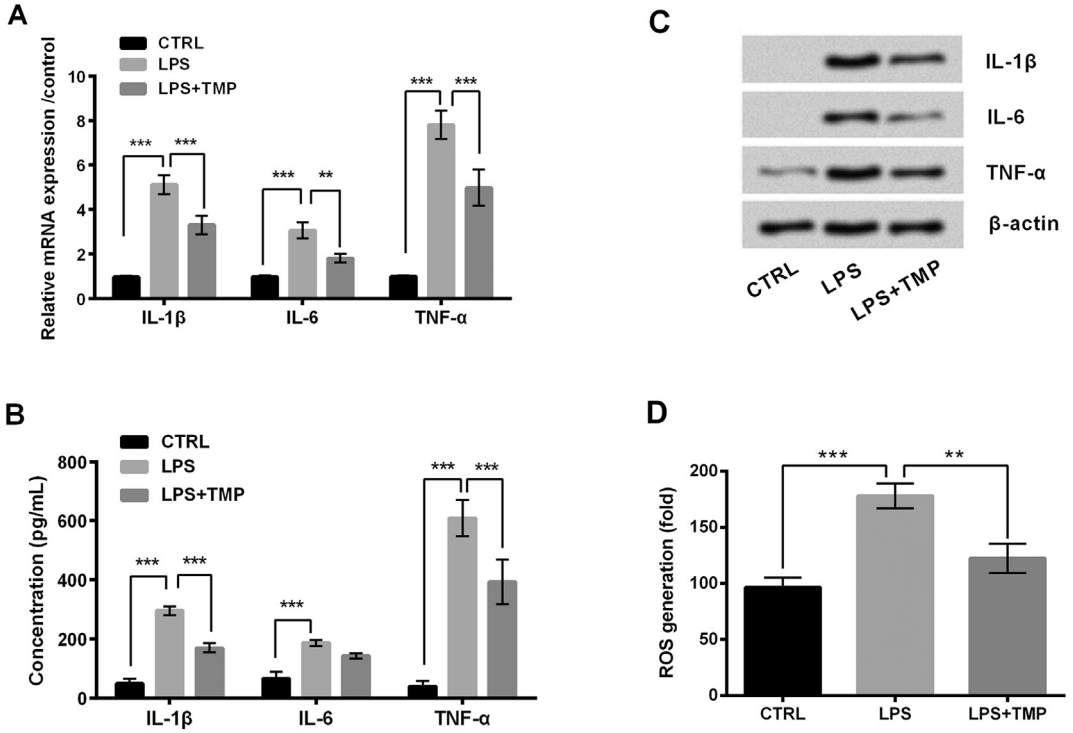

Fig. 2. Effects of TMP on cellular inflammatory factors and ROS generation.
(A) The mRNA expression of cellular inflammatory factors under TMP (24 h) and LPS (12 h) treatment was measured by qRT-PCR.
(B) The secretions of cellular inflammatory factors under TMP (24 h) and LPS (12 h) treatment were revealed by ELISA.
(C) The protein levels of cellular inflammatory factors under TMP (24 h) and LPS (12 h) treatment were measured by western blot.
(D) The ROS level under TMP (24 h) and LPS (12 h) treatment was measured by DCFH-DA staining.
**, P < .01; ***, P < .001.

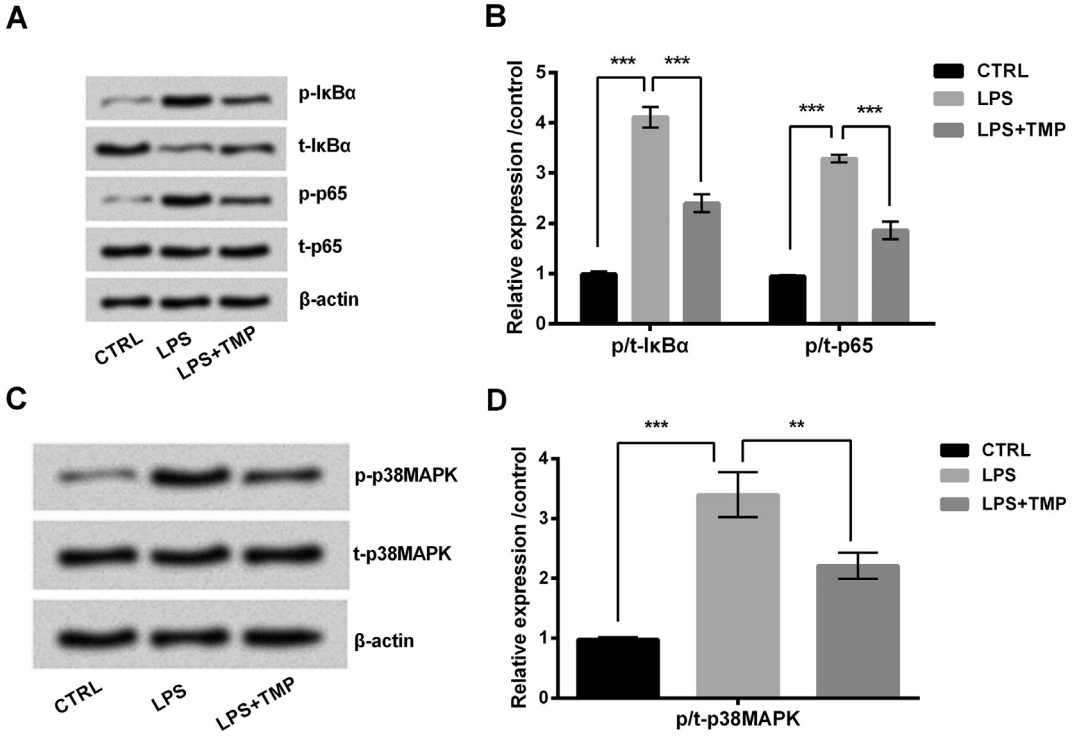

Fig. 3. Effects of TMP on NF-κB and p38MAPK pathways.
(A and B) The expression levels of p/t-IκBα and p/t-p65 under TMP (24 h) and LPS (12 h) treatment was detected by western blot.
(C and D) The expression level of p/t-p38MAPK under TMP (24 h) and LPS (12 h) treatment was detected by western blot.
**, P < .01; ***, P < .001.

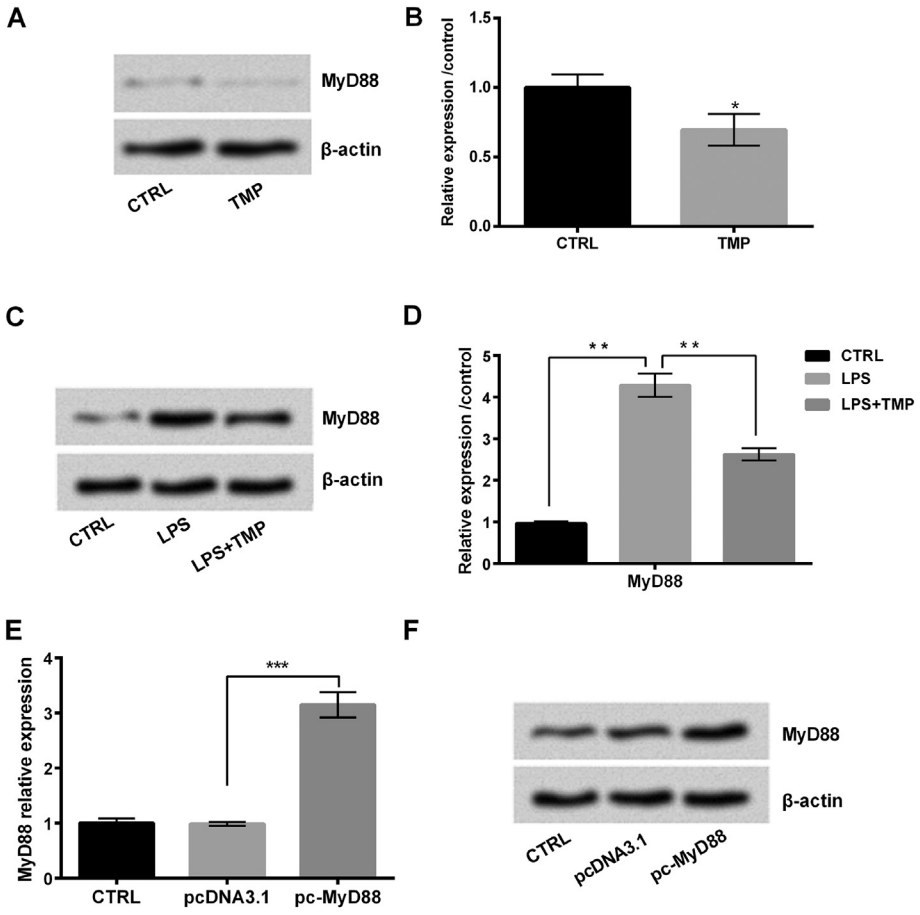

Fig. 4. Effects of TMP on MyD88 expression.
(A and B) The expression level of MyD88 under TMP (24 h) treatment was evaluated by western blot.
(C and D) The expression level of MyD88 under LPS (12 h) and TMP (24 h) treatment was measured by western blot.
(E)The mRNA expression of MyD88 after pcDNA3.1 and pc-MyD88 transfection (48 h) was investigated by qRT-PCR.
(F)The protein level of MyD88 after pcDNA3.1 and pc-MyD88 transfection (48 h) was detected by western blot.
*, P < .05; **, P < .01; ***, P < .001.

3.4. TMP down-regulated MyD88 expression in LPS-stimulated ATDC5 cells

MyD88 is an important inflammatory system adaptor protein in the TLR/MyD88/NF-κB signaling pathway and is an important node for transducing downstream signals (Qiao et al., 2019). We firstly discovered that TMP alone treatment clearly prohibited MyD88 expression in ATDC5 cells (P < .05, Fig. 4A and B). Additionally, we further investigated whether TMP modulated LPS-induced ATDC5 cells inflammatory injury via mediating MyD88 signaling pathway. Fig. 4C and D results disclosed that the protein level of MyD88 was distinctly increased under LPS treatment as relative to control group (P < .01). Under TMP pretreatment, MyD88 protein expression was down-regulated as compared to LPS treatment in ATDC5 cells (P < .01). Subsequently, we discovered that MyD88 was successfully overexpressed (P < .001) by transfection with pc-MyD88 in ATDC5 cells (Fig. 4E). Moreover, MyD88 protein expression was obviously increased by overexpression of MyD88 in ATDC5 cells (Fig. 4F). The outcomes declared that TMP cut down MyD88 protein level in LPS-stimulated ATDC5 cells, hinting that MyD88 might participate in mediating the functions of TMP in LPS-induced cell injury.

3.5. TMP attenuated LPS-induced apoptosis and cellular inflammatory factor expression and secretions by down-regulating MyD88

The above results had showed that TMP reduced LPS-induced cell apoptosis and relative mRNA expression and secretions of cellular inflammatory factors. In the following experiments, we further explored whether MyD88 took part in mediating LPS-stimulated cell apoptosis and cellular inflammatory factor expression. As shown in Fig. 5A–C, TMP-inhibited cell apoptosis and Cleaved-Caspase-3/−9 expression in LPS-treated ATDC5 cells were both overturned by overexpressed MyD88 (P < .01 or P < .001). In Fig. 6A and B, LPS enhanced mRNA expression and secretions of IL-1β, IL-6 and TNF-α were distinctly further up-regulated by overexpression of MyD88 (P < .001). After pretreatment with TMP, we discovered that LPS-triggered the elevation of mRNA expression and secretions of IL-1β, IL-6 and TNF-α were obviously prohibited by TMP (P < .05 or P < .001, Fig. 6C and D). Nonetheless, overexpressed MyD88 inverted the inhibitory impacts of TMP on IL-1β, IL-6 and TNF-α expression and production in LPS-treated ATDC5 cells (P < .05 or P < .001, Fig. 6C and D). The above data approved that TMP attenuated LPS-induced cell apoptosis and LPS-elevated cellular inflammatory factor expression and secretions by down-regulating MyD88 in ATDC5 cells.

3.6. TMP restrained LPS-prompted NF-κB and p38MAPK signaling activations through down-regulating MyD88

Above results had showed that TMP suppressed NF-κB and p38MAPK activations in LPS-stimulated ADTC5 cells. Next, we aimed to further explore whether MyD88 was involved in regulating the above phenomenon. Western blot results showed that MyD88 overexpression in LPS and TMP co-treated ATDC5 cells obviously enhanced p-lκBα, p-p65 and p-p38MAPK protein expression as compared with that in the correlative control group (P < .001, Fig. 7A–D). Altogether, these results indicated that TMP inhibited LPS-prompted the activations of NF-κB and p38MAPK via down-regulating MyD88.

4. Discussion

OA is a frequent disease in the elderly people, and the prevalence in China is quite high in 2010 (Tang et al., 2016). At present, the

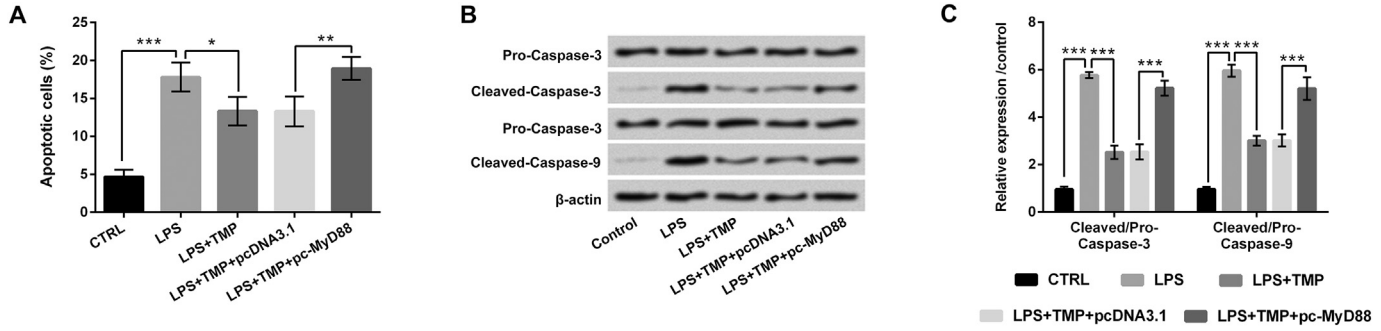

Fig. 5. Effects of MyD88 overexpression on cell apoptosis under TMP and LPS treatment.
After transfection with pcDNA3.1 and pc-MyD88 for 48 h, (A) the percentage of ATDC5 apoptotic cells under TMP (24 h) and LPS (12 h) treatment was determined by flow cytometry.
(B and C) The protein levels of Pro/Cleaved-Caspase-3 and Pro/Cleaved-Caspase-9 under TMP (24 h) and LPS (12 h) treatment was measured by western blot.
*, P < .05; **, P < .01; ***, P < .001.

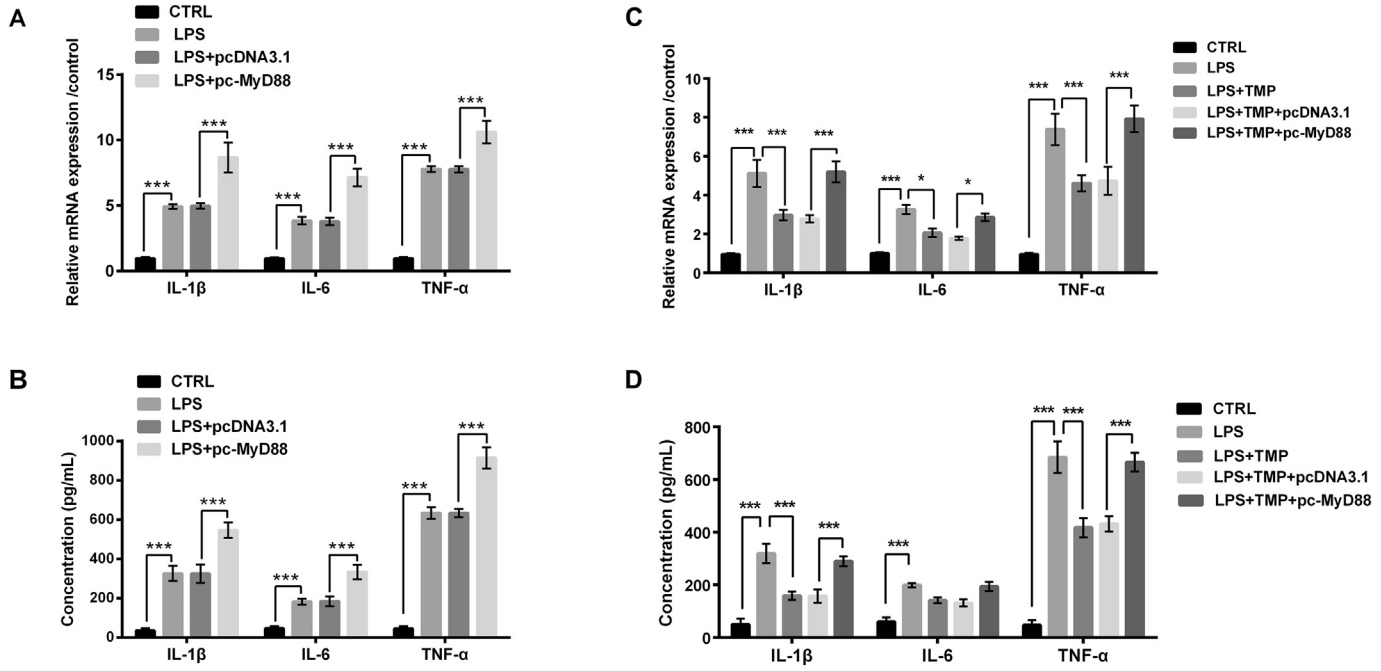

Fig. 6. Effects of MyD88 overexpression on cellular inflammatory factors.
After transfection with pcDNA3.1 and pc-MyD88 for 48 h and stimulation with LPS for 12 h, (A) The mRNA expression and (B) the concentrations of inflammatory factors (IL-1β, IL-6 and TNF-α) were evaluated by qRT-PCR and ELISA.
After transfection with pcDNA3.1 and pc-MyD88 for 48 h and co-stimulation with TMP (24 h) and LPS (12 h), (C) the mRNA expression and (D) the concentrations of inflammatory factors (IL-1β, IL-6 and TNF-α) were reassessed by qRT-PCR and ELISA.
*, P < .05; ***, P < .001.

treatment strategies for OA are still restrictive (Lohmander and Roos, 2007; Zhang et al., 2010). LPS is one of the major pathogen-associated molecules in the gram-negative bacteria, which has been proven to induce a series of inflammatory response via stimulating pro-inflammatory cytokines expression (Kim et al., 2012; Ronco, 2014). In cell and animal studies, LPS was utilized to establish OA model for analyzing the pathogenesis of OA and testing the new therapeutic target for the treatment of OA (Balaganur et al., 2014; Zhao et al., 2017). In our research, we exploited LPS to induce ATDC5 cell injury, and then the functions of TMP in LPS-injured cells were investigated. We discovered that TMP significantly alleviated LPS-triggered cell injury in ATDC5 cells. Moreover, the protective impacts were clearly overturned by MyD88 overexpression in LPS-stimulated cells. Besides, TMP hindered LPS-activated NF-κB and p38MAPK pathways via repression of MyD88.

TMP as a purified chemical component of Chinese herbal has been proven to have a wide range of pathological activities, comprising anti-apoptosis and anti-inflammation. For example, a research found that TMP could restrain LPS-induced rat peritoneal mesothelial cells apoptosis and hypoxia-induced cardiomyocyte apoptosis (Zhang et al., 2016). Additionally, TMP has been verified to extenuate cerebral ischemic damage via restraining inflammatory cell activities, intracellular inflammatory factors production, and neuronal apoptosis (Chang et al., 2007; Xiao et al., 2010). An interesting research has been reported that TMP could attenuate LPS-induced cardiomyocyte injury via improving mitochondrial function mediated by 14–3-3γ (Huang et al., 2018). Moreover, TMP could relieve LPS-induced pancreatic β-cell Min6 injury via regulation of miR-101/MKP-1 (Chen et al., 2019). Further, the protective effect of TMP on human type II alveolar epithelial cells injury triggered by LPS was also testified (Xiao-Yong and Zhou, 2017). In consistent with the predecessor's research, we also discovered that TMP prohibited LPS-induced cell apoptosis. At the same

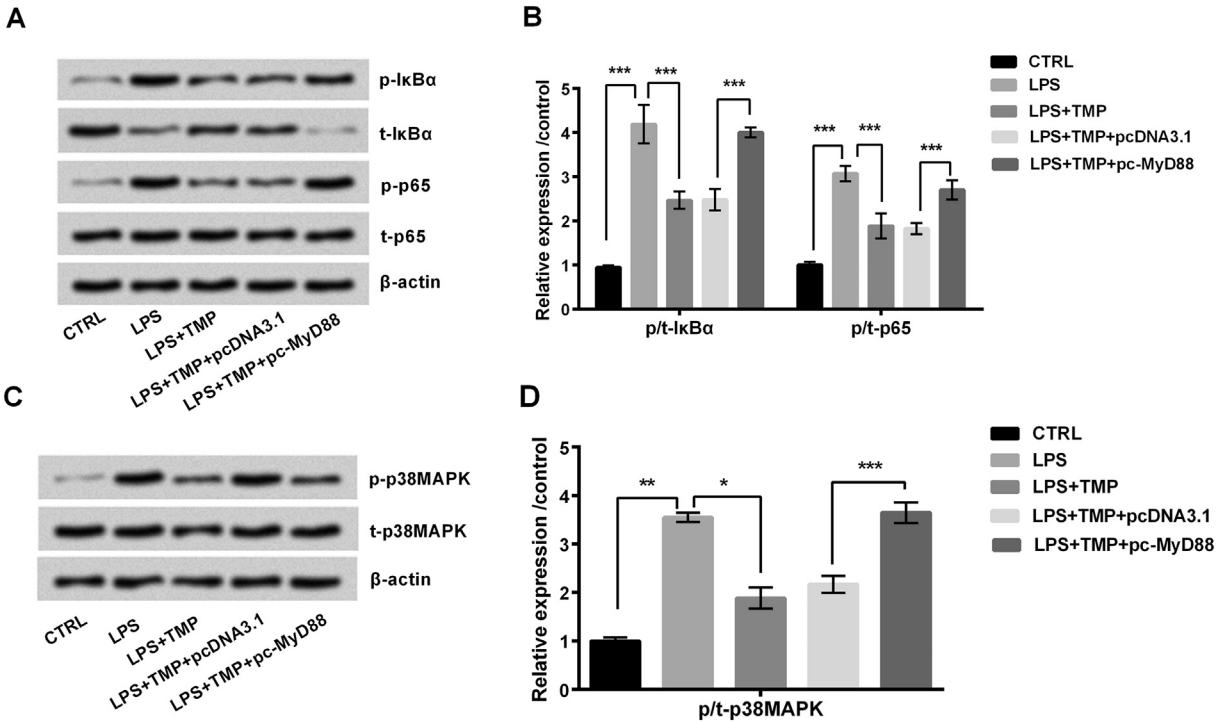

Fig. 7. Effects of MyD88 overexpression on NF-κB and p38MAPK pathways.
(A and B) The expression levels of p/t-IκBα and p/t-p65 after pc-MyD88 transfection (48 h) and LPS (12 h) + TMP (24 h) treatment were investigated by western blot.
(C and D) The expression level of p/t-p38MAPK after pc-MyD88 transfection (48 h) and LPS (12 h) + TMP (24 h) treatment was estimated by western blot.
*, P < .05; **, P < .01; ***, P < .001.

time, we found that LPS treatment promoted a series high expression of cellular inflammatory factors. More importantly, TMP pretreatment remarkably reduced the expression and secretion of these inflammatory factors under LPS treatment in ATDC5 cells. The data indicated that TMP as a protective molecule attenuated LPS-motivated apoptosis and inflammatory damage.

MyD88 is one of the general adaptor proteins in toll-like receptor 4 (TLR4) pathway, which plays a vital role in promoting the signal transduction of downstream inflammatory cytokine (Qiao et al., 2019). More and more evidences showed that various tissue damage could activate TLR4 by inducing an innate immune response, and mediating the intracellular signal transduction of downstream signaling molecule MyD88 (Kim et al., 2016; Prakash et al., 2015). In systemic inflammatory response syndrome (SIRS) research found that dioscin as a steroidal saponin from medicinal plant exerted the anti-inflammatory effects on SIRS via adjusting MyD88 signaling pathway (Zhao et al., 2018). The related research from Liu et al. disclosed that IL-1β triggered a significant up-regulation of TLR4 and MyD88 in osteoarthritic chondrocytes in vitro (Liu et al., 2014). Our result was partly similar with this research that LPS evoked an obvious elevation of MyD88 in ATDC5 cells. But, up-regulation of MyD88 triggered by LPS was clearly declined by TMP. According to this finding, we speculated that MyD88 might participate in regulating the anti-inflammatory function of TMP in LPS-injured ATDC5 cells. In our research, we discovered that MyD88 overexpression significantly induced cell apoptosis as well as increased secretions of inflammatory factors in LPS and TMP co-processed ATDC5 cells. The results showed that MyD88 might join in TMP attenuated LPS-induced ATDC5 cells injury.

NF-κB and p38MAPK pathways have been certified to participate in the regulation of inflammatory response and apoptosis in various diseases (Kim et al., 2018; Kim et al., 2017). Previous research showed that CXC195 as a TMP analog regulated the anti-inflammatory function by inhibiting the TLR4/MyD88 through activations of NF-κB and p38MAPK pathways in LPS-stimulated human hepatocellular carcinoma (HCC) cells (Wang et al., 2015b). Additionally, previous study reported that NF-κB and p38MAPK signaling pathways were both activated and participated in the pathogenesis of OA (Wang et al., 2015a). Therefore, in our experimentation, we made a thorough inquiry the influence of TMP in p38MAPK and NF-κB pathways in LPS-managed ATDC5 cells. The results manifested that TMP pretreatment reduced the activations of NF-κB and p38MAPK in LPS-treated ATDC5 cells. More interestingly, we observed that overexpressed MyD88 inverted the functions of TMP in NF-κB and p38MAPK pathways in LPS-stimulated ATDC5 cells. These findings indicated that NF-κB and p38MAPK pathways might join in mediating the protective functions of TMP in LPS-injured ATDC5 cells.

Taken together, our research firstly demonstrated that TMP attenuated LPS-induced inflammatory damage in ATDC5 cells by blocking NF-κB and p38MAPK pathways via down-regulation of MyD88. This article lays the foundation for in-depth research the function of TMP, and also provides a new target for the clinical treatment of OA.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yexmp.2019.104317.

Data availability statement

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

Funding

This study was supported by the Project of the Jilin Provincial Science and Technology Department, China (No.20191008007TC).

Declaration of competing interest

Authors declare that there is no conflict of interests.

References

Antonelli, M.C., Starz, T.W., 2012. Assessing for risk and progression of osteoarthritis: the nurse's role: understanding pathophysiology, epidemiology, and risk will aid nurses who are seeking to expand their role in management. Orthop. Nurs. 31, 98–102.

Balaganur, V., et al., 2014. Chondroprotective and anti-inflammatory effects of S-methylisothiourea, an inducible nitric oxide synthase inhibitor in cartilage and synovial explants model of osteoarthritis. J. Pharm. Pharmacol. 66, 1021–1031.

Cao, J., et al., 2015. Tetramethylpyrazine (TMP) exerts antitumor effects by inducing apoptosis and autophagy in hepatocellular carcinoma. Int. Immunopharmacol. 26, 212–220.

Cao, S., et al., 2016. Ligustrazine for the treatment of unstable angina: a meta-analysis of 16 randomized controlled trials. Evid. Based Complement. Alternat. Med. 2016, 8617062.

Chang, Y., et al., 2007. Tetramethylpyrazine suppresses HIF-1alpha, TNF-alpha, and activated caspase-3 expression in middle cerebral artery occlusion-induced brain ischemia in rats. Acta Pharmacol. Sin. 28, 327–333.

Chen, D., et al., 2019. Tetramethylpyrazine relieves LPS-induced pancreatic β-cell Min6 injury via regulation of miR-101/MKP-1. Artif. Cells Nanomed. Biotechnol. 47, 2545–2552.

Gao, H.J., et al., 2015. Ligustrazine monomer against cerebral ischemia/reperfusion injury. Neural Regen. Res. 10, 832–840.

Huang, B., et al., 2018. Tetramethylpyrazine attenuates lipopolysaccharide-induced cardiomyocyte injury via improving mitochondrial function mediated by 14-3-3γ. Eur. J. Pharmacol. 832, 67–74.

Ish-Shalom, S., Lichter, A., 2010. Analysis of fungal gene expression by real time quantitative PCR. Methods Mol. Biol. 638, 103–114.

Ju, X.D., et al., 2010. The protective effect of tetramethylpyrazine on cartilage explants and chondrocytes. J. Ethnopharmacol. 132, 414–420.

Kim, T.H., et al., 2012. Anti-inflammatory functions of purpurogallin in LPS-activated human endothelial cells. BMB Rep. 45, 200–205.

Kim, Y.K., et al., 2016. NOD-like receptors in infection, immunity, and diseases. Yonsei Med. J. 57, 5–14.

Kim, J.S., et al., 2017. PPARgamma regulates inflammatory reaction by inhibiting the MAPK/NF-kappaB pathway in C2C12 skeletal muscle cells. J. Physiol. Biochem. 73, 49–57.

Kim, E.A., et al., 2018. Anti-inflammatory effect of Apo-9′-fucoxanthinone via inhibition of MAPKs and NF-kB signaling pathway in LPS-stimulated RAW 264.7 macrophages and zebrafish model. Int. Immunopharmacol. 59, 339–346.

Koushki, D., et al., 2015. Efficacy of some non-conventional herbal medications (sulforaphane, tanshinone IIA, and tetramethylpyrazine) in inducing neuroprotection in comparison with interleukin-10 after spinal cord injury: a meta-analysis. J Spinal Cord Med. 38, 13–22.

Liu, L., et al., 2014. Protective effect of resveratrol against IL-1beta-induced inflammatory response on human osteoarthritic chondrocytes partly via the TLR4/MyD88/NF-kappaB signaling pathway: an "in vitro study". Int. J. Mol. Sci. 15, 6925–6940.

Lohmander, L.S., Roos, E.M., 2007. Clinical update: treating osteoarthritis. Lancet 370, 2082–2084.

Losina, E., et al., 2013. Lifetime risk and age at diagnosis of symptomatic knee osteoarthritis in the US. Arthritis Care Res. (Hoboken) 65, 703–711.

Mobasheri, A., et al., 2017. The role of metabolism in the pathogenesis of osteoarthritis. Nat. Rev. Rheumatol. 13, 302–311.

Prakash, P., et al., 2015. Cellular fibronectin containing extra domain A promotes arterial thrombosis in mice through platelet Toll-like receptor 4. Blood 125, 3164–3172.

Qiao, C., et al., 2019. Long noncoding RNA ANRIL contributes to the development of ulcerative colitis by miR-323b-5p/TLR4/MyD88/NF-kappaB pathway. Biochem. Biophys. Res. Commun. 508, 217–224.

Ronco, C., 2014. Lipopolysaccharide (LPS) from the cellular wall of gram-negative bacteria, also known as endotoxin, is a key molecule in the pathogenesis of sepsis and septic shock. Preface Blood Purif. 37 (Suppl. 1), 1.

Shao, H., et al., 2015. Efficacy of ligustrazine injection as adjunctive therapy for angina pectoris: a systematic review and meta-analysis. Med. Sci. Monit. 21, 3704–3715.

Tang, X., et al., 2016. The prevalence of symptomatic knee osteoarthritis in China: results from the China health and retirement longitudinal study. Arthritis Rheumatol. 68, 648–653.

Wang, D., et al., 2015a. Thymoquinone inhibits IL-1beta-induced inflammation in human osteoarthritis chondrocytes by suppressing NF-kappaB and MAPKs signaling pathway. Inflammation. 38, 2235–2241.

Wang, Y., et al., 2015b. CXC195 suppresses proliferation and inflammatory response in LPS-induced human hepatocellular carcinoma cells via regulating TLR4-MyD88-TAK1-mediated NF-kappaB and MAPK pathway. Biochem. Biophys. Res. Commun. 456, 373–379.

Xiao, X., et al., 2010. Neuroprotection and enhanced neurogenesis by tetramethylpyrazine in adult rat brain after focal ischemia. Neurol. Res. 32, 547–555.

Xiao-Yong, H.U., Zhou, F.C., 2017. Tetramethylpyrazine inhibits LPS-induced inflammation of human type II alveolar epithelial cells. Basic Clin. Med. 37, 839–844.

Yao, Y., Wang, Y., 2013. ATDC5: an excellent in vitro model cell line for skeletal development. J. Cell. Biochem. 114, 1223–1229.

Zhang, W., et al., 2010. OARSI recommendations for the management of hip and knee osteoarthritis: part III: changes in evidence following systematic cumulative update of research published through January 2009. Osteoarthr. Cartil. 18, 476–499.

Zhang, H., et al., 2016. Effect of ligustrazine on rat peritoneal mesothelial cells treated with lipopolysaccharide. Ren. Fail. 38, 961–969.

Zhang, X., et al., 2018. Intra-articular delivery of tetramethylpyrazine microspheres with enhanced articular cavity retention for treating osteoarthritis. Asian J. Pharm. Sci. 13, 229–238.

Zhao, C., et al., 2017. Knockdown of microRNA-203 alleviates LPS-induced injury by targeting MCL-1 in C28/I2 chondrocytes. Exp. Cell Res. 359, 171–178.

Zhao, X., et al., 2018. Protective effects of dioscin against systemic inflammatory response syndromevia adjusting TLR2/MyD88/NFkappab signal pathway. Int. Immunopharmacol. 65, 458–469.