

Ononin induces cell apoptosis and reduces inflammation in rheumatoid arthritis fibroblast-like synoviocytes by alleviating MAPK and NF- κ B signaling pathways

Yue Meng^{1#}, Jian Ji^{2#}, Xiao Xiao³, Minghan Li⁴, Shangbo Niu⁴, Youhua He⁴, Guojun Tong⁵ and Chenglong Pan¹✉

¹Department of Joint Surgery, The Fifth Affiliated Hospital of Southern Medical University, Guangzhou, Guangdong Province, 510900, China;

²Department of Breast Surgery, The Fifth Affiliated Hospital of Southern Medical University, Guangzhou, Guangdong Province, 510900, China;

³Department of Spine Orthopedics, Shenzhen People's Hospital, Shenzhen, Guangdong Province, 518055, China; ⁴Department of Spine Orthopedics, NanFang Hospital, Guangzhou, Guangdong Province, 510515, China; ⁵Department of Spine Orthopedics, Hangzhou Xiaoshan Hospital of TCM, Hangzhou, Zhejiang Province, 311200, China

As a kind of chronic inflammatory diseases, rheumatoid arthritis (RA) has a low cure rate and easy recurrence. It has widely reported that abnormal activation of mitogen-activated protein kinase (MAPK) and nuclear factor kappa-B (NF- κ B) signaling pathways are associated with the development of RA inflammation. Blocking the inflammatory signaling pathways of rheumatoid arthritis fibroblast-like synoviocytes (RA-FLS) can delay the development of RA. Ononin is a natural isoflavone glycoside and plays a key role in modulating inflammation related signaling pathways. However, whether Ononin exerts anti-inflammatory effects on RA inflammation remains unknown. In this study, we evaluated the therapeutic effect of Ononin on RA by establishing a tumor necrosis factor α (TNF- α)-induced RA-FLS cell model. Our data confirmed that Ononin could alleviate TNF- α -induced RA-FLS and MH7A cells viability, increase cell apoptosis, decrease the production of pro-inflammatory cytokines like interleukin-1 β (IL-1 β) and interleukin 6 (IL-6), and further inhibit the abnormal activation of NF- κ B and MAPK pathways. Our results suggested that Ononin could be a potential therapeutic agent for RA.

Key words: Rheumatoid arthritis, Ononin, rheumatoid arthritis fibroblast-like synoviocytes, MAPK, NF- κ B

Received: 27 October, 2020; revised: 15 March, 2021; accepted: 07 May, 2021; available on-line: 02 June, 2021

✉ e-mail: clpan666@163.com

#Those Authors contributed equally to this work

Acknowledgements of Financial Support: This work was supported by Research Initiative Project of Southern Medical University (Grant No.PY2017N033) and President Foundation of The Fifth Affiliated Hospital of Southern Medical University (Grant No.YZ2017MS006, Grant No.YZ2018MS002).

Abbreviations: IL-1 β , interleukin-1 β ; IL-6, interleukin 6; MAPK, mitogen-activated protein kinase; NF- κ B, nuclear factor kappa-B; RA, rheumatoid arthritis; RA-FLS, rheumatoid arthritis fibroblast-like synoviocytes; TNF- α , tumor necrosis factor α

INTRODUCTION

Rheumatoid arthritis (RA), one kind of serious chronic autoimmune and inflammatory diseases, is characterized by inflammation of the joints and parenchyma (Guo *et al.*, 2018). Previous study indicates that synovium is the main lesion site of RA, abnormal synovial hyperplasia and inflammatory cells infiltrating the synovium will eventually lead to inflammatory destruction of the joint

(Buchbender *et al.*, 2013). RA has a low cure rate and is prone to relapse, which is caused by the abnormal immune responses (Heidari, 2011). Notably, inhibiting inflammation-related pathways can delay the progression of RA (Chimenti *et al.*, 2015). Non-steroidal anti-inflammatory drugs plus anti-rheumatic medicines and corticosteroids are often used in the first-line treatment of RA, but the therapeutic effects still remains to be improved (Kumar & Banik, 2013). Previous study indicates rheumatoid arthritis fibroblast-like synoviocytes (RA-FLS) cells play a key role in the initiation and progression in synovitis induced RA (Bartok & Firestein, 2010). As the inflammation occurs, RA-FLS cells produce apoptosis resistance, excessive proliferation, migration and invasion, resulting in the destruction of the joint tissue (Shi *et al.*, 2016). Thus, exploring the apoptosis of RA-FLS caused by inflammation is of great significance for further investigating the pathogenesis of RA and improving the treatment strategy.

MAPK and NF- κ B signaling pathways are considered to be important signaling pathways involved in the development of RA inflammation (Yang *et al.*, 2017). The MAPK family contains three major classes, such as JNK1/JNK2/JNK3, ERK1/ERK2 and p38 (Johnson & Lapadat, 2002). Activated MAPKs induces a series of downstream signaling cascade including ERK, JNK and p38 cascades, which implicated in regulating various cellular processes including cell survival/apoptosis, proliferation and inflammatory responses (Kim & Choi, 2010). NF- κ B, a collective name for dimeric transcription factors including cRel, RelA (p65), RelB, NF- κ B1 and NF- κ B2, is a key factor in the transcription of many inflammatory genes (Shih *et al.*, 2015). NF- κ B can be activated by a variety of pathogenic stimulation, including bacterial or viral proteins, cytokines, radiation and oxidative stress (Karin *et al.*, 2004). Activated NF- κ B leads to the release of diverse pro-inflammatory factors, including MCP-1, cyclooxygenase-2 (COX-2), tumor necrosis factor α (TNF- α), IL-1 β and IL-6, which are essential for the inflammatory response. (Makarov, 2001) It has also demonstrated that RA is associated with the abnormal activation of the MAPK and NF- κ B pathways (Liu *et al.*, 2017).

Ononin (Formononetin 7-O-glucoside) is a natural isoflavone that widely distributed in a variety of foodborne plants such as *Astragalus membranaceus*, soybean, kudzu, lupine and broccoli (Dong *et al.*, 2017). It has

been reported that Ononin possesses anti-inflammatory effect on zebrafish model induced by lipopolysaccharide, which can reduce the production of proinflammatory cytokines like TNF- α , IL-1 β , IL-6 and IFN- γ , increase the expression of anti-inflammatory factor IL-10, and regulate the activity of MAPK pathway (Luo *et al.*, 2019). In addition, Ononin can inhibit the expression of NO, PGE2, TNF- α , IL-1 β and IL-6 by inhibiting the activation of I κ B α and MAPKs, and then plays an anti-inflammatory role in LPS stimulated macrophage RAW264.7 (Dong *et al.*, 2017). Besides, Ononin is also reported to exert inhibitory and pro-apoptotic effects on cell proliferation in breast cancer cell by down-regulating PI3K/AKT/mTOR signaling pathway (Zhang *et al.*, 2018). However, whether Ononin has anti-inflammatory and pro-apoptotic effects on RA remains unknown.

At present, we established the TNF- α -induced RA-FLS and MH7A cell model and found that Ononin alleviated the cell viability, increased cell apoptosis, decreased the production of pro-inflammatory cytokines like IL-1 β and IL-6, and further inhibited the abnormal activation of NF- κ B and MAPK pathways in TNF- α -induced RA-FLS cells. Our results indicated that Ononin was suggested to be a promising agent for the treatment of RA.

MATERIALS AND METHODS

Cell culture

RA-FLS and MH7A were cultured in DMEM medium with 10% FBS and 1% penicillin/streptomycin. Subsequently, RA-FLS and MH7A cells were randomly divided into 5 groups and stimulated by TNF- α (10 ng/ml, PeproTech) and Ononin (HY-N0270, MedChemExpress) with different concentration: 1) Control, 2-TNF- α , 3-TNF- α +Ononin (5 μ M), 4-TNF- α +Ononin (10 μ M), 5-TNF- α +Ononin (50 μ M).

MTT assay

Cell viability was detected by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Briefly, TNF- α -induced RA-FLS and MH7A cells (5 groups in total described in the part of cell culture) were randomly placed into 96-well plates (5 \times 10³/well) and incubated for 4 days. Cells were incubated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (0.5 mg/ml, Sigma) for 4 h at 37°C. Subsequently, the medium were removed and cells were washed with PBS. And then 100 μ L dimethyl sulfoxide (DMSO) was added, and the microplate reader (Bio-Rad, USA) at 570 nm was used to detect the OD value.

Flow cytometry analysis

Cell apoptosis was measured by FACS Calibur instrument (BD Biosciences, USA), equipped with CellQuest Pro 5 MacOS 9 software. Briefly, RA-FLS and MH7A (1 \times 10⁵ cells) were stimulated by TNF- α (10 ng/ml, PeproTech) and Ononin with various concentrations of 5, 10 and 50 μ M for 48 h. Then the collected cells were washed by cold PBS buffer, subsequently fixed with 70% alcohol under 4°C for 12 h and stained with propidium iodide under 37°C for 30 min prior to flow cytometry (BD Biosciences, USA) analysis.

Enzyme-linked immunosorbent assay (ELISA)

RA-FLS and MH7A cells were incubated in 6-well plates containing 2 mL containing 1 \times 10⁵ cells per well,

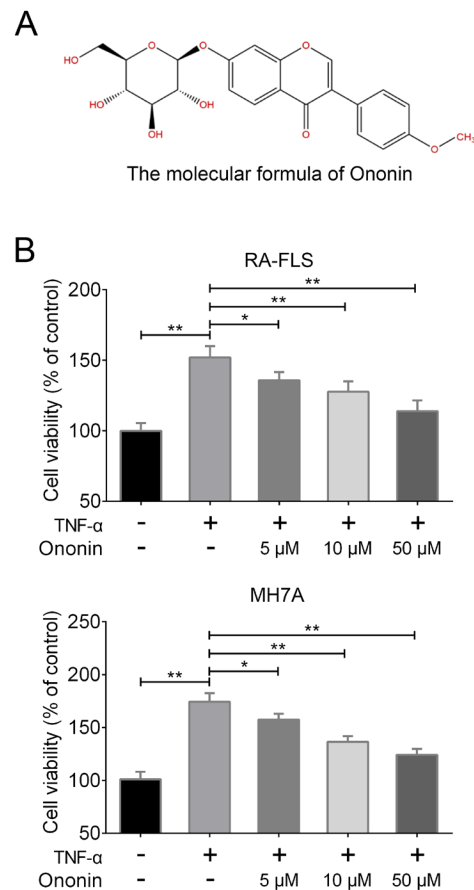


Figure 1. Ononin regulated the cell viability of TNF- α -induced RA-FLS and MH7A cells.

(A) The molecular formula of Ononin. (B) Ononin decreased the cell viability of TNF- α -induced RA-FLS and MH7A. Data were presented as means \pm S.D. * p <0.05, ** p <0.01.

and then stimulated by TNF- α (10 ng/ml, PeproTech) and Ononin with various concentrations of 5, 10 and 50 μ M for 24h. Supernatants were harvested and expression level of IL-1 β and IL-6 were analyzed by ELISA (Dakewe, Beijing, China) according to the manufacturer's instructions.

Western blot assay

Total proteins from RA-FLS and MH7A cells were collected with RIPA lysis buffer (9800, Cell Signaling, Danvers, MA) and separated by SDS-PAGE. After transferred onto PVDF membrane, membranes were blocked and subsequently incubated using the specific antibodies against GAPDH (ab9485), Bcl-2 (ab59348), Bax (ab173026), cleaved caspase-3 (ab2302), cleaved caspase-9 (ab2324), p-ERK (ab192591), ERK (ab17942), p-p38 (ab4822), p38 (ab27986), p-JNK (ab47337), JNK (ab10664), p-NF- κ B (ab86299) and NF- κ B (ab231481) purchased from Abcam (Cambridge, UK). Then the membranes were subjected to HRP-conjugated secondary antibodies for 1 h at room temperature. Finally, signals were visualized by an ECL kit.

Statistical analysis

Data are displayed as mean \pm S.D. The data was analyzed by GraphPad Prism (ver. 5.04). p <0.05 Denotes statistically significant. *Indicates p <0.05, **indicates p <0.01.

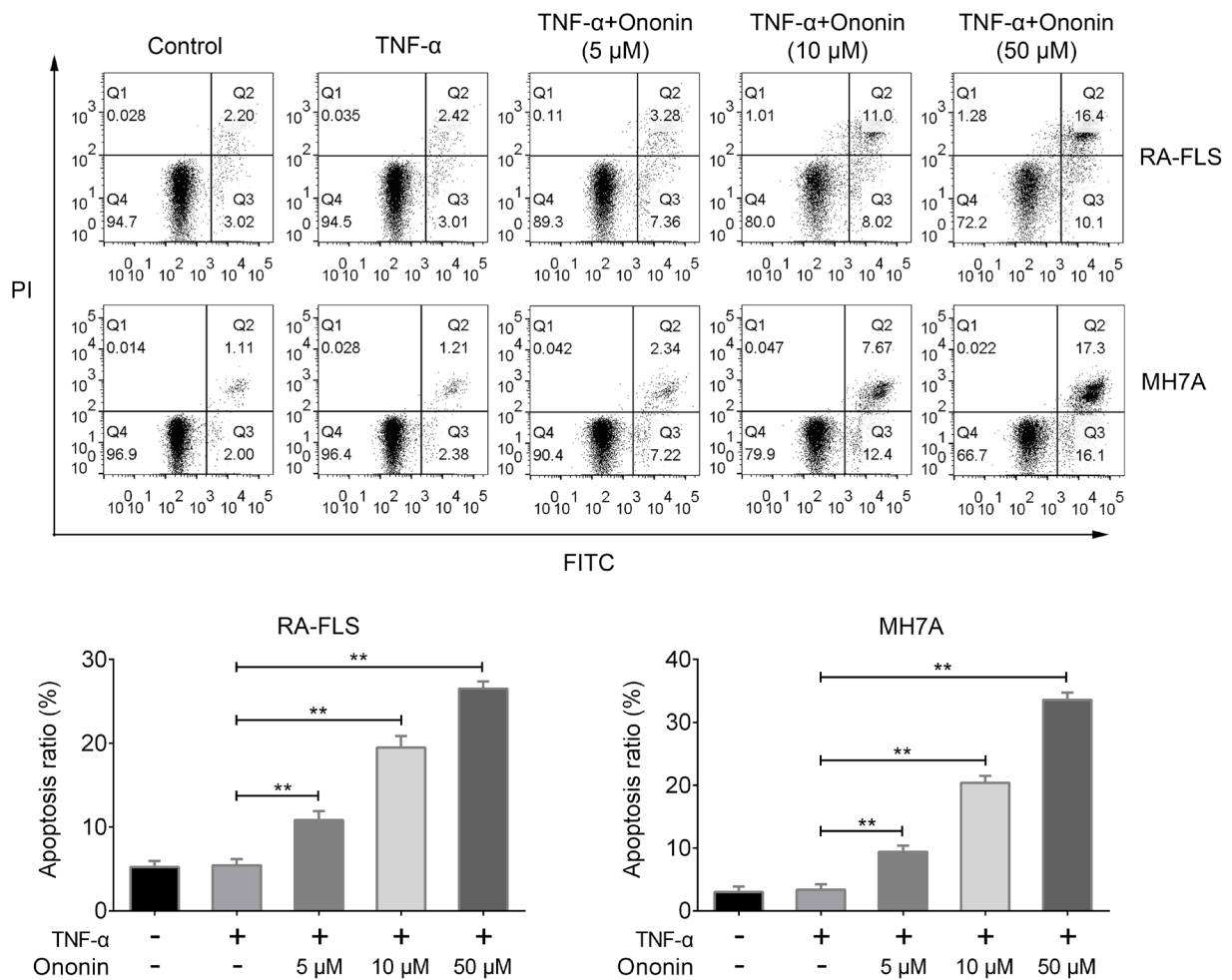


Figure 2. Ononin promoted the cell apoptosis in TNF- α -induced RA-FLS and MH7A cells. Data were presented as means \pm S.D. ** $p < 0.01$.

RESULTS

Ononin alleviated the viability in TNF- α -induced RA-FLS and MH7A cells

To evaluate the therapeutic effect of Ononin, TNF- α -induced RA-FLS and MH7A cells model was constructed. The molecular formula of Ononin was displayed in Fig. 1A. As shown in Fig. 1B, MTT assay showed that TNF- α could significantly induce the cell proliferation in RA-FLS and MH7A cells ($p < 0.01$). However, compared to TNF- α stimulation group, Ononin treatment could significantly alleviate the cell viability both in RA-FLS and MH7A cells ($p < 0.05$ and $p < 0.01$). Additionally, Ononin treatment alleviated cell viability in a concentration-dependent manner. Thus we assumed Ononin could significantly relieve TNF- α -induced RA-FLS and MH7A cells.

Ononin promoted the apoptosis in TNF- α -induced RA-FLS and MH7A cells

The cell apoptosis was assessed by flow cytometry analysis. Compared with control group, TNF- α stimulation would not affect the cell apoptosis both in TNF- α -induced RA-FLS and MH7A cells, which was consistent

with previous study (Yamaguchi *et al.*, 2004). However, after Ononin treatment, the cell apoptosis ratios were significantly increased compared to control and Ononin untreated cells (Fig. 2). These results verified Ononin may play a protective effect by attenuating the cell apoptosis in TNF- α -induced RA-FLS and MH7A cells.

Ononin regulated apoptosis protein expression in TNF- α -induced RA-FLS and MH7A cells

To further evaluate the protective effect of Ononin on TNF- α -induced cell proliferation in TNF- α -induced RA-FLS and MH7A cells, the expression of cell apoptosis-related proteins was detected by using western blot assay. Previous study indicated that apoptosis-related proteins like B-cell lymphoma 2 (Bcl-2), Bcl-2 associated X (Bax), cleaved caspase-3 and cleaved caspase-9, are modulated during the apoptosis process (Russo *et al.*, 2018). The results showed that compared to control and TNF- α -stimulated RA-FLS and MH7A cells, the apoptosis-related protein expression of Bcl-2 was reduced by Ononin treatment, and a down-regulated tendency was found with the increase of Ononin concentration. However, the expression of Bax, Cleaved caspase-3 and Cleaved caspase-9 proteins was increased by Ononin treatment, and Ononin treatment caused this phenomenon in a dose dependent manner (Fig. 3). Taken together

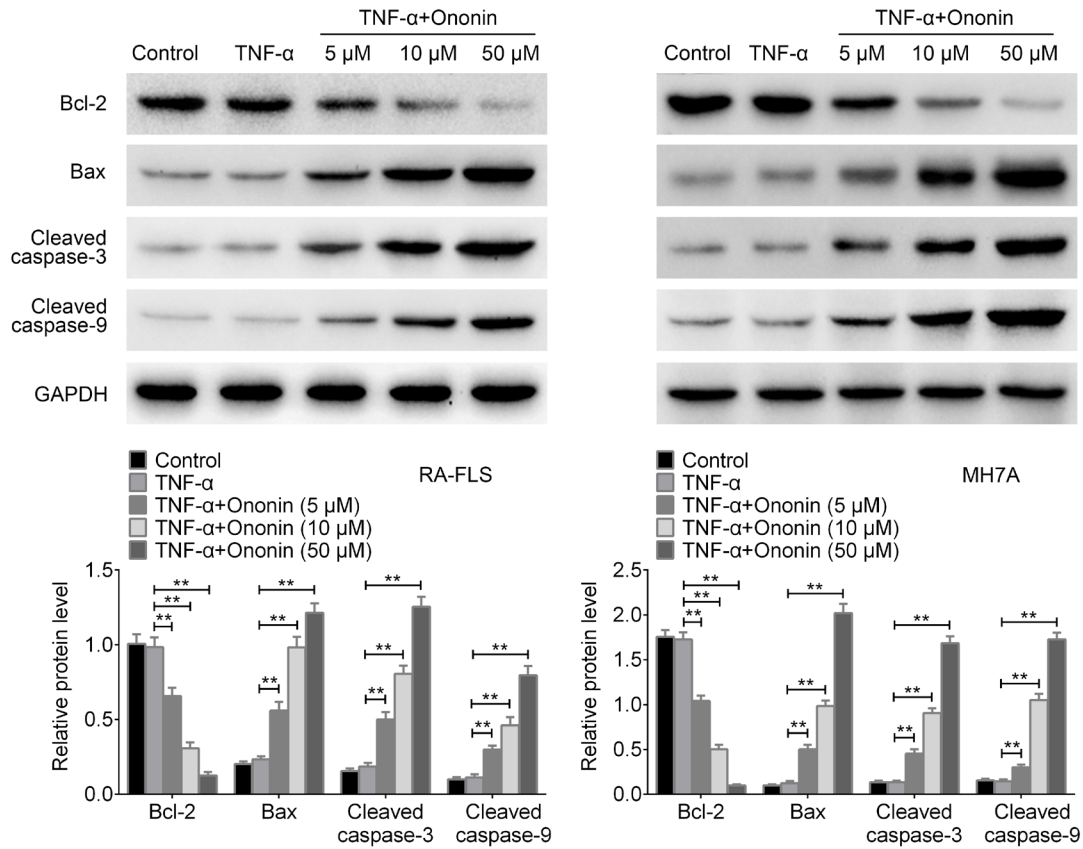


Figure 3. Ononin regulated the apoptosis-related protein expression in TNF-α-induced RA-FLS and MH7A cells. Data were presented as means ± S.D. ***p*<0.01.

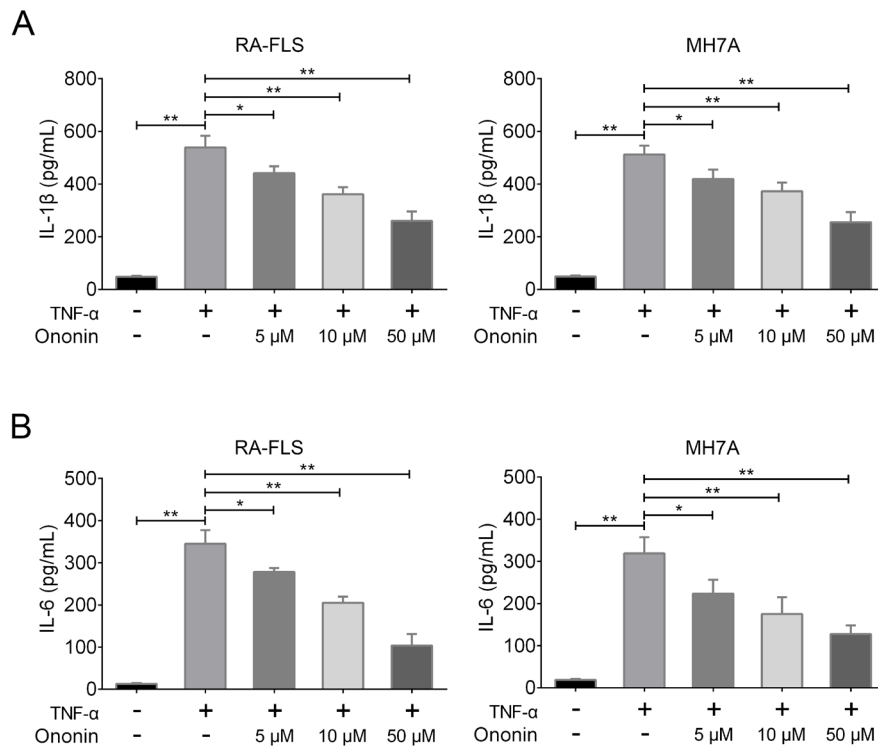


Figure 4. Ononin inhibited the levels of inflammatory cytokines in TNF-α-induced RA-FLS (A) and MH7A (B) cells. Data were presented as means ± S.D. **p*<0.05, ***p*<0.01.

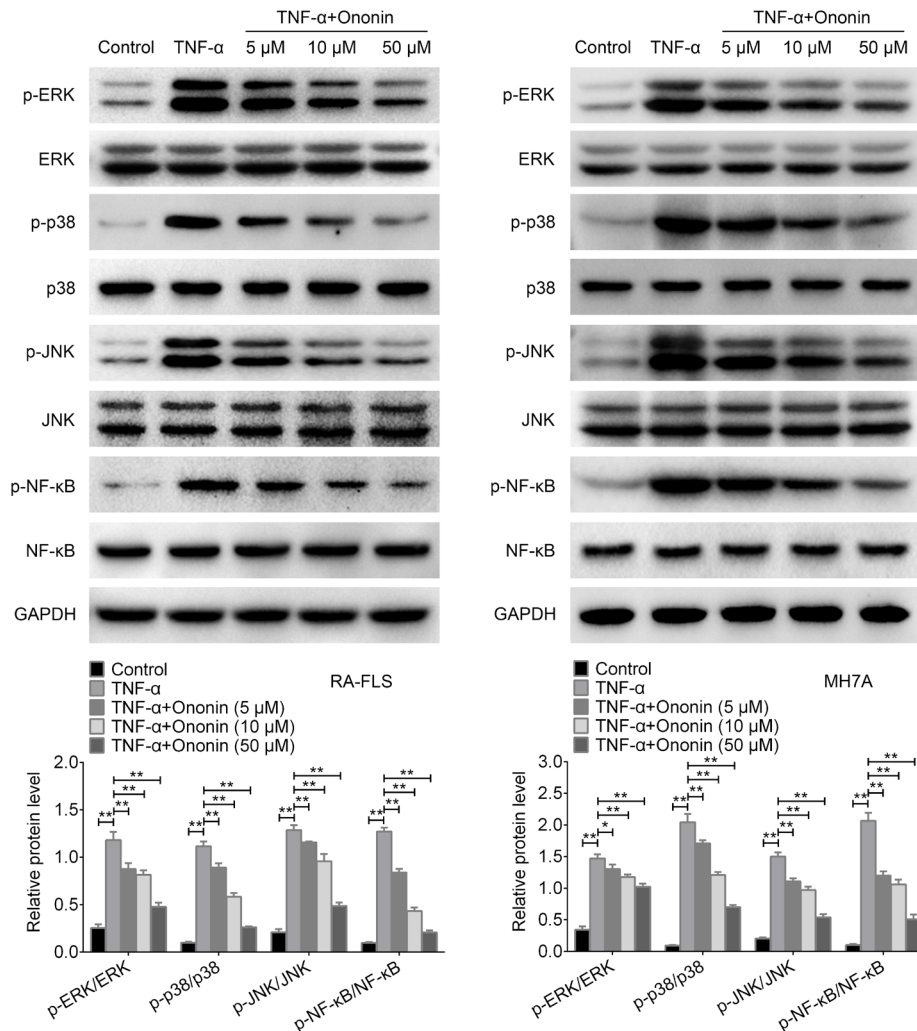


Figure 5. Ononin attenuated the activity of MAPK and NF- κ B signaling pathways. Data were presented as means \pm S.D. ** $p < 0.01$.

er, Ononin decreased the expression of apoptosis protein Bcl-2 and increased the expression of Bax, Cleaved caspase-3 and Cleaved caspase-9 proteins both in TNF- α -induced RA-FLS and MH7A cells.

Ononin reduced inflammatory cytokine production in TNF- α -induced RA-FLS and MH7A cells

Both IL-6 and IL-1 β are important pro-inflammatory cytokines responsible for the inflammation of rheumatoid arthritis (Alunno *et al.*, 2017). As shown in Fig. 4, TNF- α could significantly induce the inflammatory cytokines release of IL-1 β and IL-6 both in RA-FLS and MH7A cells. In addition, Ononin treatment significantly down-regulated the production of IL-1 β in TNF- α -induced RA-FLS and MH7A cells when compared to control and TNF- α stimulation group. And this down-regulation manner showed a downward trend with the increased of Ononin concentration (Fig. 4A). Similarly, the expression of IL-6 both in RA-FLS and MH7A cells were also reduced following Ononin challenge and displayed a dose dependent decrease (Fig. 4B). The results suggested Ononin reduced the release of inflammatory cytokines of IL-1 β and IL-6 both in TNF- α -induced RA-FLS and MH7A cells.

Ononin regulated MAPK and NF- κ B signaling pathways

Abnormal activation of MAPK and NF- κ B signaling pathways are involved in the initiation and progression of RA (Yang *et al.*, 2017). Herein, we tried to evaluate the potential therapeutic effects of Ononin on MAPK and NF- κ B signaling pathways. Previous study reported that TNF- α effectively activated MAPK and NF- κ B signaling pathways as illustrated by increasing the expression of p-p38, p-JNK, p-ERK1/ ERK2 and p-NF- κ B (p-p65) (Shi & Sun, 2018). Similarly, Ononin challenge also significantly reduced the phosphorylated level of p38 (p-p38), p-JNK, p-ERK and p-NF- κ B in TNF- α -induced RA-FLS and MH7A cells in a dose-dependent manner (Fig. 5).

DISCUSSION

As one kind of serious chronic autoimmune and inflammatory diseases, RA causes many chronic diseases including nephropathy, vasculopathy, pulmonary and cutaneous disorders as a result of the destruction of the cartilage and bone (Gupta *et al.*, 2014). At present, about 0.5-1% of the population suffer from RA, it thus has become a serious threat leading to death (Ahmed, 2010). Even though the exact pathogenesis of RA is

still unknown, the possible pathogenic factors of RA contain inflammation, genetic and environmental factors and so on (Mavrogeni *et al.*, 2014). The combination of non-steroidal drugs, immunosuppressants and biological therapies have been widely used to treat RA in clinic (Quan *et al.*, 2008). But RA is prone to relapse, above drugs for RA are difficult to meet clinical needs. Therefore, it is urgent to develop more effective therapeutic agents for RA. In this study, we developed a TNF- α -induced RA-FLS and MH7A cell model, and found Ononin could obviously attenuated inflammation and promoted apoptosis in TNF- α -induced RA-FLS and MH7A cells *via* MAPK and NF- κ B pathways. We therefore thought Ononin might be a promising agent for RA treatment.

Ononin, one kind of isoflavone, is a natural plant compound that is similar to 17- β -estradiol in chemical structure and is widely found in Fabaceae/Leguminosae family like soy beans, soy foods, and legumes (Ko, 2014). Ononin has multiple physiological functions, such as antioxidant, antimicrobial and anti-inflammatory activities (Yu *et al.*, 2016). Additionally, people also found Ononin could promote skin growth, scavenging oxygen free radicals, inhibit lipid peroxidation, maintain the concentration of nitric oxide in blood, protect ischemia-reperfusion injury and enhance immunity (Wang *et al.*, 2019). In view of its so many important physiological functions, we speculated that it had a special effect in fighting against RA. Our experimental results confirmed the significant effects of Ononin on the viability and apoptosis of TNF- α -induced RA-FLS and MH7A cells.

In this study, we noticed that Ononin induced apoptosis and attenuated inflammation in TNF- α -induced RA-FLS and MH7A cells *via* inhibiting MAPK and NF- κ B signaling pathways. Previous study suggested above signaling pathways play key roles in inflammatory diseases. For instance, Xia and others (Xia *et al.*, 2018) found inhibiting NF- κ B pathway could significantly promote cell apoptosis and inhibit cell proliferation of human FLS cells in RA. Gao *et al.*, found tetrandrine suppressed the development of RA inflammation by decreasing the release of inflammatory cytokines *via* blocking NF- κ B (Gao *et al.*, 2016). Liu *et al.*, reported that anhuinoside C could ameliorate collagen-induced RA inflammation by suppressing MAPK and NF- κ B Pathways. (Liu *et al.*, 2017) Similarly, we found Ononin reduced cell proliferation and improved apoptosis by blocking these pathways, further confirmed MAPK and NF- κ B signaling pathways were important in the progression and pathogenesis of inflammation related diseases like RA. However, the precise effects of Ononin on RA and the potential regulatory mechanisms still need further study.

In summary, we established a TNF- α -induced RA-FLS cell model and found the effects of Ononin on apoptosis in these cells. Our data confirmed that Ononin alleviated the cell viability, increased the apoptosis and decreased the cytokines release like IL-1 β and IL-6, and further inhibited the abnormal NF- κ B and MAPK pathways activation in TNF- α -induced RA-FLS and MH7A cells. We therefore thought Ononin could be used as a promising agent for RA treatment in the future.

Acknowledgements

Not applicable.

Competing interests

The authors state that there are no conflicts of interest to disclose.

Ethics approval

Not applicable.

Statement of Human and Animal Rights

Not applicable.

Statement of Informed Consent

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

Yue Meng and Jian Ji designed the study, supervised the data collection, Xiao Xiao, Minghan Li and Shangbo Niu analyzed the data, interpreted the data, Youhua He, Guojun Tong and Chenglong Pan prepare the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

REFERENCES:

- Ahmed S (2010) Green tea polyphenol epigallocatechin 3-gallate in arthritis: progress and promise. *Arthritis Res Ther* **12**: 208. <https://doi.org/10.1186/ar2982>
- Alunno A, Carubbi F, Giacomelli R, Gerli R (2017) Cytokines in the pathogenesis of rheumatoid arthritis: new players and therapeutic targets. *BMC Rheumatol* **1**: 3. <https://doi.org/10.1186/s41927-017-0001-8>
- Bartok B, Firestein GS (2010) Fibroblast-like synoviocytes: key effector cells in rheumatoid arthritis. *Immunol Rev* **233**: 233–255. <https://doi.org/10.1111/j.0105-2896.2009.00859.x>
- Buchbender C, Ostendorf B, Mattes-György K, Miese F, Wittsack HJ, Quentin M, Specker C, Schneider M, Antoch G, Müller HW, Scherer A (2013) Synovitis and bone inflammation in early rheumatoid arthritis: high-resolution multi-pinhole SPECT *versus* MRI. *Diagn Interv Radiol* **19**: 20–24. <https://doi.org/10.4261/1305-3825.DIR.5922-12.1>
- Chimenti MS, Triggianese P, Conigliaro P, Candi E, Melino G, Perricone R (2015) The interplay between inflammation and metabolism in rheumatoid arthritis. *Cell Death Dis* **6**: e1887. <https://doi.org/10.1038/cddis.2015.246>
- Dong L, Yin L, Zhang Y, Fu X, Lu J (2017) Anti-inflammatory effects of ononin on lipopolysaccharide-stimulated RAW 264.7 cells. *Mol Immunol* **83**: 46–51. <https://doi.org/10.1016/j.molimm.2017.01.007>
- Gao LN, Feng QS, Zhang XF, Wang QS, Cui YL (2016) Tetrandrine suppresses articular inflammatory response by inhibiting pro-inflammatory factors *via* NF- κ B inactivation. *J Orthop Res* **34**: 1557–1568. <https://doi.org/10.1002/jor.23155>
- Guo Q, Wang Y, Xu D, Nossent J, Pavlos NJ, Xu J (2018) Rheumatoid arthritis: pathological mechanisms and modern pharmacologic therapies. *Bone Res* **6**: 15. <https://doi.org/10.1038/s41413-018-0016-9>
- Gupta M, Sasmal S, Mukherjee A (2014) Therapeutic effects of acetone extract of *Saraca asoca* seeds on rats with adjuvant-induced arthritis *via* attenuating inflammatory responses. *ISRN Rheumatol* **2014**: 959687. <https://doi.org/10.1155/2014/959687>
- Heidari B (2011) Rheumatoid arthritis: Early diagnosis and treatment outcomes. *Caspian J Intern Med* **2**: 161–170
- Johnson GL, Lapadat R (2002) Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. *Science* **298**: 1911–1912. <https://doi.org/10.1126/science.1072682>
- Karin M, Yamamoto Y, Wang QM (2004) The IKK NF- κ B system: a treasure trove for drug development. *Nat Rev Drug Discov* **3**: 17–26. <https://doi.org/10.1038/nrd1279>
- Kim EK, Choi EJ (2010) Pathological roles of MAPK signaling pathways in human diseases. *Biochim Biophys Acta* **1802**: 396–405. <https://doi.org/10.1016/j.bbadis.2009.12.009>
- Ko KP (2014) Isoflavones: chemistry, analysis, functions and effects on health and cancer. *Asian Pac J Cancer Prev* **15**: 7001–7010. <https://doi.org/10.7314/apjcp.2014.15.17.7001>
- Kumar P, Banik S (2013) Pharmacotherapy options in rheumatoid arthritis. *Clin Med Insights Arthritis Musculoskelet Disord* **6**: 35–43. <https://doi.org/10.4137/CMAMM.S5558>

- Liu Q, Xiao XH, Hu LB, Jie HY, Wang Y, Ye WC, Li MM, Liu Z (2017) Anhuinoside C ameliorates collagen-induced arthritis through inhibition of MAPK and NF-kappaB signaling pathways. *Front Pharmacol* **8**: 299. <https://doi.org/10.3389/fphar.2017.00299>
- Luo L, Zhou J, Zhao H, Fan M, Gao W (2019) The anti-inflammatory effects of formononetin and ononin on lipopolysaccharide-induced zebrafish models based on lipidomics and targeted transcriptomics. *Metabolomics* **15**: 153. <https://doi.org/10.1007/s11306-019-1614-2>
- Makarov SS (2001) NF-kappa B in rheumatoid arthritis: a pivotal regulator of inflammation, hyperplasia, and tissue destruction. *Arthritis Res* **3**: 200–206. <https://doi.org/10.1186/ar300>
- Mavrogeni S, Dimitroulas T, Gabriel S, Sfrikakis PP, Pohost GM, Kitas GD (2014) Why currently used diagnostic techniques for heart failure in rheumatoid arthritis are not enough: the challenge of cardiovascular magnetic resonance imaging. *Rev Cardiovasc Med* **15**: 320–331. PMID: 25662926
- Quan LD, Thiele GM, Tian J, Wang D (2008) The development of novel therapies for rheumatoid arthritis. *Expert Opin Ther Pat* **18**: 723–738. <https://doi.org/10.1517/13543776.18.7.723>
- Russo A, Cardile V, Graziano ACE, Avola R, Bruno M, Rigano D (2018) Involvement of Bax and Bcl-2 in induction of apoptosis by essential oils of three Lebanese salvia species in human prostate cancer cells. *Int J Mol Sci* **19**: <https://doi.org/10.3390/ijms19010292>
- Shi DL, Shi GR, Xie J, Du XZ, Yang H (2016) MicroRNA-27a inhibits cell migration and invasion of fibroblast-like synoviocytes by targeting follistatin-like protein 1 in rheumatoid arthritis. *Mol Cells* **39**: 611–618. <https://doi.org/10.14348/molcells.2016.0103>
- Shi JH, Sun SC (2018) Tumor necrosis factor receptor-associated factor regulation of nuclear factor kappaB and mitogen-activated protein kinase pathways. *Front Immunol* **9**: 1849. <https://doi.org/10.3389/fimmu.2018.01849>
- Shih RH, Wang CY, Yang CM (2015) NF-kappaB signaling pathways in neurological inflammation: a mini review. *Front Mol Neurosci* **8**: 77. <https://doi.org/10.3389/fnmol.2015.00077>
- Wang Y, Liu L, Ma Y, Guo L, Sun Y, Liu Q, Liu J (2019) Chemical discrimination of *Astragalus mongholicus* and *Astragalus membranaceus* based on metabolomics using UHPLC-ESI-Q-TOF-MS/MS Approach. *Molecules* **24**: <https://doi.org/10.3390/molecules24224064>
- Xia ZB, Meng FR, Fang YX, Wu X, Zhang CW, Liu Y, Liu D, Li GQ, Feng FB, Qiu HY (2018) Inhibition of NF-kappaB signaling pathway induces apoptosis and suppresses proliferation and angiogenesis of human fibroblast-like synovial cells in rheumatoid arthritis. *Medicine (Baltimore)* **97**: e10920. <https://doi.org/10.1097/MD.00000000000010920>
- Yamaguchi N, Kubo C, Masuhiro Y, Lally ET, Koga T, Hanazawa S (2004) Tumor necrosis factor alpha enhances Actinobacillus actinomycetemcomitans leukotoxin-induced HL-60 cell apoptosis by stimulating lymphocyte function-associated antigen 1 expression. *Infect Immun* **72**: 269–276. <https://doi.org/10.1128/iai.72.1.269-276.2004>
- Yang G, Li S, Yuan L, Yang Y, Pan MH (2017) Effect of nobiletin on the MAPK/NF-kappaB signaling pathway in the synovial membrane of rats with arthritis induced by collagen. *Food Funct* **8**: 4668–4674. <https://doi.org/10.1039/c7fo01311f>
- Yu J, Bi X, Yu B, Chen D (2016) Isoflavones: anti-inflammatory benefit and possible caveats. *Nutrients* **8**: <https://doi.org/10.3390/nu8060361>
- Zhang HW, Hu JJ, Fu RQ, Liu X, Zhang YH, Li J, Liu L, Li YN, Deng Q, Luo QS, Ouyang Q, Gao N (2018) Flavonoids inhibit cell proliferation and induce apoptosis and autophagy through down-regulation of PI3Kgamma mediated PI3K/AKT/mTOR/p70S6K/ULK signaling pathway in human breast cancer cells. *Sci Rep* **8**: 11255. <https://doi.org/10.1038/s41598-018-29308-7>