miR-642a-5p increases glucocorticoid sensitivity by suppressing the TLR4 signalling pathway in THP-1 cells

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ABSTRACT

The incidence rate of ulcerative colitis (UC) is increasing annually, and glucocorticoid (GC) resistance (GCR) is a common cause of UC-induced remission failure. Our previous studies have shown that the expression of miR-642a-5p is downregulated in UC with GCR, suggesting that miR-642a-5p may be related to the GC response. Therefore, we investigated the mechanism by which miR-642a-5p regulates the GC response in THP-1 cells.

We found that after treatment with miR-642a-5p mimics and DEX, the expression levels of glucocorticoid receptor (GR) in the nucleus and NF-κB p65 and p50 in the cytoplasm were increased ($P < 0.05$). miR-642a-5p mimics transfected into THP-1 cells could synergize with dexamethasone (DEX) to reduce lipopolysaccharide (LPS)-induced inflammatory factor levels such as TNF-α, IL-1β, IL-6 and IL-12 ($P < 0.05$). Bioinformatics analysis and luciferase reporter assays confirmed that TLR4 is a target gene of miR-642a-5p. miR-642a-5p mimic pre-treatment enhanced the inhibitory effect of DEX on TLR4 induced by LPS and inhibited the expression of TLR4 on the cell surface ($P < 0.05$). Additionally, miR-642a-5p further prevented the nuclear import of NF-κB P65 and inhibited the phosphorylation of ERK, p38 and JNK.

These results suggest that miR-642a-5p can inhibit the inflammation by suppressing the TLR4 signalling pathway in THP-1 cells. It also highlights the TLR4 signalling pathway as a potential therapeutic target in anti-inflammation.

1. Introduction

Ulcerative colitis (UC) is a nonspecific chronic inflammatory disease of the intestinal tract with unknown aetiology. In recent years, the prevalence of UC has remained high in developed Western countries but has gradually increased in newly industrialized countries, including China, and even exceeds that in some Western countries [1–3]. Glucocorticoids (GCs) are still the most commonly used drugs to induce remission in patients with moderate or severe UC. However, reports indicate that 30%–40% of patients with acute UC have GC resistance (GCR) [4,5]. Approximately 30% of patients with severely refractory UC must undergo colectomy [6,7].

Some studies have suggested that microRNAs (miRNAs) may be involved in the GC response. miRNAs can regulate the GC response by posttranscriptional regulation of multiple mRNA targets of GC receptor (GR) signal transduction related to cell proliferation and cell death [8–11]. Studies have shown that in children with UC, miRNAs in the serum of children sensitive to GCs were detected by high-throughput sequencing, and 18 differentially expressed miRNAs were obtained [12]. miR-144–3p was most significantly upregulated in GC-sensitive UC children [13]. Chen et al. screened miRNAs in the serum of GC-sensitive, GC-resistant and healthy subjects and found that miR-195 expression was reduced in the GC-resistant group [14]. Our previous work showed that downregulated microRNAs had a significant correlation with several signal transduction pathways (the PI3K-Akt and MAPK signalling pathways) and target genes (HSP90B1, MAPK13,

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MAPK9, PIK3AP1 and TLR4) related to GC resistance. And we found that miR-642a-5p is related to GCR in UC patients for the first time, but the specific mechanism is unclear [15]. Based on the results of bioinformatics analysis, the target gene of miR-642a-5p may be TLR4, which is closely related to the GC response. And we found the expression of miR-642a-5p was significantly decreased in colonic mucosa samples from patients, and that of TLR4 was increased in the GCR group compared with the GCS group, while the expression levels of the other inflammatory factors (TNF-α, IL-1β, IL-6 and IL-12) increased accordingly (Results were not published, see supplementary material 1).

Therefore, this study aimed to elucidate that whether miR-642a-5p can increase glucocorticoid sensitivity by suppressing the TLR4 signalling pathway in THP-1 cells. This study may provide us with a new theoretical basis and target for the treatment of glucocorticoids resistant UC.

2. Material and methods

2.1. Cell culture

THP-1 cells, known to be lipopolysaccharide (LPS)-responsive through their expression of TLR4 [16], were seeded in T25 cell culture flasks and cultured in medium containing 10% FBS. The cell culture flasks were placed in an incubator at 37 °C and 5% CO_2. Cell morphology was observed daily with an inverted microscope.

2.2. miR-642a-5p mimic synthesis and transfection

We entrusted Guangzhou RiboBiotechnology Co., Ltd. to synthesize miR-642a-5p mimics, which were used to transfect THP-1 cells as described by Xiaoguang Wang et al. [17]. Configure miR-642a-5p mimics with a final concentration of 50 nM. The THP-1 cells were divided into 6 groups: the control + miR-NC group, LPS + miR-NC group, dexamethasone (DEX)+LPS + miR-NC group, control + miR-642a-5p mimics group, LPS + miR-642a-5p mimics group and DEX + LPS + miR-642a-5p mimic group. Cells were stimulated with LPS 100 ng/ml for 1 h, and DEX 1 μM, a concentration optimised in a previous study [18], for 24 h.

2.3. Bioinformatics analysis and construction of TLR4-overexpression plasmid

To elucidate the underlying mechanism by which miR-642a-5p promotes GC sensitivity, we performed in silico target prediction of miR-642a-5p target molecules as described in our previous article [15]. Targetscan (http://www.targetscan.org/vert 80/), Miranda (http://www.microrna.org/microrna/home.do) and Microcosm (http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/) were used to analyze target genes associated with glucocorticoid resistance for miR-642a-5p. Then a TLR4-overexpression plasmid (TLR4 cDNA ORF Clone, Human, C-GFPSpark® tag) containing the full-length sequence of 2520 bp was purchased from Yiqiao Shenzhou Company (see Supplementary material 2 for the sequence). Corresponding transfection reagent was used for transfection (in 20 ml of medium).

2.4. Quantitative real-time PCR analysis (qPCR)

Total RNA was extracted from THP-1 cells using TRIzol reagent (Qiagen, Hilden, Germany). Complementary DNA was synthesized using SYBR PrimeScript RT reagent kits (TaKaRa, Dalian, China) as described by Fathi et al. [19]. qPCR was performed on an ABI Prism 7900 HT sequence detection system (Applied Biosystems, Foster City, CA, USA) using the SYBR Green method. Primers specific for miR-642a-5p, U6, TLR4 and GAPDH (see Supplementary material 3 for the primers) were purchased from GeneCopoeia (Guangzhou, China). GAPDH and U6 were used as negative control. All PCR experiments were identical and run as follows: 95 °C for 10 min, 40 cycles of 95 °C for 15 s and 60 °C for 30 s, 65 °C for 5 s, and 95 °C. Relative miRNA and mRNA expression levels were calculated based on the number of PCR cycles. The comparative $2^{-\Delta CT}$ method was used to calculate the relative expression level of each target gene with GAPDH used as the internal control. Each sample was run in triplicate.

2.5. Cell proliferation assay

Cell viability was determined by 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay using a reported method [20]. The cells were seeded in a 96-well tissue culture plate (0.1×10^6 cells/well) and stimulated according to the specified experimental procedure. The absorbance at 550 nm was measured with a microplate reader. The effects of treatments on cell viability were calculated based on the ratio of the OD of the treatment group relative to the OD of the untreated control group.

2.6. Annexin V/PI detection

Cell apoptosis was analysed using an Annexin V-FITC Apoptosis Detection Kit (Xinbosheng Biotechnology) as described by Fathi et al. [19]. At last, the samples were analysed using a flow cytometer (Elite, Beckman Coulter, Fullerton, CA, USA).

2.7. ELISA

THP-1 cells were seeded into 96-well cell culture plates at a concentration of 0.1×10^6/ml, and the final experimental volume was 200 μL/well. The expression levels of the inflammatory factors IL-1, IL-6, IL-12 and TNF-α were determined with an ELISA detection kit (BD Bioscience, San Diego, CA, USA) as described by Fathi et al. [19].

2.8. Immunofluorescence staining

The cells from each experimental group were fixed with 4% paraformaldehyde (PFA), permeabilized with 0.02% Triton X-100, incubated with diluted primary antibody against GR (1:100, Santa cruz, sc-393232), antibody against NF-κB P50 (1:200, Santa cruz, sc-sc-8414). Then the cells were then incubated in diluted secondary antibody (1:300, Santa cruz, sc-2359) for 2 h at room temperature. Hoechst nuclear stain (Sigma) was added and incubated for 5 min at room temperature. The cells were mounted on coverslips using Vectashield hardset mounting medium (Vector Laboratories, Peterborough, UK). Images were acquired on a Delta Vision RT microscope using a 60 × /1.42 Plan Apo objective and a Sedat filter set (Chroma 89000). Images of DAPI- and Alexa 488-stained cells were obtained by excitation at 405 and 488 nm, respectively, using a CoolSNAP HQ (Photometrics) camera with a Z optical spacing of 0.5 μm. The original images were deconvolved using softWoRx software. ImageJ was used to process the images.

2.9. Western blotting

The protein expression of cells in different treatment groups was determined by Western blotting as described by Adibki et al. [20]. The protein content was quantified using a Bradford Assay Kit (ab102535, Abcam). SDS–PAGE gels (Tris-Gly, 4–20%, Beyotime) were used to separate the proteins, and samples containing 10 μg of protein were added to each well. After electrophoresis, the proteins were transferred to polyvinylidene difluoride membranes (Millipore) over 90 min at 4 °C. Then the membranes were blocked in 5% BSA at room temperature for 2 h. The membranes were then incubated overnight at 4 °C with the appropriate primary antibody (see Supplementary material 4 for specific information). The next day, the membranes were incubated with the secondary antibodies (1:500, Santa cruz, sc-2359) at room temperature for 1 h. Grey images showing protein expression were obtained. To

quantitatively assess the images, the 'gel analysis' function in the ImageJ program was used.

2.10. Luciferase assay

Plasmids were constructed and transfected into cells based on the experimental treatment conditions (blank group, wild-type TLR4+miR-NC group, wild-type TLR4+miR-642a-5p group, mutant TLR4+miR-NC group, and mutant TLR4+miR-642a-5p group). 48 h after plasmid transfection, the medium in the 12-well plate was discarded. Fifty microlitres of diluted $1 \times$ PLB was added to each well, and the culture plates were placed on a shaker for 20–30 min to ensure that the cells were completely lysed. Ten microlitres of supernatant was added to each well of a white, opaque 96-well microtiter plate. Then 100 μl of premixed Luciferase Assay Reagent II was added. After 2 s, the signal was measured in the dark to assess luciferase activity. After the measurement, 100 μl of premixed Stop & Glo reagent was added to each well. After standing for 2 s, the signal was measured to assess the activity of the control (Renilla luciferase). Three values were measured for each sample: RLU1-firefly luciferase reaction intensity; RLU2-endosperm Renilla luciferase reaction intensity; and the ratio of the two values, namely, RLU1/RLU2.

2.11. Flow cytometry

The cells were transferred to ice, washed with precooled FACS buffer (10 mM PBS, 10 mM HEPES and 0.25% BSA without Ca^{2+} or Mg^{2+}) and subjected to fluorescence staining with direct labeling. The cells were blocked with mouse IgG (50 g/mL) at room temperature for 10 min, then diluted mouse anti-human TLR4-FITC direct labelling antibody or mouse allotype control IgG1 was added, and the cells were incubated at 4 °C for 45 min. The cells were washed one last time with FACS buffer and fixed with CellFIX (BD Biosciences). Flow cytometry was performed

with a dual-laser FACSCaliber instrument (BD Biosciences) using Cell-Quest software (BD Biosciences) as described by Adibki et al. [20].

2.12. Statistical analyses

All statistical analyses were performed using GraphPad Prism 6.0 software. The data are presented as the mean $\pm$ SD ($\bar{x} \pm s$, n $\geq$ 3). Comparisons between two groups were performed using an independent sample t-test. Comparisons among multiple groups were performed using analysis of variance (ANOVA) and ANOVA post-hoc tests. Comparisons of individual samples were performed using the Mann–Whitney t-test. Differences for which P $<$ 0.05 were considered statistically significant.

3. Results

3.1. miR-642a-5p had no effect on the proliferation or death of THP-1 cells

After THP-1 cells were transfected with miR-642a-5p mimics, cell viability was detected by MTT assay. The results showed there was no significant difference in cell viability in the miR-642a-5p mimic transfection group compared to the blank control group or miR-NC control group (Fig. 1A). Subsequently, we further assessed whether miR-642a-5p combined with DEX would have an effect on the cells. The results showed that miR-642a-5p had no significant impact on the effects of LPS or DEX (Fig. 1B). Finally, the effect of miR-642a-5p on THP-1 cell death was detected by Annexin V/PI double staining. The results showed that there was no significant difference in cell death rate in the miR-642a-5p mimic transfection group compared to the blank control group or miR-NC control group (Fig. 1C and D).

A: The viability of the miR-642a-5p mimic transfected cells was determined by the MTT assay. B: The effects of LPS or DEX induced in
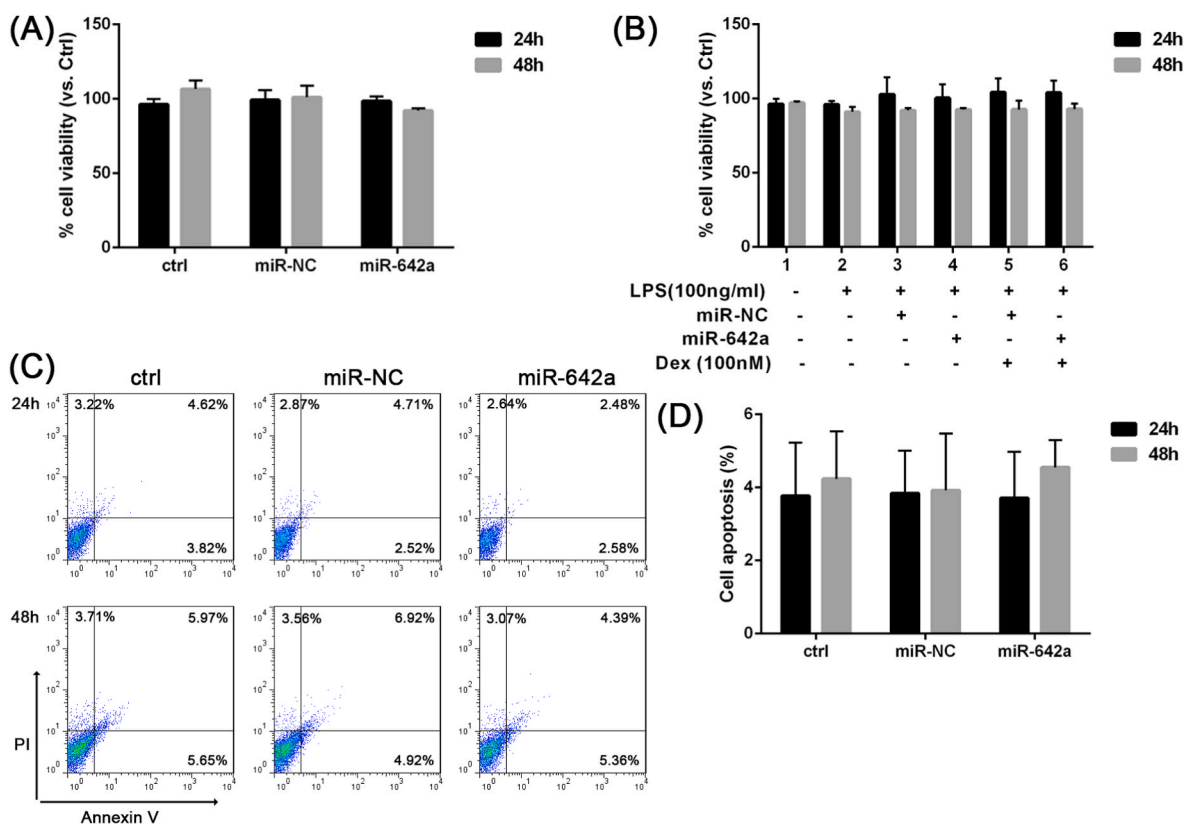

Fig. 1. miR-642a-5p had no effect on the proliferation or death of THP-1 cells.

the miR-642a-5p mimic transfected cells was determined by the MTT assay. C-D: The cell death rates between the groups were detected by Annexin V/PI double staining. n = 3.

3.2. miR-642a-5p synergized with DEX to promote GR translocation into the nucleus, then decrease the levels of LPS-induced inflammatory factors

Immunofluorescence staining showed that LPS could prevent DEX-induced GR from entering the nucleus. However, transfection of the cells with miR-642a-5p mimics alone had no significant effect on the nuclear entry of GR but promoted the effect of DEX on the nuclear entry of GR (Fig. 2A and B). We further verified the change in GR expression levels by Western blotting. Compared with that in the control group, miR-642a-5p mimic transfection slightly increased the nuclear GR level. Compared with that in the DEX group, the nuclear level of GR in the miR-642a-5p + DEX group was significantly increased, indicating that miR-642a-5p could promote the anti-inflammatory effect of DEX (Fig. 2C–E). Meanwhile, we investigated whether miR-642a-5p would exhibit anti-inflammatory effects in THP-1 cells induced with LPS. As shown in Fig. 2, LPS (100 ng/ml) treatment for 24 h significantly increased the secretion of the inflammatory factors IL-1β, IL-6, TNFα and IL-12. Pretreatment with DEX significantly reduced LPS-induced inflammatory cytokine production. After transfection of the cells with miR-642a-5p mimics, the miR-642a-5p mimics synergized with DEX to reduce the LPS-induced increase in inflammatory factors (Fig. 2F–I).

3.3. Bioinformatics analysis and luciferase reporter assays confirmed that TLR4 is the target gene of miR-642a-5p

We found by bioinformatics analysis that the miR-642a-5p seed sequence matched positions 398–404 and 1361–1367 of the 3′UTR of

TLR4 (Fig. 3A). To validate the targeting of these transcripts to miR-642a-5p, we cloned the 3′UTR of TLR4 into a dual-luciferase reporter gene vector for dual-luciferase reporter experiments. Cotransfection of luciferase plasmids with miR-642a-5p mimics resulted in a reduction in luciferase activity compared to what was observed upon transfection with control mimics. Deletion of the miR-642a-5p recognition site did not result in decreased luciferase activity following mutation of the 3′UTR of TLR4 (Fig. 3B). These data suggest that miR-642a-5p directly targets TLR4.

And transfection of miR-642a-5p mimics into cells reduced the expression level of TLR4 mRNA (Fig. 3C and D). In addition, we further examined the effect of miR-642a-5p on the expression level of TLR4 under LPS induction. The results showed that miR-642a-5p enhanced the inhibitory effect of DEX on TLR4 induced by LPS (Fig. 3E) and inhibited the expression of TLR4 on the cell surface (Fig. 3F).

3.4. miR-642a-5p regulates the MAPK and NF-κB pathways by targeting TLR4

Studies have shown that TLR4 is closely related to the MAPK and NF-κB signalling pathways [21,22] We then examined the nuclear transfer of NF-κB. As shown in Fig. 4A, LPS increased the nuclear expression of NF-κB P65 and P50. The pretreatment of cells with miR-642a-5p mimics increased the cytoplasmic expression levels of NF-κB P65 and prevented the nuclear import of NF-κB P65 (Fig. 4A and B).

Studies have shown that TLR4 is closely related to the MAPK and NF-κB signalling pathways. We then tested these pathways. The results showed that LPS could induce and upregulate the expression of TLR4, MyD88, and TAK1 and increase the phosphorylation levels of ERK, p38 and JNK, while DEX could inhibit their phosphorylation. In contrast, pretreatment of the cells with miR-642a-5p mimics further decreased
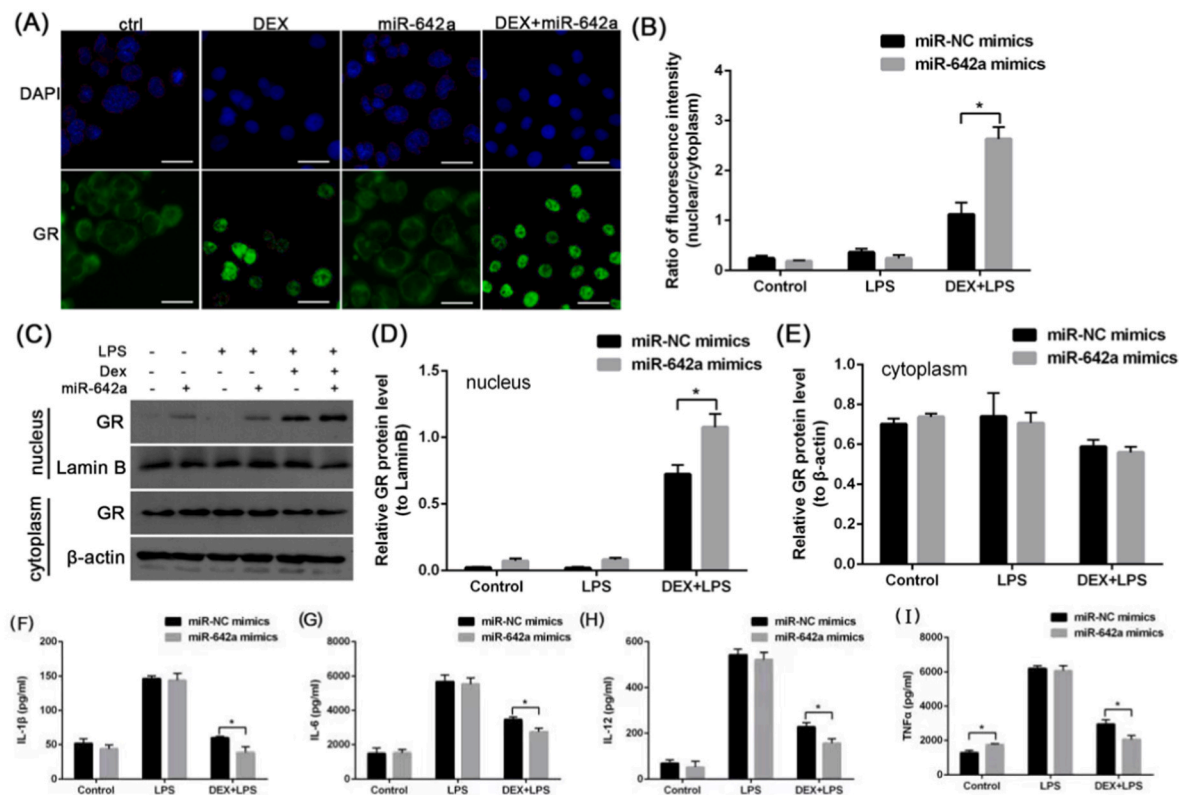

Fig. 2. Transfected miR-642a-5p mimics synergized with DEX to promote GR translocation into the nucleus in cells.
A–B: Immunofluorescence detection of the expression of GR in or out of the nucleus. C–E: Western blot detection of the expression of GR translocation into the nucleus. F–I: ELISA detection of the expression levels of the inflammatory factors TNFα, IL-1β, IL-6, and IL-12 in cells transfected with miR-642a-5p mimics. n = 3. * indicates P < 0.05.
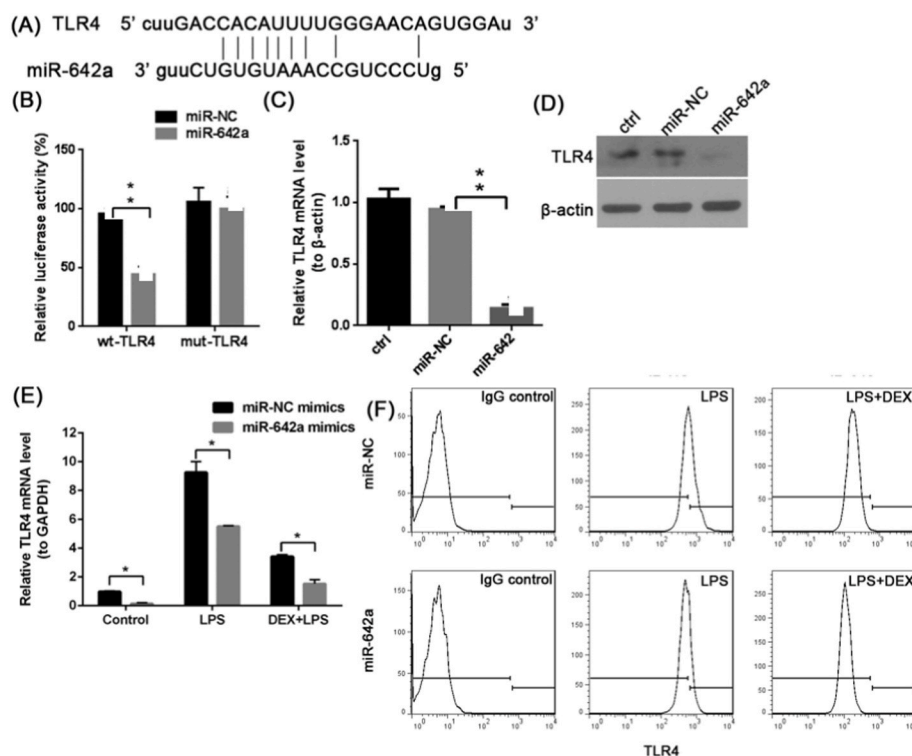
Fig. 3. Validation that TLR4 is the target gene of miR-642a-5p. A: Bioinformatics analysis for the TLR4 and miR-642a-5p. B: Luciferase experiments of the wt-TLR4 and miR-642a-5p. C: qPCR detection of the level of the TLR4 mRNA in miR-642a-5p mimic-transfected cells. D: Western Blot detection of the expression of the TLR4 protein in miR-642a-5p mimic-transfected cells. E: qPCR detection of the level of the TLR4 mRNA in miR-642a-5p mimic-transfected cells induced by LPS or DEX. F: Flow cytometry detection of the protein expression of TLR4 on the surface of the miR-642a-5p mimic-transfected cells induced by LPS or DEX. **n = 3.** * indicates P < 0.05.

phosphorylation levels (Fig. 4C and D).

4. Discussion

miRNAs have been found to play an important role in the GC response in IBD. However, there are few studies on the role of miRNAs in GC-resistant UC. The present findings suggested that miR-642a-5p may have suppressive roles in several cancers [17,23,24], and only one research reported that miR-642a-5p contributed to LPS-induced hyperpermeability and apoptosis of pulmonary microvascular endothelial cells [25]. In this study, miR-642a-5p affected LPS-induced expression of members of the TLR4 signal pathway for inhibition. miR-642a-5p decreased NF-κB expression and nuclear entry which promoted the anti-inflammatory effect of GR in the nucleus, thus synergizes with GCs to inhibit inflammation.

Previous studies have shown that complex inflammatory responses are related to the pathogenesis of UC [26,27]. Bioinformatics software predicted that TLR4 is a target gene of miR-642a-5p. Current studies suggest that the TLR4/NF-κB pathway is closely related to regulation of the expression of inflammatory cytokines [28–30]. Increased expression of IL-1β, IL-6, IL-12, and TNF-α has been detected in active UC, and this increased expression is associated with the severity of inflammation [31–33]. A number of studies have shown that TLR4 is the main mediator of the LPS response [34–36], and TLR4 signal transduction can promote intestinal injury in DSS-induced or TNBS-induced colitis [37–39]. We had verified the increased expression of TLR4 in clinical intestinal mucosa specimens from patients with GC-resistant UC, which was negatively correlated with the expression level of miR-642a-5p [15]. And increased expressions of IL-1β, IL-6, IL-12, and TNF-α have been detected in miR-642a-5p mimic-pretreated cells in this study. Thus we speculate that miR-642a-5p may increase glucocorticoid sensitivity by suppressing the TLR4 signalling pathway.

However, there have been few studies on TLR4 directly related to GCR in UC patients. Studies in other systems, such as drug resistance in asthma, suggest that gram-negative bacilli activate the TLR4/TAK1 signal pathway and play a role in altering cellular responses to GCs [40].

In rheumatic diseases, TLR4 gene polymorphisms are closely related to the GC response [41]. Many studies have shown that NF-κB activation through various pathways can inhibit the production of anti-inflammatory factors by preventing GR from binding specific DNA regions of the GRE, the first step in the GC response, leading to GC resistance [42–44]. Our study also found miR-642a-5p mimic enhanced the inhibitory effect of DEX on TLR4, promoted GR translocation into the nucleus, and decreased the levels of LPS-induced inflammatory factors.

TLR4 binds to MyD88's TIR domain by recognizing bacterial LPS and activates MAPK and JNK pathways, leading to the activation of NF-κB, which induces a series of inflammatory responses [21,45]. Our research showed that LPS could upregulate the expression of TLR4, MyD88, TAK1, increase the phosphorylation levels of ERK, P38 and JNK. DEX + miR-642a-5p mimics inhibited their phosphorylation. miR-642a-5p mimic-pretreated increased cytoplasmic expression of NF-κB P65 and P50, prevented NF-κB entry into the nucleus, thus synergizes with GCs to inhibit inflammation. These are consistent with previous studies [21,42, 45].

Thus, this study demonstrates for the first time that miR-642a-5p exerts a protective effect against LPS-induced inflammation by regulating the production of cytokines corresponding to the TLR4 signal pathway. The synergistic activities of miR-642a-5p and GCs can reduce the body's inflammatory response and promote sensitivity to GCs. The above conclusions will be verified again in animal experiments. It may also be necessary to re-verify whether miR-642a-5p has other mechanisms of action besides TLR4.
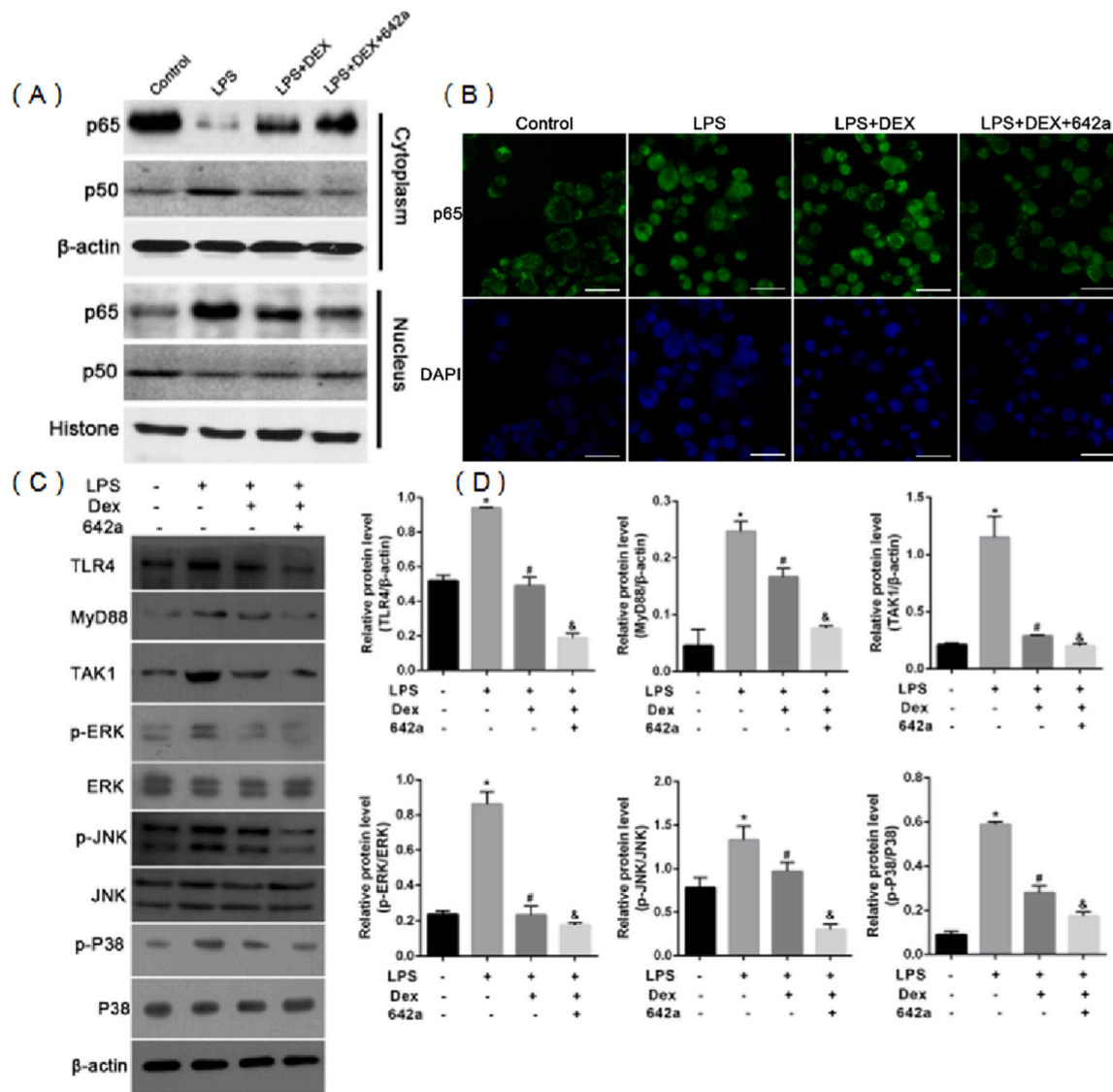
Author contribution

Fig. 4. miR-642a-5p cooperated with DEX to prevent the nuclear entry of NF-κB P65 and P50. A: Western blot analysis of the nuclear entry of NF-κB P65 and P50. B: Immunofluorescence staining to detect the nuclear entry of NF-κB P65. C–D: Western blotting was used to detect the expression and phosphorylation of TLR4, MyD88, TAK1, ERK, p38 and JNK. **n = 3.** *, p < 0.05 vs. no-treatment; #, p < 0.05 vs. LPS treatment; &, p < 0.05 vs. LPS + Dex treatment.

Availability of data and materials

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

Ethics and consent to participate declarations

Not applicable.

Consent for publication

All authors approved to submit this version to this publication.

Declaration of competing interest

Juan Luo, Yijie Wang, Xiangqian Dong, Wen Wang, Yanju Mu, Yang Sun, Fengrui Zhang and Yinglei Miao declare that they have no confict of interest.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2022.101356.

References

[1] S.C. Ng, H.Y. Shi, N. Hamidi, F.E. Underwood, W. Tang, E.I. Benchimol, R. Panaccione, S. Ghosh, J. Wu, F. Chan, J. Sung, G.G. Kaplan, Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies, Lancet 390 (2017) 2769–2778.

[2] S.C. Ng, G.G. Kaplan, W. Tang, R. Banerjee, B. Adigopula, F.E. Underwood, D. Tanyingoh, S.C. Wei, W.C. Lin, H.H. Lin, J. Li, S. Bell, O. Niewiadomski, M. A. Kamm, Z. Zeng, M. Chen, P. Hu, D. Ong, C.J. Ooi, K.L. Ling, Y. Miao, J. Miao, D. S.H. Janaka, M. Niriella, S. Aniwan, J. Limsrivilai, P. Pisespongsa, K. Wu, H. Yang, K.K. Ng, H.H. Yu, Y. Wang, Q. Ouyang, M. Abdullah, M. Simadibrata, J. Gunawan, I. Hilmi, G.K. Lee, Q. Cao, H. Sheng, A. Ong-Go, V.H. Chong, J. Ching, J. Wu, F. Chan, J. Sung, Population density and risk of inflammatory bowel disease: a prospective population-based study in 13 countries or regions in Asia-pacific, Am. J. Gastroenterol. 114 (2019) 107–115.

[3] J. Niu, J. Miao, Y. Tang, Q. Nan, Y. Liu, G. Yang, X. Dong, Q. Huang, S. Xia, K. Wang, Y. Miao, Identification of environmental factors associated with inflammatory bowel disease in a Southwestern Highland region of China: a nested case-control study, PLoS One 11 (2016), e0153524.

[4] S. De Iudicibus, R. Franca, S. Martelossi, A. Ventura, G. Decorti, Molecular mechanism of glucocorticoid resistance in inflammatory bowel disease, World J. Gastroenterol. 17 (2011) 1095–1108.

[5] P. Hoffmann, C. Wehling, J. Krisam, J. Pfeiffenberger, N. Belling, A. Gauss, Performance of tacrolimus in hospitalized patients with steroid-refractory acute severe ulcerative colitis, World J. Gastroenterol. 25 (2019) 1603–1617.

[6] D. Turner, C.M. Walsh, A.H. Steinhart, A.M. Griffiths, Response to corticosteroids in severe ulcerative colitis: a systematic review of the literature and a meta-regression, Clin. Gastroenterol. Hepatol. 5 (2007) 103–110.

[7] H. Eronen, T. Ilus, A. Jussila, H. Huhtala, P. Collin, P. Oksanen, Long-term outcome of patients with acute ulcerative colitis after first course of intravenous corticosteroids, Scand. J. Gastroenterol. 56 (2021) 234–238.

[8] Y. Hong, J. Wu, J. Zhao, H. Wang, Y. Liu, T. Chen, X. Kan, Q. Tao, X. Shen, K. Yan, Z. Zhai, miR-29b and miR-29c are involved in Toll-like receptor control of glucocorticoid-induced apoptosis in human plasmacytoid dendritic cells, PLoS One 8 (2013), e69926.

[9] S.K. Pore, A. Choudhary, B. Rathore, A. Ganguly, P. Sujitha, C.G. Kumar, S. B. Agawane, J.M. Kumar, V. Scaria, B. Pillai, R. Banerjee, Hsp90-targeted miRNA-liposomal formulation for systemic antitumor effect, Biomaterials 34 (2013) 6804–6817.

[10] A. Palagani, D.B.K. Op, S. Naulaerts, J. Diddens, C.C. Sekhar, G. Van Camp, K. Laukens, K. Heyninck, S. Gerlo, P. Mestdagh, J. Vandesompele, W.V. Berghe, Ectopic microRNA-150-5p transcription sensitizes glucocorticoid therapy response in MM1S multiple myeloma cells but fails to overcome hormone therapy resistance in MM1R cells, PLoS One 9 (2014), e113842.

[11] Y.N. Liang, Y.L. Tang, Z.Y. Ke, Y.Q. Chen, X.Q. Luo, H. Zhang, L.B. Huang, MiR-124 contributes to glucocorticoid resistance in acute lymphoblastic leukemia by promoting proliferation, inhibiting apoptosis and targeting the glucocorticoid receptor, J. Steroid Biochem. Mol. Biol. 172 (2017) 62–68.

[12] S. De Iudicibus, M. Lucafo, N. Vitulo, S. Martelossi, R. Zimbello, F. De Pascale, C. Forcato, S. Naviglio, A. Di Silvestre, M. Gerdol, G. Stocco, G. Valle, A. Ventura, M. Bramuzzo, G. Decorti, High-throughput sequencing of microRNAs in glucocorticoid sensitive paediatric inflammatory bowel disease patients, Int. J. Mol. Sci. 19 (2018).

[13] L. McBeth, A.C. Nwaneri, M. Grabnar, J. Demeter, A. Nestor-Kalinoski, T.J. Hinds, Glucocorticoid receptor beta increases migration of human bladder cancer cells, Oncotarget 7 (2016) 27313–27324.

[14] G. Chen, S. Cao, F. Liu, Y. Liu, miR-195 plays a role in steroid resistance of ulcerative colitis by targeting Smad7, Biochem. J. 471 (2015) 357–367.

[15] J. Luo, Y. Wang, D. Lan, J. Niu, J. Miao, X. Dong, G. Yang, F. Zhang, Y. Cao, K. Wang, Y. Miao, Differential expression of serum microRNAs in glucocorticoid-resistant patients with ulcerative colitis, Int. J. Clin. Exp. Pathol. 11 (2018) 936–946.

[16] L.L. Silva, T.V. Luu, B. De Haan, M. Faas, P. De Vos, TLR2 and TLR4 activity in monocytes and macrophages after exposure to amoxicillin, ciprofloxacin, doxycycline and erythromycin, J. Antimicrob. Chemother. (2022).

[17] X. Wang, Z. Song, B. Hu, Z. Chen, F. Chen, C. Cao, MicroRNA642a5p inhibits colon cancer cell migration and invasion by targeting collagen type I alpha1, Oncol. Rep. 45 (2021) 933–944.

[18] E. Goleva, L.P. Jackson, J.K. Harris, C.E. Robertson, E.R. Sutherland, C.F. Hall, J. J. Good, E.W. Gelfand, R.J. Martin, D.Y. Leung, The effects of airway microbiome on corticosteroid responsiveness in asthma, Am. J. Respir. Crit. Care Med. 188 (2013) 1193–1201.

[19] E. Fathi, S. Vandghanooni, S. Montazersaheb, R. Farahzadi, Mesenchymal stem cells promote caspase-3 expression of SH-SY5Y neuroblastoma cells via reducing telomerase activity and telomere length, Iran J Basic Med Sci 24 (2021) 1583–1589.

[20] K. Adibkia, A. Ehsani, A. Jodaei, E. Fathi, R. Farahzadi, M. Barzegar-Jalali, Silver nanoparticles induce the cardiomyogenic differentiation of bone marrow derived mesenchymal stem cells via telomere length extension, Beilstein J. Nanotechnol. 12 (2021) 786–797.

[21] S. He, C. Wang, Y. Huang, S. Lu, W. Li, N. Ding, C. Chen, Y. Wu, Chlamydia psittaci plasmid-encoded CPSIT_P7 induces macrophage polarization to enhance the antibacterial response through TLR4-mediated MAPK and NF-kappaB pathways, Biochim. Biophys. Acta Mol. Cell Res. 1869 (2022), 119324.

[22] S. Zhang, L. Xiong, C. Cui, H. Zhao, Y. Zhang, Z. Tian, W. Guan, F. Chen, Maternal supplementation with Artemisia annua L. ameliorates intestinal inflammation via inhibiting the TLR4/NF-kappaB and MAPK pathways and improves the oxidative stability of offspring, Food Funct. 13 (18) (2022) 9311–9323.

[23] Y. Liu, Y. Wang, X. Shen, C. Chen, H. Ni, N. Sheng, M. Hua, Y. Wu, Down-regulation of lncRNA PCGEM1 inhibits cervical carcinoma by modulating the miR-642a-5p/LGMN axis, Exp. Mol. Pathol. 117 (2020) 104561.

[24] D.J. Beveridge, K.L. Richardson, M.R. Epis, R. Brown, L.M. Stuart, A.J. Woo, P. J. Leedman, The tumor suppressor miR-642a-5p targets Wilms Tumor 1 gene and cell-cycle progression in prostate cancer, Sci. Rep. 11 (2021), 18003.

[25] L. Fei, G. Sun, Q. You, miR-642a-5p partially mediates the effects of lipopolysaccharide on human pulmonary microvascular endothelial cells via eEF2, FEBS Open Bio 10 (2020) 2294–2304.

[26] P.T. Santana, S. Rosas, B.E. Ribeiro, Y. Marinho, H. de Souza, Dysbiosis in inflammatory bowel disease: pathogenic role and potential therapeutic targets, Int. J. Mol. Sci. 23 (2022).

[27] Q.Q. Li, H.H. Zhang, S.X. Dai, New insights and Advances in pathogenesis and treatment of very early onset inflammatory bowel disease, Front Pediatr 10 (2022), 714054.

[28] Y. Yamamoto, R.B. Gaynor, Therapeutic potential of inhibition of the NF-kappaB pathway in the treatment of inflammation and cancer, J. Clin. Invest. 107 (2001) 135–142.

[29] D. Chen, S. Xu, R. Jiang, Y. Guo, X. Yang, Y. Zhang, L. Zhou, X. Ge, J. Han, X. Guo, H. Yang, IL-1beta induced by PRRSV co-infection inhibited CSFV C-strain proliferation via the TLR4/NF-kappaB/MAPK pathways and the NLRP3 inflammasome, Vet. Microbiol. 273 (2022), 109513.

[30] B. Kong, H. Fu, Z. Xiao, Y. Zhou, W. Shuai, H. Huang, Gut microbiota dysbiosis induced by a high-fat diet increases susceptibility to atrial fibrillation, Can. J. Cardiol. (2022).

[31] R. Wiest, A. Krag, A. Gerbes, Spontaneous bacterial peritonitis: recent guidelines and beyond, Gut 61 (2012) 297–310.

[32] J.B. Swann, Y. Hayakawa, N. Zerafa, K.C. Sheehan, B. Scott, R.D. Schreiber, P. Hertzog, M.J. Smyth, Type I IFN contributes to NK cell homeostasis, activation, and antitumor function, J. Immunol. 178 (2007) 7540–7549.

[33] M. Coskun, O.H. Nielsen, Tumor necrosis factor inhibitors for inflammatory bowel disease, N. Engl. J. Med. 369 (2013) 2561–2562.

[34] T. Xiong, X. Zheng, K. Zhang, H. Wu, Y. Dong, F. Zhou, B. Cheng, L. Li, W. Xu, J. Su, J. Huang, Z. Jiang, B. Li, B. Zhang, G. Lv, S. Chen, Ganluyin ameliorates DSS-induced ulcerative colitis by inhibiting the enteric-origin LPS/TLR4/NF-kappaB pathway, J. Ethnopharmacol. 289 (2022), 115001.

[35] B. Wei, Q. Wu, X. Yang, C. Lai, Z. Su, Z. Liang, Effect of TRAF6 in acute pancreatitis-induced intestinal barrier injury via TLR4/NF-kappaB signal pathway, Tissue Cell 76 (2022), 101792.

[36] Y.W. Chung, J.H. Choi, T.Y. Oh, C.S. Eun, D.S. Han, Lactobacillus casei prevents the development of dextran sulphate sodium-induced colitis in Toll-like receptor 4 mutant mice, Clin. Exp. Immunol. 151 (2008) 182–189.

[37] X. He, Z. Wei, J. Wang, J. Kou, W. Liu, Y. Fu, Z. Yang, Alpinetin attenuates inflammatory responses by suppressing TLR4 and NLRP3 signaling pathways in DSS-induced acute colitis, Sci. Rep. 6 (2016), 28370.

[38] H. Xiong, L. Tian, Z. Zhao, S. Chen, Q. Zhao, J. Hong, Y. Xie, N. Zhou, Y. Fu, The sinomenine enteric-coated microspheres suppressed the TLR/NF-kappaB signaling in DSS-induced experimental colitis, Int. Immunopharm. 50 (2017) 251–262.

[39] Y. Sugitani, A. Nishida, O. Inatomi, M. Ohno, T. Imai, M. Kawahara, K. Kitamura, A. Andoh, Sodium absorption stimulator prostasin (PRSS8) has an anti-inflammatory effect via downregulation of TLR4 signaling in inflammatory bowel disease, J. Gastroenterol. 55 (2020) 408–417.

[40] M.A. Sacta, B. Tharmalingam, M. Coppo, D.A. Rollins, D.K. Deochand, B. Benjamin, L. Yu, B. Zhang, X. Hu, R. Li, Y. Chinenov, I. Rogatsky, Gene-specific mechanisms direct glucocorticoid-receptor-driven repression of inflammatory response genes in macrophages, Elife 7 (2018).

[41] P.J. Barnes, I.M. Adcock, Glucocorticoid resistance in inflammatory diseases, Lancet 373 (2009) 1905–1917.

[42] G. Wang, Z. Hu, Q. Fu, X. Song, Q. Cui, R. Jia, Y. Zou, C. He, L. Li, Z. Yin, Resveratrol mitigates lipopolysaccharide-mediated acute inflammation in rats by inhibiting the TLR4/NF-kappaBp65/MAPKs signaling cascade, Sci. Rep. 7 (2017), 45006.

[43] G. Gallucci, A. Diaz, R. Fernandez, B. Bongiovanni, M. Imhoff, E. Massa, N. Santucci, D. Bertola, S. Lioi, M.L. Bay, O. Bottasso, L. D'Attilio, Differential expression of genes regulated by the glucocorticoid receptor pathway in patients with pulmonary tuberculosis, Life Sci. 301 (2022), 120614.

[44] Z. Li, N. Trakooljul, F. Hadlich, K. Wimmers, E. Murani, Transcriptome analysis of porcine PBMCs reveals lipopolysaccharide-induced immunomodulatory responses and crosstalk of immune and glucocorticoid receptor signaling, Virulence 12 (2021) 1808–1824.

[45] H. Gao, Y. Cui, N. Kang, X. Liu, Y. Liu, Y. Zou, Z. Zhang, X. Li, S. Yang, J. Li, C. Wang, Q.M. Xu, X. Chen, Isoacteoside, a dihydroxyphenylethyl glycoside, exhibits anti-inflammatory effects through blocking toll-like receptor 4 dimerization, Br. J. Pharmacol. 174 (2017) 2880–2896.