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Down-regulation of miR-215 attenuates lipopolysaccharide-induced inflammatory injury in CCD-18co cells by targeting GDF11 through the TLR4/NF-κB and JNK/p38 signaling pathways

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Summary. Ulcerative colitis (UC) is a risk factor for carcinogenesis of colorectal cancer, which is associated with disruption of the epithelial barrier and disorder of the inflammatory response. It has been reported that the expression of microRNA (miR)-215 is upregulated in patients with long-term UC. The present study aimed to investigate the effects of miR-215 on lipopolysaccharide (LPS)-induced inflammatory injury in CCD-18Co cells, as well as to identify the underlying possible molecular mechanisms. CCD-18Co cells were treated with 1 μg/ml LPS to induce inflammatory injury. Reverse transcription-quantitative PCR was performed to determine the expression of miR-215 in LPS-treated CCD-18Co cells. Moreover, a dual luciferase reporter system assay was used to evaluate the interaction of miR-215 and growth differentiation factor 11 (GDF11) in CCD-18Co cells. The expression of miR-215 was significantly upregulated in LPS-treated CCD-18Co cells. Knockdown of miR-215 significantly alleviated the inflammatory response and oxidative stress in LPS-treated CCD-18Co cells. In addition, GDF11 was identified as a direct binding target of miR-215 in CCD-18Co cells. Knockdown of miR-215 significantly increased the expression of GDF11, but decreased the expression levels of Toll-like receptor (TLR)4, phosphorylated (p)-p65, iNOS, p-p38 and p-JNK in LPS-treated CCD-18Co cells. Collectively, the present findings indicated that knockdown of miR-215 alleviated oxidative stress and inflammatory response in LPS-treated CCD-18Co cells by upregulating GDF11 expression and inactivating the TLR4/NF-κB and JNK/p38 signaling pathways.

Key words: Ulcerative colitis, miR-215, GDF11, TLR4/NF-κB p65 signaling pathway, JNK/MAPK signaling pathway

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Introduction

Ulcerative colitis (UC) is recognized as a chronic inflammatory disorder of the colon, which is a well-established risk factor for carcinogenesis of colorectal cancer (Farraye et al., 2010; Chen et al., 2014b). UC is categorized into chronic, idiopathic and relapse, and the repetition of epithelial cells impairment and repair is the main cause of developing colorectal carcinoma (Terzic et al., 2010). The primary aim of the treatment of UC is to rapidly improve the symptoms of patients with UC and maintain the improvement of the disease in the long term. Steroids, hormones, immunosuppressants or their derivatives are major therapeutic methods for patients with UC; however, modest effects and serious side effects are observed. In recent years, 5-aminosalicylates

(5-ASAs) are considered as a potential standard therapeutic method for improving the symptoms of patients with mild-moderate active UC (Hanauer, 2016). However, 80% of patients with UC are moderate-to-severe. Although there is an increasing number of studies investigating the etiology of UC, the molecular mechanisms of pathogenesis of UC remain unknown. Emerging evidence has has revealed that the expression levels of inflammatory cytokines, including IL-1β, IL-6 and TNF-α, in colon tissues, regulated by the Toll-like receptor 4 (TLR4)/NF-κB p65 signaling pathway or the JNK/MAPK signaling pathway, serve vital roles in the development of UC (Zhang et al., 2015; Min-Seok et al., 2018; Wang et al., 2019a).

MicroRNAs (miRNAs/miRs), a class of non-coding transcripts <25 nucleotides, serve essential roles in a variety of biological processes by regulating the expression of targets genes by binding to their 3'-utranslated regions (UTRs) (Bartel, 2009; Zhang and Li, 2018). Aberrant expression of miRNAs has been reported to be involved in immune and inflammatory responses (Fabbri et al., 2012; Chen et al., 2014a). For instance, inflammatory factors and lipopolysaccharide (LPS) can induce the upregulation of miRNAs, including miR-155 and miR-146a, by modulating the activation of the NF-κB signaling pathway (Taganov et al., 2006; Williams et al., 2008).

It has been revealed that miRNAs exert critical roles in the pathogenesis of UC (Qu et al., 2019). For example, Feng et al observed that miR-126 was significantly upregulated in patients with UC, and overexpression of miR-126 could activate the NF-κB signaling pathway (Feng et al., 2012). In addition, high expression of miR-15 in patients with UC has been shown, and upregulation of miR-15 induced nuclear translocation of p-65 and increased the expression levels of inflammatory cytokines, such as IL-8 and IFN-γ (Zhang and Li, 2018). Moreover, miR-215 is upregulated in patients with long-standing UC and the abnormal expression of miR-215 is associated with UC-related colorectal cancer (Pekow et al., 2018). However, the role of miR-215 in the pathogenesis and progression of UC is yet to be fully elucidated (Tan et al., 2013; Pekow et al., 2018). Therefore, the present study was conducted to determine the role of miR-215 in the progression of UC and investigate its underlying molecular mechanisms, in order to provide novel targets for treatment of UC.

Materials and methods

Cell culture

The normal human colon epithelial cell line, CCD-18Co, was obtained from American Type Culture Collection. CCD-18Co cells were cultured in Eagle's Minimum Essential Medium (MEM; Gibco; Thermo Fisher Scientific, Inc.) with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.).

Cell transfection

miR-215 agomir, miR-215 antagomir and GDF11 small interfering (si)RNA were purchased from Shanghai GenePharma Co., Ltd. The miR-215 agomir, miR-215 antagomir or GDF11 siRNA were transfected into CCD-18Co cells using Lipofectamine 2000 (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocols. The culture medium containing 10% FBS was changed at 6 h after transfection, and the cells were then incubated for 42 h at 37°C.

Inflammatory analysis

The culture media of CCD-18Co cells was collected and centrifuged at 1,400 rpm/min to obtain the cell-free supernatant. Expression levels of IL-6, TNF-α, IL-10, IL-1β, SOD and GSH were detected using ELISA, in accordance with the manufacturer's instructions (ELK Biotechnology, Wuhan, China).

A Lipid Peroxidation (MDA) Assay Kit (NanJing JianCheng Bioengineering Institute, Nanjing, China) was used to detect the level of MDA in CCD-18Co cells.

Real-time quantitative reverse transcription PCR (RT-qPCR)

TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) was used to extract total RNAs from CCD-18Co cells. Subsequently, the total RNAs were used for reverse transcription with a QuantiTect Reverse Transcription kit (Qiagen, NY, USA). qPCR was conducted using the SuperRT One Step RT-PCR kit (CWBIO, Beijing, China). The relative expression of miR-215 was evaluated via the $2^{-\Delta\Delta Cq}$ method, and the expression of U6 was used as the internal control. Primers were used as follow: miR-215-forward: 5'-GGGACCTATGAATTGAGACC-3', miR-215-reverse: 5'- CTCAACTGGTGTCGTGGAGTC -3'; U6-forward: 5'- CTCGCTTCGGCACAT-3', U6-reverse: 5'-AACG CTTCACGAATTTGCGT -3'.

Dual-luciferase reporter system

The 3'UTR sequence of GDF11, including wild-type (WT) or mutant (MT) miR-215 binding site, was sub-cloned into pmirGLO vectors (Promega Corporation). The miR-215 or NC was co-transfected with pmirGLO-GDF11-WT or pmirGLO-GDF11-MT vector into CCD-18Co cells using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). Subsequently, luciferase activity was detected at 48 h using a dual-luciferase assay kit (Promega Corporation) according to the manufacturer's instructions.

Western blotting

Cells were collected, washed three times with 1X

PBS and suspended with RIPA buffer to extract proteins. Then, the concentration of protein was detected with a BCA Protein Assay (Bio-Rad Laboratories, Inc.), and 30 μg proteins of each sample were separated by electrophoresis. Western blotting was performed as previously described (Luan et al., 2018). The primary antibodies were used as follows: GDF11 (1:2,000; cat. no. ab239515; Abcam), TLR4 (1:1,000; cat. no. ab22048; Abcam), phosphorylated (p)-p65 (1:500; cat. no. ab53489; Abcam), p65 (1:1,000; cat. no. ab16502; Abcam), iNOS (1:1,000; cat. no. ab213987; Abcam), p-p38 (1:1,000; cat. no. 4511; Cell Signaling Technology, Inc.), p38 (1:1,000; cat. no. 8690c; Cell Signaling Technology, Inc.), p-JNK (1:1,000; cat. no. 9255; Cell Signaling Technology, Inc.), JNK (1:1,000; cat. no. 3708; Cell Signaling Technology, Inc.) and β-actin (1:1,000; cat. no. 4970; Cell Signaling Technology, Inc.)

Reactive oxygen species (ROS) analysis

ROS generation was assessed by staining cells with 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate (DCFHDA; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol.

Data statistics

All of the data in the present study are presented as the mean ± standard deviation. Data analysis was conducted using SPSS 22.0 software (IBM Corp.). $P<0.05$ was considered to indicate a statistically significant difference. Results were obtained from at least three independent experiments.

Results

Downregulation of miR-215 inhibited LPS-induced inflammatory response in CCD-18Co cells

To investigate the role of miR-215 in the progression of UC, the expression of miR-215 in CCD-18Co cells treated with LPS was detected. As shown in Fig. 1A, the expression of miR-215 was significantly increased in LPS-treated CCD-18Co cells, compared with the control

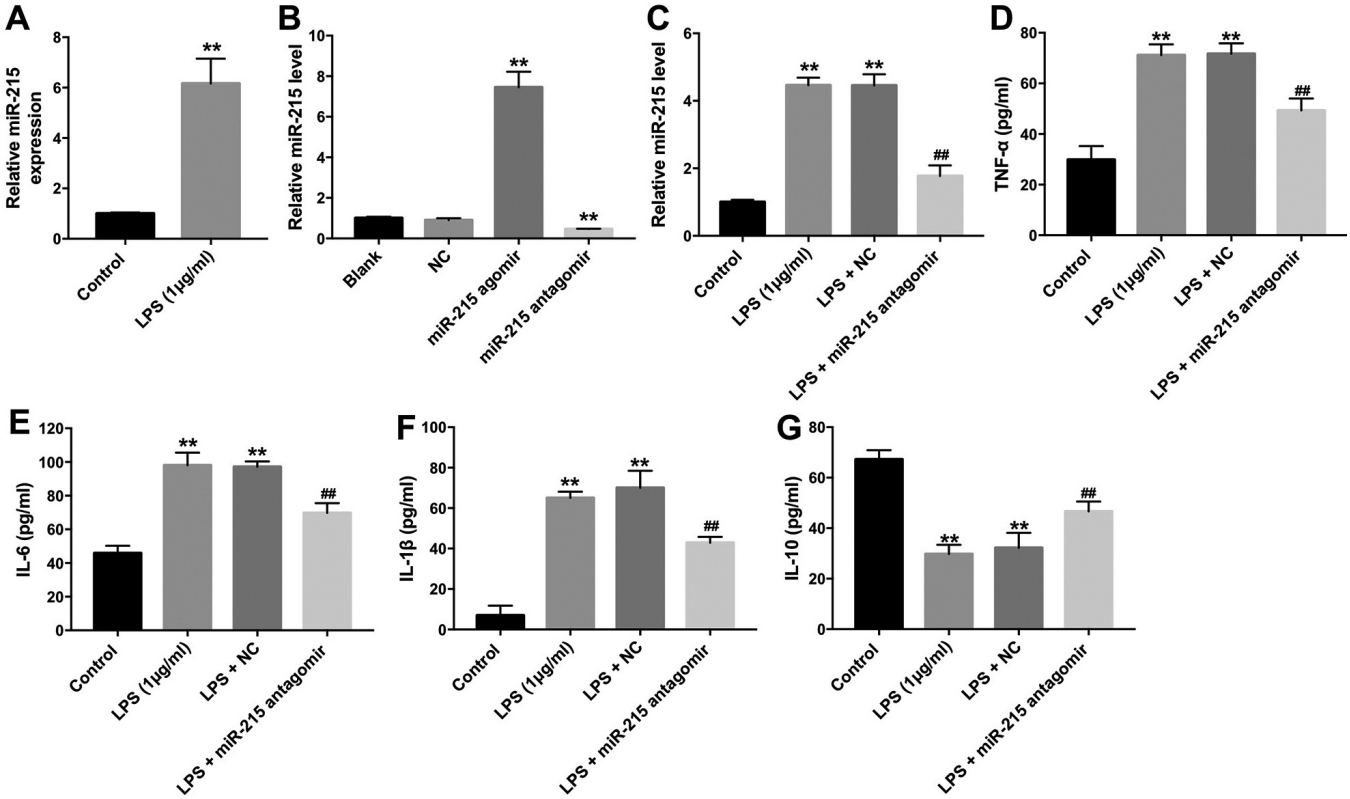

Fig. 1. Downregulation of miR-215 inhibited LPS-induced inflammatory response in CCD-18Co cells. **A.** CCD-18Co cells were treated with 1μg/ml LPS for 48 h. The level of miR-215 in CCD-18Co cells was detected by RT-qPCR. **B.** CCD-18Co cells were transfected with NC, miR-215 agomir and miR-215 antagomir for 48 h. The level of miR-215 in CCD-18Co cells was detected by RT-qPCR. **C.** CCD-18Co cells were transfected with miR-215 antagomir for 48 h, then exposed to 1μg/ml LPS for 24 h. The level of miR-215 in CCD-18Co cells was detected by RT-qPCR. **D-G.** CCD-18Co cells were transfected with miR-215 antagomir for 48 h, then exposed to 1μg/ml LPS for 24 h. The levels of TNF-α, IL-6, IL-1b and IL-10 in the cells-free supernatant was determined using ELISA kit. **P<0.01 vs. control group. ##P<0.01 vs. LPS + NC group.

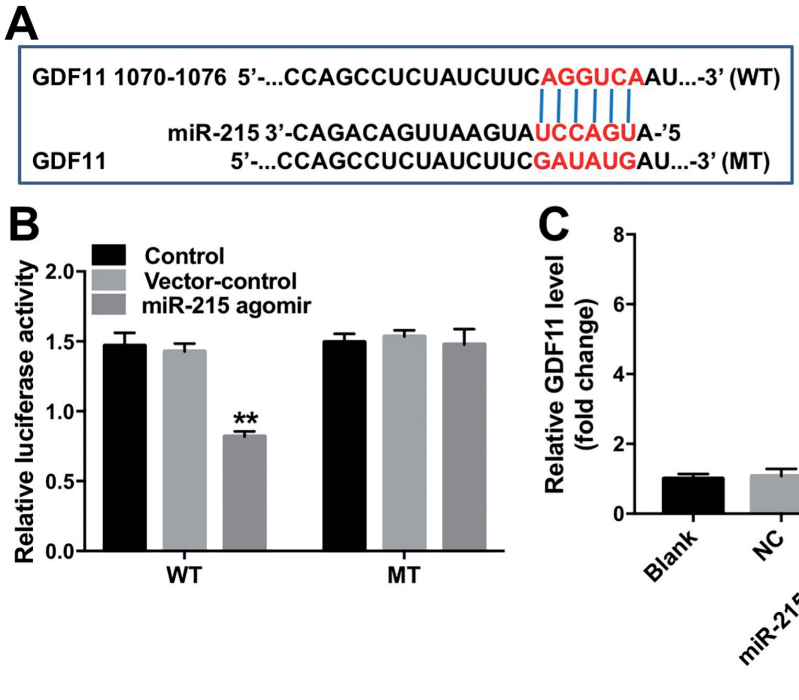

Fig. 2. GDF11 was a direct binding target of miR-215. **A.** Gene structure of GDF11 at the position of 1070-1076 indicates the predicted target site of miR-215 in its 3'UTR. **B.** Luciferase activity was detected following co-transfecting with WT/MT GDF11 plasmid and miR-215 in CCD-18Co cells using the dual luciferase reporter assay. **P<0.01 vs. vector-control group. **C.** CCD-18co cells were transfected with NC, miR-215 agomir and miR-215 antagomir for 48 h. The level of GDF11 in CCD-18Co cells was detected by RT-qPCR. **P<0.01 vs. NC group.

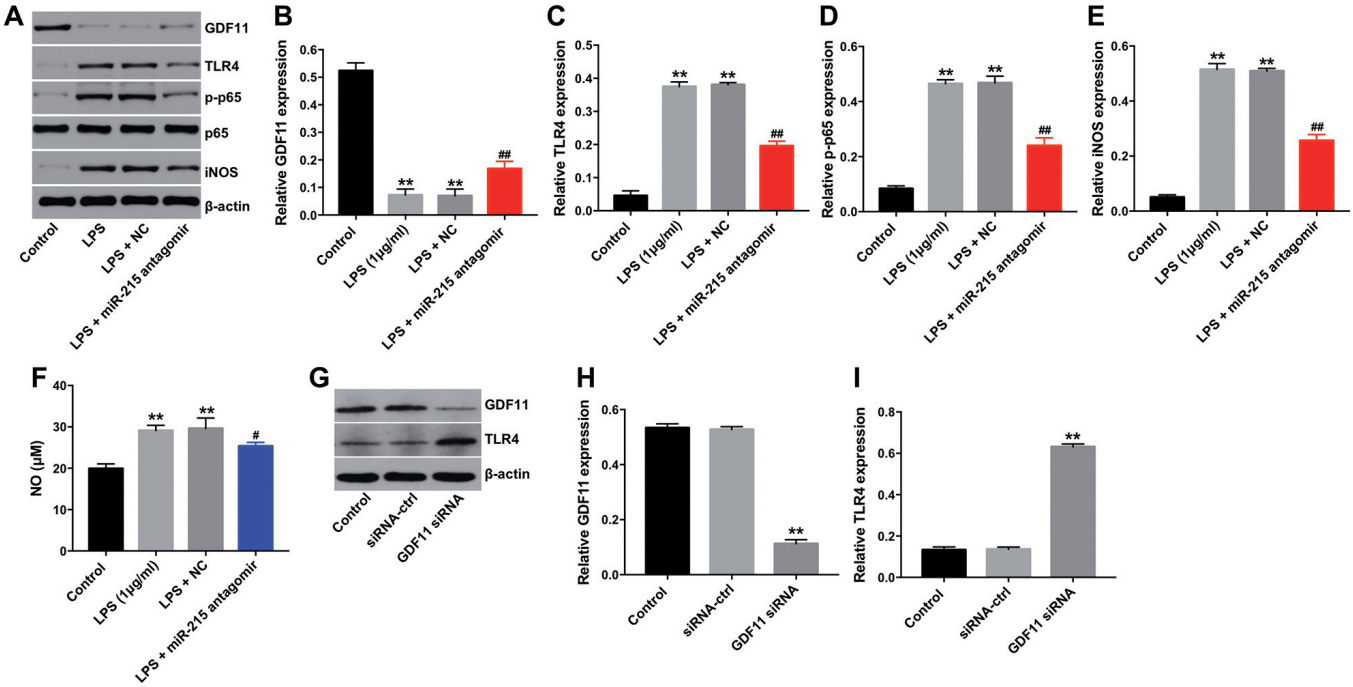

Fig. 3. Downregulation of miR-215 inhibited LPS-induced inflammatory response in CCD-18Co cells via regulation of GDF11/TLR4/NF-κB p65 signaling. **A.** CCD-18Co cells were transfected with miR-215 antagomir for 48 h, then exposed to 1 μg/ml LPS for 24 h. Expression levels of GDF11, TLR4, p-p65, iNOS in CCD-18Co cells were detected with western blotting. β-actin was used as an internal control. **B-E.** The relative expression of GDF11, TLR4 and iNOS in CCD-18Co cells was normalized to β-actin; The relative expression of p-p65 in CCD-18Co cells was normalized to p65. **F.** ELISA was adopted to determine the level of NO in the cell-free supernatant. **G.** CCD-18Co cells were transfected with GDF11 siRNA for 48 h. Expression levels of GDF11 and TLR4 in CCD-18Co cells were detected with western blotting. β-actin was used as an internal control. **H, I.** The relative expression of GDF11 and TLR4 in CCD-18Co cells were normalized to β-actin. **P<0.01 vs. control group. #P<0.05, ##P<0.01 vs. LPS + NC group.

group. In addition, overexpression of miR-215 markedly increased miR-215 expression in CCD-18Co cells, while knockdown of miR-215 notably decreased the expression of miR-215 in cells (Fig. 1B).

Knockdown of miR-215 significantly reduced the expression of miR-215 in LPS-stimulated CCD-18Co cells, compared with LPS + NC group (Fig. 1C). In addition, LPS significantly increased the expression levels of TNF-α, IL-6 and IL-β, while decreasing the expression of IL-10 in CCD-18Co cells. However, these LPS-induced changes were reversed in the presence of miR-215 antagomir (Fig. 1D-G). The results suggested that downregulation of miR-215 could inhibit LPS-induced inflammatory response in CCD-18Co cells.

GDF11 was a direct binding target of miR-215

Next, the present study predicted the target gene of miR-215 using Starbase 2.0. As presented in Fig. 2A, GDF11 was identified as a potential target of miR-215. A dual luciferase assay was conducted to confirm the direct interaction between miR-215 and GDF11. As shown in Fig. 2B, miR-215 agomir suppressed the luciferase activity of GDF11-WT, but it did not affect the luciferase activity of GDF11-MT. In addition, overexpression of miR-215 significantly inhibited the expression of GDF11 in CCD-18Co cells, while knockdown of miR-215 notably upregulated the expression of GDF11 in cells (Fig. 2C). Thus, GDF11 was a direct binding target of miR-215.

Downregulation of miR-215 inhibited LPS-induced inflammatory response in CCD-18Co cells via regulation of GDF11/TLR4/NF-κB p65 signaling

To determine whether the TLR4/NF-κB p65 signaling pathway exerts a role in the progression of UC mediated by the miR-215/GDF11 axis, western blotting was performed. As shown in Fig. 3A-E, LPS significantly reduced the expression of GDF11, but increased the expression levels of TLR4, p-p65 and iNOS in CCD-18Co cells. However, these LPS-induced changes were reversed in the presence of miR-215 antagomir. Additionally, LPS-induced NO production in CCD-18Co cells was reversed by miR-215 knockdown (Fig. 3F). It was found that GDF11 knockdown markedly downregulated the expression of GDF11, but upregulated the expression of TLR4 in CCD-18Co cells (Fig. 3G-I). These results demonstrated that downregulation of miR-215 inhibited the LPS-induced inflammatory response in CCD-18Co cells via inactivation of TLR4/NF-κB p65 signaling by targeting GDF11.

Downregulation of miR-215 inhibited LPS-induced inflammatory response in CCD-18Co cells via inactivation of JNK/p38 signaling pathway

To examine whether the JNK/p38 signaling pathway contributes to the development of UC, the phosphorylation and expression levels of JNK and p38

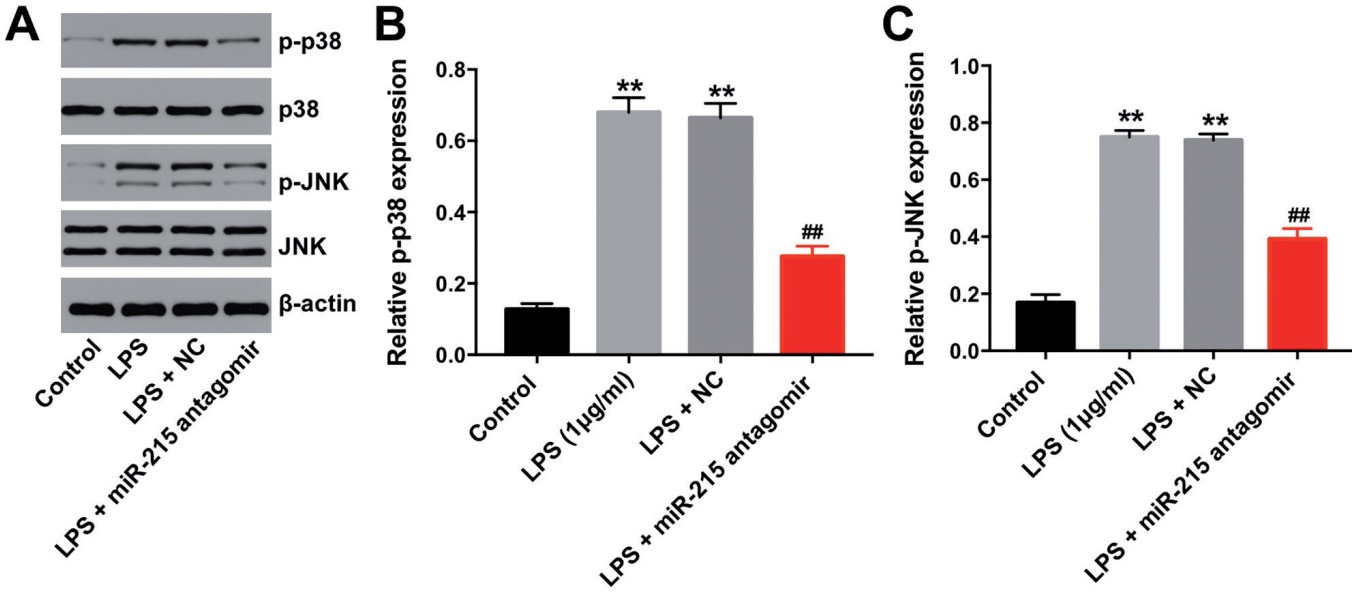

Fig. 4. Downregulation of miR-215 inhibited LPS-induced inflammatory response in CCD-18Co cells via inactivation of JNK/p38 signaling pathway. CCD-18Co cells were transfected with miR-215 antagomir for 48 h, then exposed to 1 μg/ml LPS for 24 h. **A.** Expression levels of p-p38 and p-JNK in CCD-18Co cells were detected with western blotting. β-actin was used as an internal control. **B.** The relative expression of p-p38 in CCD-18Co cells was normalized to p38. **C.** The relative expression of p-JNK in CCD-18Co cells was normalized to JNK. **P<0.01 vs. control group. ##P<0.01 vs. LPS + NC group.

were detected using western blot analysis. As shown in Fig. 4A-C, treatment with LPS resulted in increased phosphorylation of p38 and JNK proteins in CCD-18Co cells, but these LPS-induced changes were reversed by miR-215 knockdown. Therefore, it was revealed that knockdown of miR-215 was able to inhibit LPS-induced inflammatory response in CCD-18Co cells via inactivation of the JNK/p38 signaling pathway.

Downregulation of miR-215 alleviated LPS-induced oxidative stress in CCD-18Co cells

Next, the present study investigated the effects of miR-215 on LPS-induced oxidative stress by detecting intracellular ROS generation. As shown in Fig. 5A, treatment with LPS significantly increased ROS generation in CCD-18Co cells. However, knockdown of miR-215 reversed the production of ROS caused by LPS (Fig. 5A). Furthermore, LPS treatment significantly increased the level of MDA, but decreased the activities of SOD and GSH in CCD-18Co cells (Fig. 5B-D), but these changes were markedly reversed by miR-215 knockdown. Collectively, the results suggested that knockdown of miR-215 could alleviate LPS-induced oxidative stress in CCD-18Co cells.

Discussion

It has been reported that miRNAs are closely associated with the development and progression of UC (Polytarchou et al., 2015; Yang et al., 2019). Ando et al. found that the expression of miR-21 was downregulated in CD3 T cells during the remission phase of UC, indicating that miR-21 may be a novel target candidate to trigger remission in UC (Ando et al., 2016). In addition, knockdown of miR-223 alleviates intestinal barrier in inflammatory bowel disease (IBD) mice (Wang et al., 2016; Li et al., 2020). Meng et al also indicated that miR-129 could ameliorate intestinal inflammation in trinitrobenzene sulphonic acid-induced colitis mice by targeting FBW7 and inhibition of the NF-ϰB pathway (Meng et al., 2020). The present results indicated that knockdown of miR-215 upregulated the expression of GDF11, resulting in the inactivation of

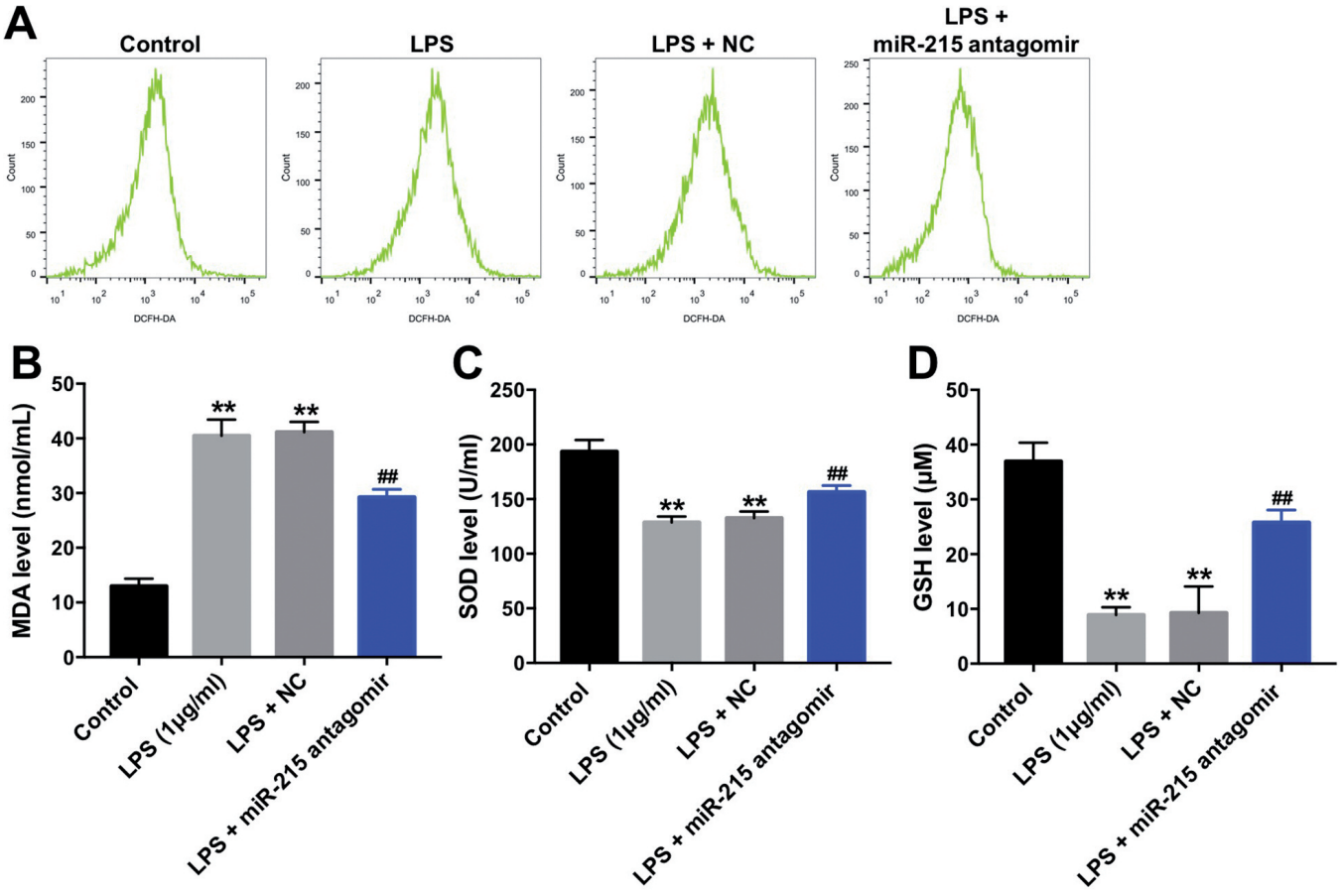

Fig. 5. Downregulation of miR-215 alleviated LPS-induced oxidative stress in CCD-18Co cells. CCD-18Co cells were transfected with miR-215 antagomir for 48 h, then exposed to 1 μg/ml LPS for 24 h. **A.** Flow cytometry was used to detect the generation of ROS. **B-D.** The production of MDA, SOD and GSH in cell-free supernatant was detected by ELISA. **P<0.01 vs. control group. ##P<0.01 vs. LPS + NC group.

TLR4/NF-κB signaling pathway and amelioration of inflammatory response in LPS-treated CCD-18Co cells. Moreover, knockdown of miR-215 abolished the activation of the JNK/p38 signaling pathway in LPS-treated CCD-18Co cells. The present study demonstrated that miR-215 serves an essential role in the progression of UC.

Increasing evidence has revealed that miR-215 is involved in the development of a variety of cancer types. For instance, increased expression of miR-215 is associated with the poor prognosis of patients with high-grade glioma, and overexpression of miR-215 significantly promotes the proliferation of glioma cells by inhibiting the expression of RB1 (Meng and Xi, 2017). In addition, miR-215 is considered an oncogene in gastric cancer (Li et al., 2016). However, it has been reported that miR-215 also acts as a tumor suppressor in cancer types, including breast cancer, colon cancer and non-small cell lung cancer (Cai et al., 2017; Gao et al., 2019a; Ullmann et al., 2019). Nonetheless, the complex function of miR-215 in the progression of cancer, its roles in inflammatory disease, are yet to be fully elucidated. The differential expression of miR-215 has been reported in multiple inflammatory diseases, including diabetic nephropathy, hepatitis and UC (Huang et al., 2019; Wang et al., 2019b). Previous studies have also identified the upregulation of miR-215 in patients with UC, while its function on the progression of UC and the underlying molecular mechanisms remain unknown.

In the present study, it was found that the expression of miR-215 was significantly upregulated in LPS-treated CCD-18Co cells. In addition, GDF11 was identified as a binding target of miR-215. It has been reported that GDF11 is involved in embryogenesis, metabolic disturbance and a variety of cancer types (Shimajiri et al., 2011; Sinha et al., 2014). Moreover, a recent study revealed that treatment with GDF11 could decrease the levels of pro-inflammatory factors in apolipoprotein E-null mice and inhibit neuroinflammation in Alzheimer's disease (Mei et al., 2016; Zhang et al., 2018). Upregulation of GDF11 can suppress inflammatory response in inflammatory arthritis and psoriasis by inactivating the NF-κB signaling pathway (Li et al., 2019; Wang et al., 2019c). In DSS-induced colitis, GDF11 significantly suppresses the secretion of IL-1β and inactivates NLRP3 inflammation by inhibiting the activation of the TLR4/NF-κB p65 signaling pathway (Wang et al., 2018). In the present study, it was demonstrated that knockdown of miR-215 significantly increased the expression GFD11, but decreased the expression levels of TLR4 and NF-κB p65 in LPS-treated CCD-18Co cells. Consistent with a previous study (Wang et al., 2018), the current results suggested that knockdown of GDF11 could increase the expression of TLR4 in CCD-18Co cells. Therefore, these data indicated that knockdown of miR-215 could alleviate LPS-induced inflammatory response in CCD-18Co cells via regulating the GDF11/TLR4/NF-κB p65 signaling pathway.

Emerging evidence has demonstrated the vital roles of the JNK/MAPK signaling pathway in the pathogenesis of UC (Gao et al., 2018). Activation of the JNK/MAPK signaling pathway is involved in the regulation of inflammatory response, apoptosis and oxidative stress (Panahi et al., 2018). It has also been shown that increasing the phosphorylation level of JNK/MAPK signaling transduction and overproduction of malondialdehyde (MDA), iNOS and TNF-α, IL-1β, and IL-6 were responsible for the progression of UC (Shi et al., 2016). Another study revealed that treatment with chlorogenic acid could attenuate the development of UC induced by dextran sodium sulfate by inhibiting the activation of the JNK/MAPK signaling pathway, as well as decreasing the level of MDA and increasing the activities of SOD and GSH (Gao et al., 2019b). Consistent with previous studies, the present findings demonstrated that treatment with LPS significantly activated the JNK/MAPK signaling pathway, enhanced the level of MDA and decreased the activities of SOD and GSH in CCD-18Co cells. However, these changes were markedly reversed by miR-215 knockdown.

In conclusion, the present results suggest that knockdown of miR-215 inhibited the inflammatory response and oxidative stress in LPS-stimulated CCD-18Co cells by inactivating the TLR4/NF-κB p65 and JNK/p38 signaling pathways. Therefore, miR-215 may be a potential biomarker and therapeutic target for the treatment of UC.

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Author's contribution. Study design, literature research, experimental study was performed by Boyang Sun and Kai Xing. Data acquisition, data analysis and statistical analysis was performed by Chen Qi and Ke Yan. Yan Xu was responsible for guarantor of integrity of entire study, manuscript preparation and manuscript editing.

Ethics approval and consent to participate. The study was carried out in accordance with the World Medical Association Declaration of Helsinki was and approved by the Ethics Committee at The Affiliated Stomatological Hospital of Nanjing Medical University.

Competing interests. The authors declare that they have no competing interests.

Data Availability. The data sets used in this study are available from the corresponding author on reasonable request.

References

Ando Y., Mazzurana L., Forkel M., Okazaki K., Aoi M., Schmidt P.T., Mjösberg J. and Bresso, F. (2016). Downregulation of microRNA-21 in colonic CD3+ T cells in UC remission. Inflamm. Bowel. Dis. 22, 2788-2793.

Bartel D.P. (2009). MicroRNAs: target recognition and regulatory functions. Cell 136, 215-233.

Cai X., Peng D., Wei H., Yang X., Huang Q., Lin Z., Xu W., Qian M., Yang C., Liu T., Yan W. and Zhao, J. (2017). miR-215 suppresses

proliferation and migration of non-small cell lung cancer cells. Oncol. Lett. 13, 2349-2353.

Chen J., Jiang S., Cao Y. and Yang Y. (2014a). Altered miRNAs expression profiles and modulation of immune response genes and proteins during neonatal sepsis. J. Clin. Immunol. 34, 340-348.

Chen L., Wang W., Zhou R., Ng S.C., Li J., Huang M., Zhou F., Wang X., Shen B., Kamm M.A., Wu K. and Xia B. (2014b). Characteristics of fecal and mucosa-associated microbiota in Chinese patients with inflammatory bowel disease. Medicine (Baltimore) 93, e51.

Fabbri M., Paone A., Calore F., Galli R., Gaudio E., Santhanam R., Lovat F., Fadda P., Mao C., Nuovo G.J., Zanesi N., Crawford M., Ozer G.H., Wernicke D., Alder H., Caligiuri M.A., Sinkam P.N., Perrotti D. and Croce C.M. (2012). MicroRNAs bind to Toll-like receptors to induce prometastatic inflammatory response. Proc. Natl. Acad. Sci. USA 109, E2110-2116.

Farraye F.A., Odze R.D., Eaden J. and Itzkowitz S.H. (2010). AGA technical review on the diagnosis and management of colorectal neoplasia in inflammatory bowel disease. Gastroenterology 138, 746-774.

Feng X., Wang H., Ye S., Guan J., Tan W., Cheng S., Wei G., Wu W., Wu F. and Zhou Y. (2012). Up-regulation of microRNA-126 may contribute to pathogenesis of ulcerative colitis via regulating NF-kappaB inhibitor IkappaBalpha. PLoS One 7, e52782.

Gao Z., Yu C., Liang H., Wang X., Liu Y., Li X., Ji K., Xu H., Yang M., Liu K., Qi D. and Fan H. (2018). Andrographolide derivative CX-10 ameliorates dextran sulphate sodium-induced ulcerative colitis in mice: Involvement of NF-kappaB and MAPK signalling pathways. Int. Immunopharmacol. 57, 82-90.

Gao J.B., Zhu M.N. and Zhu X.L. (2019a). miRNA-215-5p suppresses the aggressiveness of breast cancer cells by targeting Sox9. FEBS Open Bio. 9, 1957-1967.

Gao W., Wang C., Yu L., Sheng T., Wu Z., Wang X., Zhang D., and Lin Y. and Gong Y. (2019b). Chlorogenic acid attenuates dextran sodium sulfate-induced ulcerative colitis in mice through MAPK/ERK/JNK pathway. Biomed. Res. Int. 2019, 6769789.

Hanauer S.B. (2016). Oral or topical 5-ASA in ulcerative colitis. Dig. Dis. 34, 122-124.

Huang X., He Y., Zheng L., Feng H., Li Y., Li H., Yang F. and Li J. (2019). Identification of hepatitis B virus and liver cancer bridge molecules based on functional module network. World J. Gastroenterol. 25, 4921-4932.

Kim M.S., Han J.Y., Kim S.H., Kim H.Y., Jeon D. and Lee K. (2018). Polyhexamethylene guanidine phosphate induces IL-6 and TNF-α expression through JNK-dependent pathway in human lung epithelial cells. J. Toxicol. Sci. 43, 485-492.

Li N., Zhang Q., Zou J., Li Z., Tian T., Dong B., Liu X., Ge S., Zhu Y., Gao J. and Shen L. (2016). miR-215 promotes malignant progression of gastric cancer by targeting RUNX1. Oncotarget 7, 4817-4828.

Li W., Wang W., Liu L., Qu R., Chen X., Qiu C., Li J., Hayball J., Liu L., Chen J., Wang X., Pan X. and Zhao Y. (2019). GDF11 antagonizes TNF-alpha-induced inflammation and protects against the development of inflammatory arthritis in mice. FASEB. J. 33, 3317-3329.

Li M., Zhao J., Cao M., Liu R., Chen G., Li S., Xie Y., Xie J., Cheng Y., Huang L., Su M., Xu Y., Zheng M., Zou K., Geng L., Xu W. and Gong S. (2020). Mast cells-derived MiR-223 destroys intestinal barrier function by inhibition of CLDN8 expression in intestinal epithelial cells. Biol. Res. 53, 12.

Luan W., Zhou Z., Zhu Y., Xia Y., Wang J. and Xu B. (2018). miR-137 inhibits glutamine catabolism and growth of malignant melanoma by targeting glutaminase. Biochem. Biophys. Res. Commun. 495, 46-52.

Mei W., Xiang G., Li Y., Li H., Xiang L., Lu J., Xiang L., Dong J. and Liu M. (2016). GDF11 protects against endothelial injury and reduces atherosclerotic lesion formation in apolipoprotein e-null mice. Mol. Ther. 24, 1926-1938.

Meng Q., Wu W., Pei T., Xue J., Xiao P., Sun L., Li L. and Liang D. (2020). miRNA-129/FBW7/NF-κB, a novel regulatory pathway in inflammatory bowel disease. Mol. Ther. Nucleic Acids 19, 731-740.

Meng X. and Shi B. (2017). miR-215 functions as an oncogene in high-grade glioma by regulating retinoblastoma 1. Biotechnol. Lett. 39, 1351-1358.

Panahi G., Pasalar P., Zare M., Rizzuto R. and Meshkani R. (2018). High glucose induces inflammatory responses in HepG2 cells via the oxidative stress-mediated activation of NF-kappaB, and MAPK pathways in HepG2 cells. Arch. Physiol. Biochem. 124, 468-474.

Pekow J., Meckel K., Dougherty U., Haider H. I., Deng Z., Hart J., Rubin D.T. and Bissonnette M. (2018). Increased mucosal expression of miR-215 precedes the development of neoplasia in patients with long-standing ulcerative colitis. Oncotarget 9, 20709-20720.

Polytarchou C., Hommes D. W., Palumbo T., Hatziapostolou M., Koutsioumpa M., Koukos G., van der Meulen-de Jong A.E., Oikonomopoulos A., van Deen W.K., Vorvis C., Serebrennikova O.B., Birli E., Choi J., Chang L., Anton P. A., Tsichlis P.N., Pothoulakis C., Verspaget H.W. and Iliopoulos D. (2015). MicroRNA214 is associated with progression of ulcerative colitis, and inhibition reduces development of colitis and colitis-associated cancer in mice. Gastroenterology 149, 981-992.

Qu S., Shen Y., Wang M., Wang X. and Yang Y. (2019). Suppression of miR-21 and miR-155 of macrophage by cinnamaldehyde ameliorates ulcerative colitis. Int. Immunopharmacol. 67, 22-34.

Shi L., Lin Q., Yang T., Nie Y., Li X., Liu B., Shen J., Liang Y., Tang Y. and Luo F. (2016). Oral administration of Lentinus edodes beta-glucans ameliorates DSS-induced ulcerative colitis in mice via MAPK-Elk-1 and MAPK-PPARgamma pathways. Food Funct. 7, 4614-4627.

Shimajiri Y., Kosaka Y., Scheel D.W., Lynn F.C., Kishimoto N., Wang J., Zhao S. and German M.S. (2011). A mouse model for monitoring islet cell genesis and developing therapies for diabetes. Dis. Model Mech. 4, 268-276.

Sinha M., Jang Y.C., Oh J., Khong D., Wu E. Y., Manohar R., Miller C., Regalado S. G., Loffredo F.S., Pancoast J. R., Hirshman M.F., Lebowitz J., Shadrach J.L., Cerletti M., Kim M.J., Serwold T., Goodyear L.J., Rosner B., Lee R.T. and Wagers A.J. (2014). Restoring systemic GDF11 levels reverses age-related dysfunction in mouse skeletal muscle. Science 344, 649-652.

Taganov K.D., Boldin M.P., Chang K.J. and Baltimore D. (2006). NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proc. Natl. Acad. Sci. USA 103, 12481-12486.

Tan Y.G., Zhang Y.F., Guo C.J. Yang M. and Chen M.Y. (2013). Screening of differentially expressed microRNA in ulcerative colitis related colorectal cancer. Asian Pac. J. Trop. Med. 6, 972-976.

Terzic J., Grivennikov S., Karin E. and Karin M. (2010). Inflammation and colon cancer. Gastroenterology 138, 2101-2114 e2105.

Ullmann P., Nurmik M., Schmitz M., Rodriguez F., Weiler J., Qureshi-Baig K., Felten P., Nazarov P. V., Nicot N., Zuegel N., Haan S. and

Letellier E. (2019). Tumor suppressor miR-215 counteracts hypoxia-induced colon cancer stem cell activity. Cancer Lett. 450, 32-41.

Wang H., Chao K., Ng S.C., Bai A.H., Yu Q., Yu J., Li M., Cui Y., Chen M., Hu J. and Zhang S. (2016). Pro-inflammatory miR-223 mediates the cross-talk between the IL23 pathway and the intestinal barrier in inflammatory bowel disease. Genome Biol. 17, 58.

Wang L., Wang Y., Wang Z., Qi Y., Zong B., Liu M., Li X., Zhang X., Liu C., Cao R. and Ma Y. (2018). Growth differentiation factor 11 ameliorates experimental colitis by inhibiting NLRP3 inflammasome activation. Am. J. Physiol. Gastrointest Liver Physiol. 315, G909-G920.

Wang C., Li W., Wang H., Ma Y., Zhao X., Zhang X., Yang H., Qian J. and Li J. (2019a). *Saccharomyces boulardii* alleviates ulcerative colitis carcinogenesis in mice by reducing TNF-alpha and IL-6 levels and functions and by rebalancing intestinal microbiota. BMC Microbiol. 19, 246.

Wang L.P., Gao Y., Song B., Yu G., Chen H., Zhang Z., Feng C., Pan Y. and Yu X. (2019b). MicroRNAs in the progress of diabetic nephropathy: A systematic review and meta-analysis. Evid. Based Complement. Alternat. Med. 2019, 3513179.

Wang W., Qu R., Wang X., Zhang M., Zhang Y., Chen C., Chen X., Qiu C., Li J., Pan X., Li W. and Zhao Y. (2019c). GDF11 Antagonizes psoriasis-like skin inflammation via suppression of NF-kappaB signaling pathway. Inflammation 42, 319-330.

Williams A. E., Perry M. M., Moschos S. A., Larner-Svensson H. M. and Lindsay M. A. (2008). Role of miRNA-146a in the regulation of the innate immune response and cancer. Biochem. Soc. Trans. 36, 1211-1215.

Yang L., Bian Y., Li Z., Yan Y., Li J., Li W. and Zeng L. (2019). Identification of potential biomarkers and pathways in ulcerative colitis with combined public mRNA and miRNA expression microarray data analysis. J. Gastrointest. Oncol. 10, 847-858.

Zhang H. and Li W. (2018). microRNA-15 Activates NF-kappaB pathway via down regulating expression of adenosine a2 receptor in ulcerative colitis. Cell Physiol. Biochem. 51, 1932-1944.

Zhang J., Cao L., Wang H., Cheng X., Wang L., Zhu L., Yan T., Xie Y., Wu Y., Zhao M., Ma S., Wu M., Wang G. and Zhao M. (2015). Ginsenosides regulate PXR/NF-κB signaling and attenuate dextran sulfate sodium–induced colitis. Drug Metab. Dispos. 43, 1181-1189.

Zhang W., Guo Y., Li B., Zhang Q., Liu J.H., Gu G.J., Wang J.H., Bao R.K., Chen Y.J. and Xu J.R. (2018). GDF11 rejuvenates cerebrovascular structure and function in an animal model of Alzheimer's disease. J. Alzheimers Dis. 62, 807-819.

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