European Review for Medical and Pharmacological Sciences 2018; 22: 5284-5294

MicroRNA-19b alleviates lipopolysaccharide-induced inflammatory injury in human intestinal cells by up-regulation of Runx3

C.-X. QIAO[1], S. XU[2], D.-D. WANG[1], S.-Y. GAO[3], S.-F. ZHAO[4], M.-L. ZHANG[1], B. YU[1], Q. YIN[1], G. ZHAO[1]

[1]Department of Anorectal, The Affiliated Hospital of Qingdao University, Qingdao, Shandong, China
[2]Department of Anorectal, Yancheng Hospital of Traditional Chinese Medicine, Yancheng, Jiangsu, China
[3]Department of Anorectal, The Affiliated Hospital of Shandong University of TCM, Jinan, Shandong, China
[4]Department of Oncology, The Affiliated Hospital of Qingdao University, Qingdao, Shandong, China

Cuixia Qiao and Su Xu contributed equally to this work

Abstract. – OBJECTIVE: Ulcerative colitis (UC) is an unexplained inflammatory disease in bowel. Some studies reported that microRNA-19b (miR-19b) was closely related to cell inflammatory response. We aimed to explore the molecular mechanism of miR-19b on lipopolysaccharide (LPS)-induced human intestinal cell inflammatory injury.

MATERIALS AND METHODS: Caco2 cells were treated with 10 ng/ml LPS to induce inflammatory injury. The expression of miR-19b and runt-related transcription factor 3 (Runx3) was changed in Caco2 cells by cell transfection. Then, the viability, apoptosis and pro-inflammatory factors expressions of transfected cells were assessed using trypan blue exclusion assay, flow cytometry, qRT-PCR, Western blotting and enzyme-linked immunosorbent assay (ELISA), respectively, after LPS treatment. At last, the expressions of key factors involved in nuclear factor kappa B (NF-κB) and phosphatidylinositol 3-kinase/protein kinase 3 (PI3K/AKT) pathways were evaluated using Western blotting.

RESULTS: LPS significantly induced Caco2 cell inflammatory injury, down-regulated miR-19b expression and activated NF-κB and PI3K/AKT pathways. Suppression of miR-19b enhanced the LPS-induced Caco2 cell inflammatory injury, as well as NF-κB and PI3K/AKT pathways activation. Overexpression of miR-19b had opposite effects. In addition, miR-19b regulated the expression of Runx3 in Caco2 cells. Overexpression of Runx3 reversed the miR-19b knockdown-induced Caco2 cell viability inhibition, apoptosis enhancement, inflammatory factors expressions and NF-κB and PI3K/AKT signaling pathways activation.

CONCLUSIONS: Our study demonstrated that miR-19b alleviated LPS-induced Caco2 cell inflammatory injury via up-regulation of Runx3 and deactivation of NF-κB and PI3K/AKT signaling pathways.

Key Words:
Ulcerative colitis, MicroRNA-19b, Runt-related transcription factor 3, NF-κB pathway, PI3K/AKT pathway, Inflammatory injury.

Introduction

Ulcerative colitis (UC) is a kind of idiopathic inflammatory bowel disease (IBD) with unknown reason[1]. UC lesions mainly confined on the mucosa of the colon, manifested as inflammation or ulcers, involving the rectum and distal colon, but can be extended to the proximal, and even throughout the colon[2]. Its clinical features are mostly persistent or repeated mucus bloody stool and abdominal pain with varying degrees of systemic symptoms[3]. At present, there is no any particularly effective treatment method to cure UC. It is mainly treated with drugs, such as mesalazine, corticosteroids, and immunosuppressive drugs[4], and some patients would like to choose surgical treatment[2]. At the same time, patients with UC have an increasing risk of colorectal cancer[5]. Hence, it is urgently needed to study the mechanism of inflammatory response in colon cells.

microRNAs (miRNAs) are a class of non-coding RNA fragments encoded by endogenous genes with an average length of 19-24 nucleotides[6,7]. miRNAs can lead to gene silencing by inhibiting the transla-

Corresponding Author: Gang Zhao, MD; e-mail: robertrose6655@gmail.com

tion process or degrading the target gene by complementarily binding to the target gene, thereby participating in gene transcription and post-transcriptional expression regulation[8]. miRNAs have been aroused more and more attention in recent years due to their critical regulatory roles in hematological malignancies and lymphoma[9,10]. Furthermore, some researches[11-13] have confirmed that many miRNAs are related to the development and progression of various inflammatory diseases. By using bioinformatics analysis, miRNA-19b (miR-19b) is predicted to be a potential target for the regulation of inflammatory factors in immune-inflammatory diseases[14]. Evidence[15] has shown that miR-19b plays pivotal roles in cell inflammatory response by regulating the expression of toll-like receptor 2 (TLR2) and releasing interleukin 6 (IL-6) and matrix metalloproteinases 3 (MMP3). Ashraf et al[16] proved that miR-19b regulated Japanese encephalitis virus (JEV)-induced inflammatory response in the Japanese encephalitis. Ye et al[17] revealed that miR-19b reduced the airway remodeling, airway inflammation and degree of oxidative stress in a mouse model of asthma. However, the role of miR-19b in the regulation of inflammatory injury in human colorectal mucosa cells is still unclear.

Runt-related transcription factor 3 (Runx3) is a transcription factor of runt-domain family[18]. It has been reported to regulate cell proliferation and apoptosis in various cancers[19,20]. In recent years, some investigations[18,21,22] revealed that Runx3 was closely related to the inflammation of human diseases. Therefore, Runx3 has an important function in the immune regulation of inflammatory diseases.

This study aimed to investigate the molecular mechanism of miR-19b on lipopolysaccharide (LPS)-stimulated human colorectal mucosa cells, to provide a new theoretical basis for deeply exploring the pathogenesis of UC.

Materials and Methods

Cell Culture and Treatment

Human colorectal Caco2 cells were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA) and seeded in Dulbecco's Modified Eagle's Medium (DMEM; Solarbio, Beijing, China) supplemented with 25 mM N'-a-hydroxythylpiperazine-N'-ethanesulfanic acid (HEPES; Solarbio, Beijing, China), 15% fetal bovine serum (FBS; Sijiqing, Hangzhou, China), 100 U/ml penicillin, 100 μg/ml streptomycin, 0.25 mg/ml amphotericin B (all from Sigma-Aldrich, St. Louis, MO, USA). LPS (10 ng/ml, Solarbio, Beijing, China) was used to stimulate Caco2 cells in this research[23]. All cells were cultured at 37°C in 5% CO_2 atmosphere.

Cell Transfection

Cells were transfected with miR-19b mimic, miR-19b inhibitor and the negative controls (NC) (Biomics, Jiangsu, China), respectively. The full-length Runx3 sequence was constructed into pcDNA3.1 (GenePharma, Shanghai, China), which was considered as pc-Runx3. According to the manufacturer's protocols, the Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA, USA) was used for cell transfection. The stably transfected cells were selected by the culture medium including 0.5 mg/ml G418 (Sigma-Aldrich, St. Louis, MO, USA). After approximately 4 weeks, G418-resistant Caco2 cell lines were established and collected directly for the following experiments.

Cell Viability Assay

Caco2 cells (1×10^6 /ml) were collected and trypsinized by using 0.5 % trypsin (Thermo Fisher Scientific, Waltham, MA, USA). The viable cells in each group were calculated using cell counting chamber under microscope (Leica, Weitzlar, Germany).

Apoptosis Assay

Flow cytometry analysis was performed to identify and quantify the apoptotic cells post-transfection by using Annexin V-FITC/PI Apoptosis Detection Kit (Beijing Biosea Biotechnology, Beijing, China). The cells were collected from G418-resistant stably transfected cell lines. The differentiation of apoptotic cells (Annexin-V positive and PI-negative) and necrotic cells (Annexin-V and PI-positive) were detected by flow cytometry (Beckman Coulter, Coulter, CA, USA).

Enzyme-Linked Immunosorbent Assay (ELISA)

Supernatant of culture medium was collected from 24-well plates after relevant treatment or transfection. The concentrations of interleukin 1 (IL-1) and tumor necrosis factor α (TNF-α) were measured using ELISA kit (R&D Systems, Abingdon, UK) following manufacturer's instructions.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

The total RNA was extracted from transfected cells by using TRIzol reagent (Life Technologies

Corporation, Carlsbad, CA, USA) and treated with 150 U/ml DNaseI (Tiangen, Beijing, China). For the expression level of IL-1, TNF-α and Runx3 detection, 5 μg of total RNA were reverse transcribed by using mRNA Selective PCR kit (TaKaRa, Dalian, China), and the One Step SYBR® PrimeScript™ PLUS RT-PCR Kit (TaKaRa, Dalian, China) was used for the qRT-PCR analysis. For miR-19b level detection, the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) was used to synthetize cDNA from 5 μg of total RNA, and qRT-PCR was performed by using the TaqMan Universal Master Mix II (Applied Biosystems, Foster City, CA, USA). The expressions of U6 and GAPDH were acted as endogenous controls. Data were calculated by $2^{-\Delta\Delta Ct}$ method[24].

Western Blot Assay

RIPA Lysis Buffer (Beyotime Biotechnology, Shanghai, China) supplemented with protease inhibitors (Roche, Basel, Switzerland) and BCA™ Protein Assay Kit (Pierce Biotechnology, Waltham, MA, USA) were used to extract and quantify the total proteins in cells, respectively. Anti-GAPDH (G9545), anti-cleaved-caspase-9 (C7729), and anti-AKT (SAB4500797) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Anti-Bcl-2 (ab59348), anti-Bax (ab53154), anti-pro-caspase-3 (ab32150), anti-cleaved-caspase-3 (ab13585), anti-pro-caspase-9 (ab135544), anti-IL-1 (ab8320), anti-TNF-α (ab6671), anti-p65 (ab16502), anti-inhibitor of nuclear factor kappa B (IκBα) (ab5076), anti-p-IκBα (ab64813), anti-phosphatidylinositol 3 kinase (PI3K) (ab86714), and anti-p-P13K (ab182651) were purchased from Abcam Biotechnology (Cambridge, MA, USA). Anti-p-p65 (#3033) and anti-p-AKT (#4056) were obtained from Cell Signaling Technology (Danvers, MA, USA). Primary antibodies were prepared in 1% blocking buffer (1:1,000) and incubated with the membrane at 4°C overnight, washed and incubated with secondary antibody marked by horseradish peroxidase (HRP) for 1 h at room temperature. After rinsing, the polyvinylidene difluoride (PVDF) membrane carried blots and antibodies were transferred into the Bio-Rad ChemiDoc™ XRS system, and then 200 μl Immobilon Western chemiluminescent horseradish peroxidase (HRP) substrate (Millipore, Billerica, MA, USA) was added to cover the membrane surface. The signals of proteins were captured and the intensity of the bands was quantified using Image Lab™ Software (Bio-Rad, Hercules, CA, USA).

Statistical Analysis

The results from three repeated experiments were estimated using SPSS 19.0 statistical software (SPSS Inc., Chicago, IL, USA) and presented as mean ± standard deviation (SD). The *p*-values were calculated using one-way analysis of variance (ANOVA) with Sidak post-hoc test. $p < 0.05$ was described as statistically significant result.

Results

LPS Induced Inflammatory Injury in Caco-2 Cells

A research[25] has shown that LPS induces inflammatory injury in multiple cells. In our study, we found that the viability of Caco2 cells was decreased in LPS treatment group ($p < 0.05$, Figure 1A), while apoptotic cells were increased significantly ($p < 0.01$, Figure 1B). The results of Western blot analysis showed that the expression of Bcl-2 was down-regulated, and Bax, cleaved-caspase-3 and cleaved-caspase-9 expressions were up-regulated in response to LPS exposure in Caco2 cells (Figure 1C). Data in Figure 1D and 1E showed that the expressions of inflammatory cytokines IL-1 and TNF-α in Caco2 cells were overexpressed at mRNA and protein levels in response to LPS exposure ($p < 0.05$). Besides, ELISA assay results showed that the concentrations of IL-1 and TNF-α in culture supernatant of Caco2 cells were also raised significantly in LPS group ($p < 0.05$, Figure 1F and 1G).

Effects of miR-19b on LPS-Induced Inflammatory Injury in Caco-2 Cells

qRT-PCR was performed to measure the expression of miR-19b in Caco2 cells after LPS treatment. We found that the level of miR-19b was down-regulated after LPS treatment ($p < 0.05$, Figure 2).

Then, we speculated that miR-19b might be involved in the LPS-induced Caco2 cell inflammatory injury. To verify this hypothesis, miR-19b mimic and miR-19b inhibitor were respectively transfected into Caco2 cells to overexpress and silence miR-19b expression. Figure 3A showed that the expression of miR-19b was notably up-regulated in miR-19b mimic transfection group and down-regulated in miR-19b inhibitor transfection group ($p < 0.01$). Figure 3B and 3C presented that LPS-induced decrease of cell viability and increase of cell apoptosis were both exacerbated by miR-19b silence, and inhibited by miR-19b overexpression ($p <$

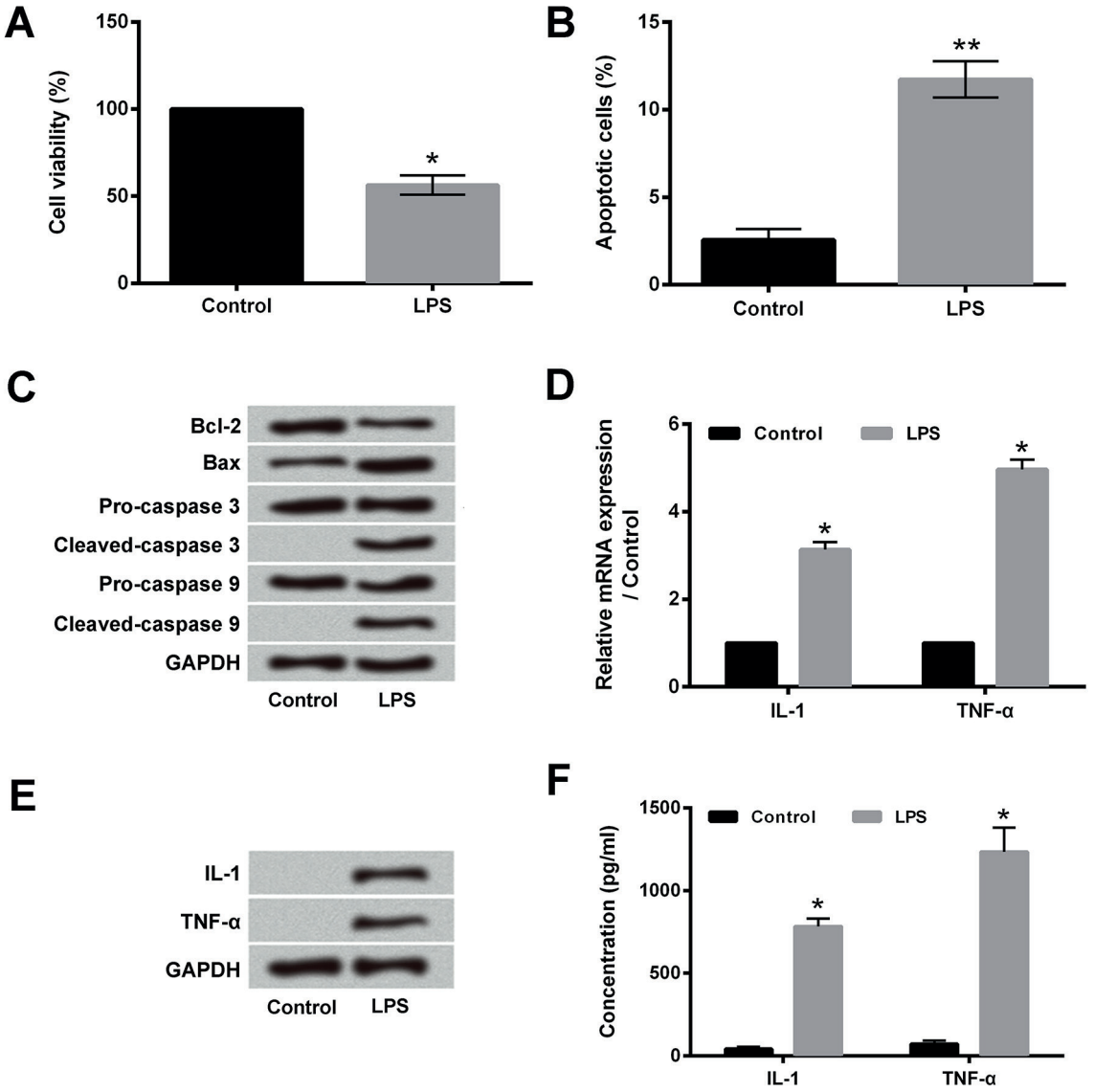

Figure 1. LPS-induced inflammatory injury in Caco2 cells. (A-B) Viability and apoptosis of Caco2 cells after LPS treatment were measured, respectively. (C) The expressions of apoptosis-related proteins in Caco2 cells after LPS treatment were detected using Western blot analysis. (D-E) mRNA and protein levels of inflammatory cytokines IL-1 and TNF-α in Caco2 cells after LPS treatment were detected using qRT-PCR and Western blot analysis, respectively. (F) Concentrations of IL-1 and TNF-α in culture supernatant of Caco2 cells after LPS treatment were detected using ELISA. *$p < 0.05$, **$p < 0.01$.

0.05 or $p < 0.01$). Western blot analysis showed that LPS-induced down-regulation of Bcl-2, and up-regulations of Bax, cleaved caspase-3 and cleaved caspase-9 were further exacerbated by silence miR-19b expression and alleviated by miR-19b overexpression (Figure 3D). Furthermore, the effects of miR-19b on the production of inflammatory cytokines were also examined. According to the results of qRT-PCR, ELISA and Western blot analysis, the level of IL-1 and TNF-α were increased by miR-19b silence, and were decreased by miR-19b overexpression after LPS treatment ($p < 0.05$ or $p < 0.01$, Figure 3E-3H). Summed up these results, we suggested that miR-19b silence aggravated LPS-induced inflammatory injury in Caco2 cells. On the contrary, miR-19b overexpression alleviated it.

miR-19b Deactivated NF-κB and PI3K/AKT Signaling Pathways in Caco2 Cells

Then, the regulatory effects of LPS and miR-19b on NF-κB and PI3K/AKT signaling pathways in

Caco2 cells were investigated. Western blot results suggested that LPS treatment up-regulated the protein levels of p-p65, p-IκBα, p-PI3K and p-AKT in Caco2 cells. Silence of miR-19b enhanced the effects of LPS and overexpression of miR-19b attenuated the effects of LPS (Figure 4A and 4B).

miR-19b alleviated LPS-Induced Inflammatory Injury in Caco2 Cells by Up-Regulation of Runx3

By using qRT-PCR and Western blot analysis, we found that Runx3 expression level was down-regulated in miR-19b inhibitor transfection group compared with NC group, but notably up-regulated in miR-19b mimic transfection group ($p< 0.05$, Figure 5A and 5B). This result revealed that miR-19b could regulate the expression of Runx3 in Caco2 cells.

Then, we explored the effects of Runx3 overexpression on LPS-induced inflammatory injury in Caco2 cells when miR-19b was silenced. Figure 6A and 6B suggested that Runx3 expression level was significantly up-regulated in pc-Runx3 group compared to pcDNA3.1 group ($p < 0.01$). Next, we found that the decrease of cell viability and increase of cell apoptosis induced by LPS exposure combined with miR-19b silence were both attenuated by addition of pc-Runx3 ($p < 0.05$, Figure 6C and 6D). From Western blot analysis, we also found that the addition of pc-Runx3 mitigated LPS-stimulation and silence miR-19b expression led to up-regulation of Bcl-2 and down-regulations of Bax, cleaved caspase-3 and cleaved caspase-9 (Figure 6E). In addition, we also examined the effects of Runx3 on inflammatory cytokines when miR-19b was silenced and LPS-stimulation. As shown in Figure 6F-6I, the levels of IL-1 and TNF-α were raised in miR-19b inhibitor+pcDNA3.1 group, and reduced in miR-19b inhibitor+pc-Runx3 group after LPS treatment ($p < 0.05$). These above results indicated that miR-19b alleviated LPS-induced Caco2 cell inflammatory injury by up-regulation of Runx3.

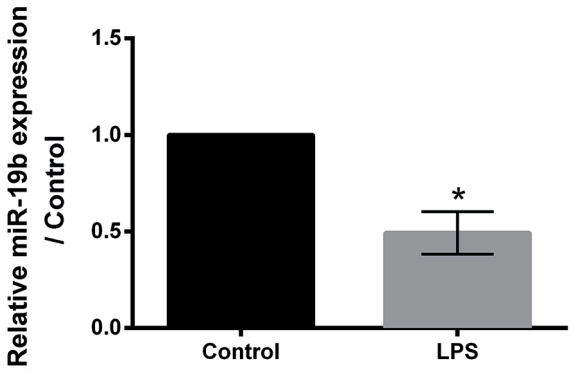

Figure 2. miR-19b was down-regulated in LPS-induced Caco2 cells. Relative level of miR-19b in Caco2 cells after LPS treatment was examined using qRT-PCR. *$p < 0.05$.

miR-19b Regulated NF-κB and PI3K/AKT Signaling Pathways in Caco2 Cells by Modulating Runx3 Expression

Western blot analysis was conducted to analyze the effects of Runx3 on NF-κB and PI3K/AKT pathways in Caco2 cells. The results revealed that the protein levels of p-p65, p-IκBα, p-PI3K and p-AKT were down-regulated after LPS+miR-19b inhibitor +pc-Runx3 group, compared to LPS+-miR-19b inhibitor+pcDNA3.1 group (Figure 7A and 7B).

Discussion

UC is a chronic disease with high incidence and prevalence rate in North America and Northern Europe[2]. Due to the Westernization of diet, the incidence of UC increased significantly in China[26]. However, there are no particularly good medical methods to cure UC. Patients often need long-term medication to maintain, and often endure, the recurrence of UC[2]. In recent years, the molecular mechanism researches of disease were popular in order to know the pathogenesis of diseases and find new therapeutic regimens. Some studies[27,28] have showed that miRNAs play an important regulatory role in IBD. Cheng et al[29] suggested that miR-19b was significantly down-regulated in Crohn's disease (CD), which is another type of IBD. However, the effects of miR-19b on the regulation of inflammatory injury in UC are still unclear. We found that miR-19b exhibited protective functions in LPS-stimulated Caco2 cell inflammatory injury, as evidenced by the increase of cell viability, decrease of cell apoptosis, and inhibition of inflammatory cytokines release. There was a positive correlation between the expression of miR-19b and Runx3. Inflammatory injury induced by LPS plus miR-19b silence was partially attenuated when Runx3 was overexpressed. Besides, we found that miR-19b overexpression deactivated NF-κB and PI3K/AKT signaling pathways possibly via up-regulation of Runx3.

From the previous study[30], we found that LPS could induce cell inflammatory response. Wang et al[31] reported miR-19b inhibited the expression

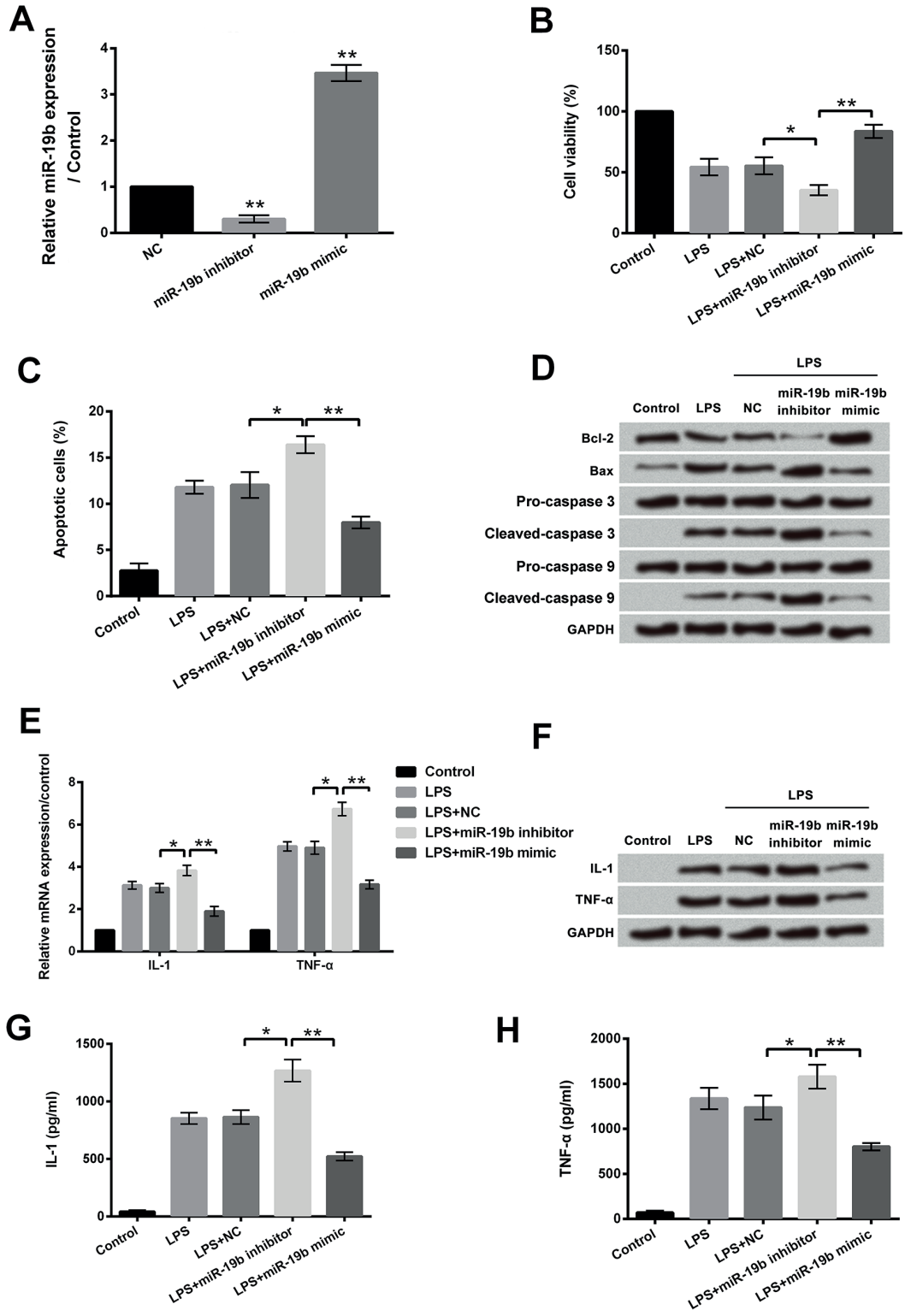

Figure 3. Overexpression of miR-19b alleviated LPS-induced inflammatory injury in Caco2 cells. Caco2 cells were transfected with miR-19b mimic or miR-19b inhibitor and then exposed to LPS. (A) The expression of miR-19b was assessed. (B-C) Cell viability and apoptosis were measured, respectively. (D) The expressions of apoptosis-related proteins were detected using Western blot analysis. (E-F) mRNA and protein levels of inflammatory cytokines IL-1 and TNF-α were examined using qRT-PCR and Western blot analysis, respectively. (G-H) Concentrations of IL-1 and TNF-α in the culture supernatant of Caco2 cells were detected using ELISA. *$p < 0.05$, **$p < 0.01$.

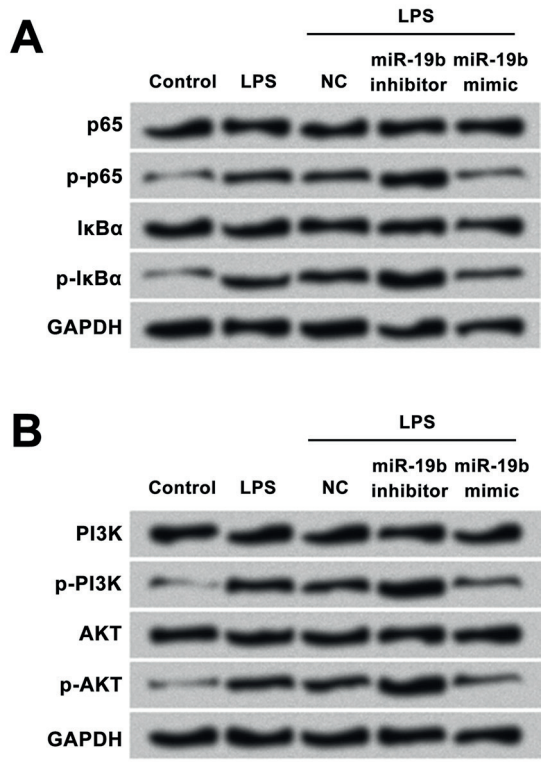

Figure 4. miR-19b deactivated NF-κB and PI3K/AKT signaling pathways in Caco2 cells. Caco2 cells were transfected with miR-19b mimic or miR-19b inhibitor and then exposed to LPS. The protein expressions of main factors involved in (A) NF-κB and (B) PI3K/AKT signaling pathways were measured using Western blot analysis.

of p47phox in macrophages, thereby reduced the LPS-induced inflammatory response. Therefore, in this study, we used LPS to induce inflammatory injury in Caco2 cells. We found that LPS treatment significantly decreased Caco2 cell viability, increased cell apoptosis and promoted the expression of inflammatory factors in Caco2 cells. These results were consistent with the previous studies.

TNF-α is a very important immune-regulatory factor in cells[32]. Right amount of TNF-α is beneficial to the body's immune response, but excessive TNF-α could cause some abnormal symptoms, such as inflammation, septic shock and multiple organ damage[33]. According to the previous researches, TNF-α plays an important role in the treatment of UC, which affected by signal transduction pathways and increased in UC patients[2,28]. 7 UC patients are examined by colonoscopy, and the concentration of IL-1 was significantly increased[34]. Accordingly, we detected the concentrations of the inflammatory cytokines IL-1 and TNF-α in Caco2 cells by ELISA and the results suggested that miR-19b silence accelerated the release of IL-1 and TNF-α, and overexpression of miR-19b suppressed the release.

Runx3 has an important function in the immune system of inflammatory diseases, which is commonly expressed in B cells and T cells[35]. Relevant research evidenced that the occurrence and development of various diseases are related to the deactivation of Runx3, including colon cancer, intraepithelial neoplasia and gastric cancer[36]. In addition, Runx3 could promote cell apoptosis and control cell proliferation[37]. In our study, we found that overexpression of miR-19b up-regulated Runx3 expression, and miR-19b silencing results in the opposite direction. Further investigations indicated that miR-19b silence promoted release of inflammatory cytokines by down-regulation of Runx3 expression. Thus, we inferred that miR-19b could affect the inflammatory injury in Caco2 cells by regulating the expression of Runx3.

According to related study, activation of TLRs triggers innate and adaptive immune responses in the gastrointestinal disease, leading to the activation of NF-κB[2], which could regulate the survival

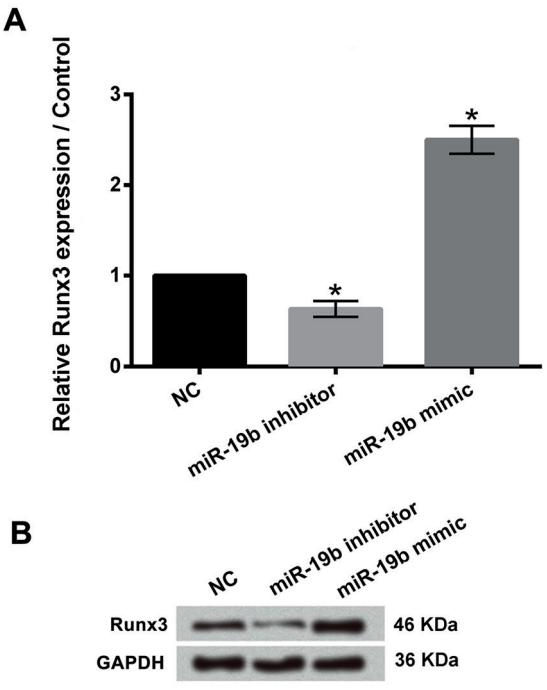

Figure 5. miR-19b regulated Runx3 expression in Caco2 cells. Caco2 cells were transfected with miR-19b mimic or miR-19b inhibitor, respectively. (A) mRNA and (B) protein levels of Runx3 were detected by qRT-PCR and Western blot analysis, respectively. *$p < 0.05$.

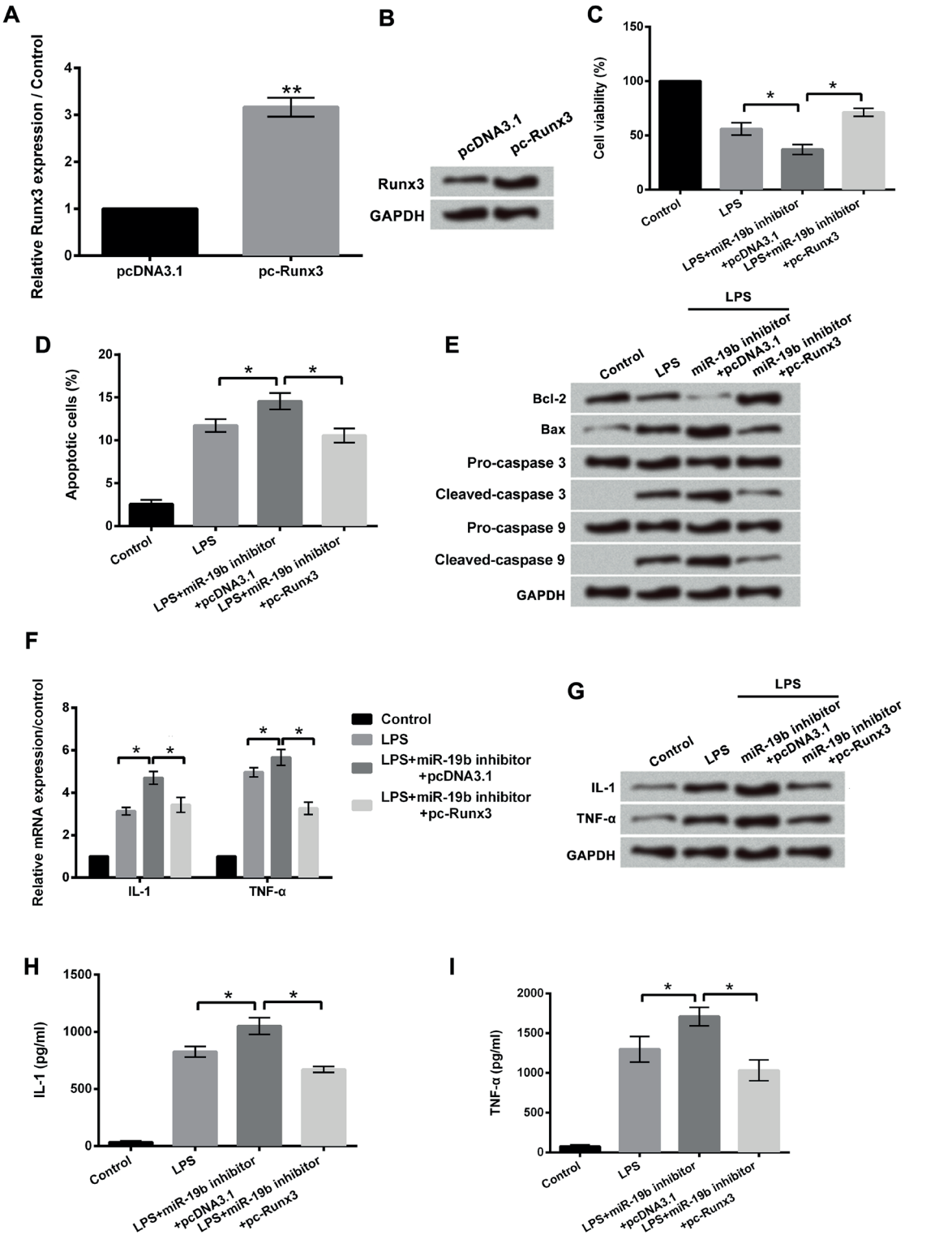

Figure 6. miR-19b alleviated LPS-induced inflammatory injury in Caco2 cells via up-regulation of Runx3. Caco2 cells were co-transfected with miR-19b inhibitor and Runx3 expressing vector (pc-Runx3). (A-B) mRNA and protein levels of Runx3 were detected by qRT-PCR and Western blot analysis, respectively. (C) Cell viability, (D) apoptosis were measured, respectively. (E) The expressions of apoptosis-related proteins were measured using Western blot analysis. (F-G) mRNA and protein levels of inflammatory cytokines IL-1 and TNF-α were examined using qRT-PCR and Western blot analysis, respectively. (H-I) Concentrations of IL-1 and TNF-α in the culture supernatant of Caco2 cells were detected using ELISA. $*p < 0.05$, $**p < 0.01$.

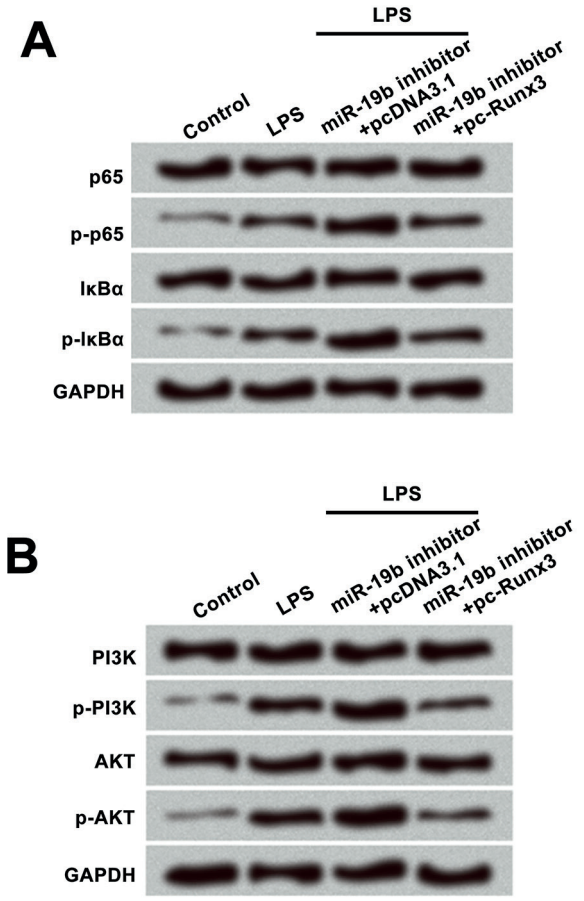

Figure 7. miR-19b deactivated NF-κB and PI3K/AKT signaling pathways in Caco2 cells via up-regulation of Runx3. Caco2 cells were co-transfected with miR-19b inhibitor and Runx3 expressing vector (pc-Runx3). The protein expressions of main factors involved in (A) NF-κB and (B) PI3K/AKT signaling pathways were measured using Western blot analysis.

function of cells, promote the growth of tumor growth factor (TGF) receptor, and induce inflammatory protein expression, cause kinase cascade[2,38]. AKT belongs to the serine/threonine protein kinase, plays an important role in cell function, and could activate cell survival, cell proliferation, differentiation and metabolism[39]. Activation of the PI3K/AKT signaling pathway could suppress LPS-activated MAPK and NF-κB pathways, resulting in reduced cellular inflammatory factors[40]. In order to explore the mechanism of which miR-19b expression affected LPS-induced inflammatory injury in Caco2 cell, we focused on NF-κB and PI3K/AKT signaling pathways. The results revealed that miR-19b silencing activated NF-κB and PI3K/AKT signaling pathways in Caco2 cells by down-regulating Runx3 expression.

Conclusions

We demonstrated that miR-19b overexpression mitigated LPS-induced inflammatory injury in Caco2 cells. Further results indicated that miR-19b exerted protective function possibly via up-regulation of Runx3 and thus deactivation of NF-κB and PI3K/AKT signaling pathways. The findings of this study provide a novel viewpoint regarding regulatory effects of miR-19b on UC inflammatory response.

Acknowledgement

This work was supported by Yancheng Hospital of Traditional Chinese Medicine Research Project (YB201608).

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Podolsky DK. Selective adhesion-molecule therapy and inflammatory bowel disease--a tale of Janus? N Engl J Med 2005; 353: 1965-1968.
2) Ordás I, Eckmann L, Talamini M, Baumgart DC, Sandborn WJ. Ulcerative colitis. Lancet 2012; 380: 1606-1619.
3) da Silva BC, Lyra AC, Rocha R, Santana GO. Epidemiology, demographic characteristics and prognostic predictors of ulcerative colitis. World J Gastroenterol 2014; 20: 9458-9467.
4) Kane SV. Systematic review: adherence issues in the treatment of ulcerative colitis. Aliment Pharmacol Ther 2006; 23: 577-585.
5) Pekow J, Meckel K, Dougherty U, Huang Y, Chen X, Almoghrabi A, Mustafi R, Ayaloglu-Butun F, Deng Z, Haider HI, Hart J, Rubin DT, Kwon JH, Bissonnette M. miR-193a-3p is a key tumor suppressor in ulcerative colitis-associated colon cancer and promotes carcinogenesis through up-regulation of IL17RD. Clin Cancer Res 2017; 1-11.
6) Zhou B, Li Z, Yang H, He N. Extracellular miRNAs: origin, function and biomarkers in hepatic diseases. J Biomed Nanotechnol 2014; 10: 2865-2890.
7) Wang ZM, Wan XH, Sang GY, Zhao JD, Zhu QY, Wang DM. miR-15a-5p suppresses endometrial cancer cell growth via Wnt/beta-catenin signaling pathway by inhibiting WNT3A. Eur Rev Med Pharmacol Sci 2017; 21: 4810-4818.
8) Zeng Y, Yi R, Cullen BR. MicroRNAs and small interfering RNAs can inhibit mRNA expression by similar mechanisms. Proc Natl Acad Sci U S A 2003; 100: 9779-9784.
9) Malumbres R, Sarosiek KA, Cubedo E, Ruiz JW, Jiang X, Gascoyne RD, Tibshirani R, Lossos IS. Differentia-

tion stage-specific expression of microRNAs in B lymphocytes and diffuse large B-cell lymphomas. Blood 2009; 113: 3754-3764.

10) Calin GA, Ferracin M, Cimmino A, Di Leva G, Shimizu M, Wojcik SE, Iorio MV, Visone R, Sever NI, Fabbri M, Iuliano R, Palumbo T, Pichiorri F, Roldo C, Garzon R, Sevignani C, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M, Croce CM. A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. N Engl J Med 2005; 353: 1793-1801.

11) Ranjha R, Paul J. Micro-RNAs in inflammatory diseases and as a link between inflammation and cancer. Inflamm Res 2013; 62: 343-355.

12) Galicia JC, Naqvi AR, Ko CC, Nares S, Khan AA. MiRNA-181a regulates Toll-like receptor agonist-induced inflammatory response in human fibroblasts. Genes Immun 2014; 15: 333-337.

13) Kishore A, Borucka J. Novel insights into miRNA in lung and heart inflammatory diseases. 2014; 2014: 259131.

14) McKenna LB, Schug J, Vourekas A, McKenna JB, Bramswig NC, Friedman JR, Kaestner KH. MicroRNAs control intestinal epithelial differentiation, architecture, and barrier function. Gastroenterology 2010; 139: 1654-1664.

15) Philippe L, Alsaleh G, Suffert G, Meyer A, Georgel P, Sibilia J, Wachsmann D, Pfeffer S. TLR2 expression is regulated by microRNA miR-19 in rheumatoid fibroblast-like synoviocytes. J Immunol 2012; 188: 454-461.

16) Ashraf U, Zhu B, Ye J, Wan S, Nie Y, Chen Z, Cui M, Wang C, Duan X, Zhang H, Chen H, Cao S. MicroRNA-19b-3p Modulates Japanese encephalitis virus-mediated inflammation via targeting RNF11. J Virol 2016; 90: 4780-4795.

17) Ye L, Mou Y, Wang J, Jin ML. Effects of microRNA-19b on airway remodeling, airway inflammation and degree of oxidative stress by targeting TSLP through the Stat3 signaling pathway in a mouse model of asthma. Oncotarget 2017; 8: 47533-47546.

18) Fu D, Song X, Hu H, Sun M, Li Z, Tian Z. Downregulation of RUNX3 moderates the frequency of Th17 and Th22 cells in patients with psoriasis. Mol Med Rep 2016; 13: 4606-4612.

19) Chen HX, Wang S, Wang Z, Zhang ZP, Shi SS. Overexpression of RUNX3 inhibits malignant behaviour of Eca109 cells in vitro and vivo. Asian Pac J Cancer Prev 2014; 15: 1531-1537.

20) Heng L, Jia Z, Bai J, Zhang K, Zhu Y, Ma J, Zhang J, Duan H. Molecular characterization of metastatic osteosarcoma: differentially expressed genes, transcription factors and microRNAs. Mol Med Rep 2017; 15: 2829-2836.

21) Fu D, Yu W, Li M, Wang H, Liu D, Song X, Li Z, Tian Z. MicroRNA-138 regulates the balance of Th1/Th2 via targeting RUNX3 in psoriasis. Immunol Lett 2015; 166: 55-62.

22) Renzi TA, Rubino M, Gornati L, Garlanda C, Locati M, Curtale G. MiR-146b mediates endotoxin tolerance in human phagocytes. Mediators Inflamm 2015; 2015: 145305.

23) Couto MR, Goncalves P, Catarino TA, Martel F. The effect of inflammatory status on butyrate and folate uptake by tumoral (Caco-2) and non-tumoral (IEC-6) intestinal epithelial cells. Cell J 2017; 19: 96-105.

24) Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25: 402-408.

25) Vessichelli M, Mariggi Ograve S, Varone A, Zizza P, Di Santo A, Amore C, Dell'Elba G, Cutignano A, Fontana A, Cacciapuoti C, Di Costanzo G, Zannini M, de Cristofaro T, Evangelista V, Corda D. The natural phosphoinositide-derivative glycerophosphoinositol inhibits the lipopolysaccharide-induced inflammatory and thrombotic responses. J Biol Chem 2017; 292: 12828-12841.

26) He XY, Tang ZP. Diet and ulcerative colitis. Shi Jie Hua Ren Xiao Hua Za Zhi 2007; 15: 3826-3829.

27) Fasseu M, Treton X, Guichard C, Pedruzzi E, Cazals-Hatem D, Richard C, Aparicio T, Daniel F, Soule JC, Moreau R, Bouhnik Y, Laburthe M, Groyer A, Ogier-Denis E. Identification of restricted subsets of mature microRNA abnormally expressed in inactive colonic mucosa of patients with inflammatory bowel disease. PLoS One 2010; 5(10). pii: e13160.

28) Dalal SR, Kwon JH. The role of MicroRNA in inflammatory bowel disease. Gastroenterol Hepatol (NY) 2010; 6: 714-722.

29) Cheng X, Zhang X, Su J, Zhang Y, Zhou W, Zhou J, Wang C, Liang H, Chen X, Shi R, Zen K, Zhang CY, Zhang H. miR-19b downregulates intestinal SOCS3 to reduce intestinal inflammation in Crohn's disease. Sci Rep 2015; 5: 10397.

30) Huang P, Wang J, Lin X, Yang FF, Tan JH. Effects of IL-10 on iron metabolism in LPS-induced inflammatory mice via modulating hepcidin expression. Eur Rev Med Pharmacol Sci 2017; 21: 3469-3475.

31) Wang T, Liu YP, Wang T, Xu BQ, Xu B. ROS feedback regulates the microRNA-19-targeted inhibition of the p47phox-mediated LPS-induced inflammatory response. Biochem Biophys Res Commun 2017; 489: 361-368.

32) Chen Y, Zhao Y. Curative efficacy of penehyclidine combined with edaravone on acute cerebral infarction and their effects on serum TNF-alpha and NDS score in rats. Eur Rev Med Pharmacol Sci 2018; 22: 223-228.

33) Carpagnano GE, Spanevello A, Curci C, Salerno F, Palladino GP, Resta O, Di Gioia G, Carpagnano F, Foschino Barbaro MP. IL-2, TNF-alpha, and leptin: local versus systemic concentrations in NSCLC patients. Oncol Res 2007; 16: 375-381.

34) Meister D, Ghosh S. Effect of fish oil enriched enteral diet on inflammatory bowel disease tissues in organ culture: differential effects on ulcerative colitis and Crohn's disease. World J Gastroenterol 2005; 11: 7466-7472.

35) Yagi R, Junttila IS, Wei G, Urban JF, Jr., Zhao K, Paul WE, Zhu J. The transcription factor GATA3 actively represses RUNX3 protein-regulated production of interferon-gamma. Immunity 2010; 32: 507-517.

36) Puthanveetil P, Chen S, Feng B, Gautam A, Chakrabarti S. Long non-coding RNA MALAT1 regulates hyperglycaemia induced inflammatory process in the endothelial cells. J Cell Mol Med 2015; 19: 1418-1425.

37) Li H, Li D, Meng N. Effects of RUNX3 mediated Notch signaling pathway on biological characteristics of colorectal cancer cells. Int J Oncol 2017; 50: 2059-2068.

38) Cheng CY, Hsieh HL, Sun CC, Lin CC, Luo SF, Yang CM. IL-1 beta induces urokinase-plasminogen activator expression and cell migration through PKC alpha, JNK1/2, and NF-kappaB in A549 cells. J Cell Physiol 2009; 219: 183-193.

39) Li B, Wu N, Zhang XJ, Wei ZL, Shang LX. MicroRNA-409 inhibits the proliferative ability of cervical carcinoma cells by regulating AKT. Eur Rev Med Pharmacol Sci 2018; 22: 936-942.

40) Androulidaki A, Iliopoulos D, Arranz A, Doxaki C, Schworer S, Zacharioudaki V, Margioris AN, Tsichlis PN, Tsatsanis C. The kinase Akt1 controls macrophage response to lipopolysaccharide by regulating microRNAs. Immunity 2009; 31: 220-231.