Upregulation of microRNA-219-5p relieves ulcerative colitis through balancing the differentiation of Treg/Th17 cells

Xinhua Li, Lijuan Sun, Li Chen, Yonghong Xu and Xinjuan Kong

Objective: This study aimed to investigate the specific regulatory roles of microRNA-219-5p (miR-219-5p) on ulcerative colitis (UC), and reveal the potential mechanisms relating with the differentiation of Treg/Th17 cells.
Methods: The mouse model of chronic UC was established by oral administration of 3% dextran sodium sulfate for three cycles. After intravenous injected with lentivirus (LV)-miR-219-5p for 24 h, the disease activity index (DAI), colon length, as well as the serum levels of Interleukin (IL)-6, -17A, -21, and -23 were measured. In addition, the histopathological changes in colon tissues were observed by Hematoxylin-eosin staining. The differentiation of Treg/Th17 cells was detected by Flow cytometry, and the expression of retinoic acid-related orphan receptor (RORrt), signal transducer and activator of transcription 3 (STAT3), and forkhead box p3 (Foxp3) were detected by quantitative real-time PCR and Western blot.
Results: MiR-219-5p was downregulated in colonic mucosal tissues of UC mice ($P < 0.05$). UC mice injected with LV-miR-219-5p exhibited significantly relieved histopathological changes of colon tissues, increased colon length, decreased DAI, as well as decreased serum levels of IL-6, -17A, -21, and -23 ($P < 0.05$). In addition, the injection of LV-miR-219-5p significantly increased the percentage of Treg cells via upregulating Foxp3, and decreased the percentage of Th17 cells via downregulating RORrt and STAT3 in UC mice ($P < 0.05$).
Conclusion: The upregulation of miR-219-5p relieved the colonic damage and inflammation of UC through balancing the differentiation of Treg/Th17 cells. Eur J Gastroenterol Hepatol 32: 813–820
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Introduction

Ulcerative colitis (UC) is an inflammatory bowel disease that characterized by inflammation and ulcers of the colon [1]. The overall incidence of UC is about 1.2–20.3/100 000 each year in the world, with a prevalence of 7.6–245/100 000 each year [2]. UC patients are usually accompanied with the intestinal symptoms of bloody diarrhea and abdominal pain, as well as high risk of colorectal cancer [3]. In clinic practice, the treatment of UC mainly includes the stepwise application of mesalazine, corticosteroids, and immunomodulators or surgery [4]. In addition, tumor necrosis factor α (TNF-α) antagonists, such as adalimumab, etanercept, and infliximab emerge as effective therapeutic agents for UC [5,6]. However, the long-term application of TNF-α antagonists may lead to severe infection, autoimmune diseases, and malignancy

[7]. Therefore, novel therapeutic strategy for UC with high efficiency and safety is urgently needed.

MicroRNAs (miRs) is a type of short, non-coding RNAs that play important roles in the pathogenesis of UC [8]. Previous studies have been proved that some miRs are upregulated in UC, such as miR-16, -21, -23a, -24, -29a, -155, -126, -195, -203, -206, and -214, and some are downregulated in UC, such as miR-124, -192, -320, -375, and -422b [9–11]. Since UC is a severe inflammatory process, these miRs exert diverse regulatory roles in the inflammatory process of UC. For example, miR-206 promotes the inflammatory process by downregulating adenosine A3 receptor and activating nuclear factor-kappa B signaling, thereby enhancing the severity of dextran sodium sulfate (DSS)-induced UC [12]. The downregulation of miR-124 promotes the inflammation of UC by activating signal transducer and activator of transcription 3 (STAT3) [13], while the downregulation of miR-214 inhibits the inflammation of UC by increasing levels phosphatase and tensin homolog and PDZ and LIM domain 2 [14]. MiR-219-5p is known as a tumor suppressor that also involved in the regulation of inflammation. A previous study has proved that miR-219-5p relieves spinal cord injury (SCI) by inhibiting neurogenic differentiation 2-regulated inflammation [15]. However, researches on the specific regulatory roles of miR-219-5p on UC are greatly limited.

The abnormal intestinal inflammatory response of UC is closely associated with the imbalance of Treg/Th17 cell differentiation [16]. The activation of Th17 cells and over-production of cytokines directly contribute to the occurrence and development of UC [17]. Emerging researches

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have proved that diverse therapeutic agents against UC are involved in the regulation of Treg/Th17 cell balance, such as resveratrol [16], mesalazine [18], Norisoboldine [19], and Heme oxygenase-1 [20]. In addition, the balance of Treg/Th17 cell differentiation can also be regulated by diverse miRs, such as miR-16 [21], 21 [22], -155 [23], -141, -200a [24], and -384 [25]. However, the regulatory roles of miR-219-5p on Treg/Th17 cell balance are still unclear.

In this study, the mouse model of chronic UC was first established. The specific regulatory effects of miR-219-5p on the colonic damage and inflammation of UC were evaluated. In addition, the regulatory effects and mechanisms of miR-219-5p on the differentiation of Treg/Th17 cells were analyzed. Our findings may open up new insights into the underlying mechanisms of inflammation in UC, and provide a potential therapeutic target for UC.

Materials and methods

Establishment of mouse model of chronic ulcerative colitis

A total of 40 BALB/c mice (male, 7 weeks, specific pathogen free grade) were purchased from the Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). All mice were fed in a specific pathogen-free grade animal room with free access to water and food. The mouse model of chronic UC was established by oral administration of 3% DSD (dissolved in drinking water) for three cycles. Each cycle consisted of 3% DSS for 5 days followed by drinking water (without DSS) for 14 days. The modeling lasted for 50 days. The disease activity index (DAI) of UC was determined by weight loss, stool consistency, and intestinal bleeding [26]. All animal assays were approved by the local ethics committee.

Treatments

Lentivirus miR-219-5p negative control (LV-miR-NC) and LV-miR-219-5p were purchased from Guangzhou Ruibo Biotechnology Co., Ltd. (Guangzhou, China). Mice (10 weeks) were randomly divided into four groups (10 mice each group), including Control, normal mice; UC, UC mice; UC + LV-miR-NC, UC mice intravenous injected with 200 μl LV-miR-NC (5×10^7 TU/ml) through vena caudalis; and UC + LV-miR-219-5p, UC mice intravenous injected with 200 μl LV-miR-219-5p (5×10^7 TU/ml) through vena caudalis. After 24 h of treatment, mice were anesthetized by intraperitoneal injection of 10% chloral hydrate (300 mg/kg, bodyweight), and the blood samples were collected from the abdominal aorta. All mice showed no signs of peritonitis following administration of 10% chloral hydrate. After blood collection, mice were immediately sacrificed by cervical dislocation followed by decapitation, and the colonic tissues were collected.

Hematoxylin-eosin staining

The colonic tissues were fixed in 10% formaldehyde, dehydrated in graded ethanol, paraffin-embedded, and sliced. Then the tissue sections were dewaxed in xylene, rehydrated in graded ethanol, and stained with Hematoxylin for 1 min and Eosin for 1 min. After dehydrated in graded ethanol and vitrificated in dimethylbenzene, the tissue sections were observed under microscope (Olympus, Tokyo, Japan).

ELISA

The serum levels of Interleukin (IL)-6, -17A, -21, and -23 were detected in mice by using ELISA kits (Boster, Wuhan, China) in accordance with manufacturers' instructions. The optical density (OD) at 450 nm was measured by a Microplate Reader (BIO-RAD, Hercules, California, USA).

Flow cytometry

Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples by 25 min of ficoll-hypaque density gradient centrifugation at 2000 rpm/min, and then resuspended in Roswell Park Memorial Institute-1640 medium containing 10% fetal bovine serum. PBMCs were treated with 25 ng/ml phorbol myristate acetate combined with 1 μg/ml ionomycin for 4 h, and monensin for 2 h. For detection of Treg cell differentiation, PBMCs were incubated with allophycocyanin (APC)-conjugated anti-CD25 (1:1000; Bioss, Beijing, China) for 30 min, and phycoerythrin (PE)-conjugated anti-forkhead box p3 (Foxp3) (1:1000; Bioss) for 20 min at 4°C in darkness. For detection of Th17 cell differentiation, PBMCs were incubated with APC-conjugated anti-CD4 (1:1000; Bioss) for 30 min, and PE-conjugated anti-IL-17A (1:1000; Bioss) for 20 min at 4°C in darkness. Cells were finally analyzed on a Flow cytometry (BD, San Jose, California, USA).

Quantitative real-time PCR

Total RNA was extracted from colonic mucosal tissues using TRIzol-A (Thermo Fisher Scientific, Waltham, Massachusetts, USA), and reverse-transcribed into cDNA using a cDNA Reverse Transcription Kit (Thermo Fisher Scientific) in accordance with manufacturers' instructions. Quantitative real-time PCR (qRT-PCR) was performed on ABI 7500 (Applied Biosystems, Foster City, California, USA) by using specific primers (Table 1). U6 and GAPDH were used as internal controls. The PCR program included 95°C for 10 min, 40 cycles at 95°C for 10 s, 60°C for 20 s, and 72°C for 34 s. The relative expression of target genes was calculated according to the $2^{-\Delta\Delta Ct}$ method [27].

Western blot

Colonic mucosal tissues were lysed in RIPA Lysis buffer (Beyotime, Shanghai, China). Total proteins were separated by 10% SDS-polyacrylamide gel electrophoresis, and transferred to polyvinylidenefluoride membrane. The membrane was blocked with 5% skim milk in Tris-buffered saline Tween (TBST) for 2 h, and incubated with specific primary antibodies (rabbit anti-mouse; anti-RORγt, #23109R; anti-STAT3, #1141R; anti-Foxp3, #10211R; anti-GAPDH, #0755R; 1:1000; Bioss) overnight at 4°C. Then the membrane was washed with TBST for three times, and incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (goat anti-rabbit, #1141R, 1:2000; Bioss) for 1 h at 25°C. The protein bands were visualized using a HRP color development kit (Thermo Fisher Scientific).

Table 1. The sequences of specific primers used in quantitative real-time PCR

Primers	Sequences	
	Forward	Reverse
miR-219-5p	5′-GGTGATTGTCCAAACGG-3′	5′-CAGTGCGTGTCGTGGA-3′
U6	5′-GCTTCGGCAGCACATATACTAAAAT-3′	5′-CGCTTCACGAATTTGCGTGTCAT-3′
Foxp3	5′-TGAGCTGGCTGCAATTCTGG-3′	5′-ATCTAGCTGCTCTGCATGAGGTGA-3′
RORγt	5′-GCTGCTGACCCCAGTATGAA-3′	5′-AAAACCCCTCCCTCCCTCT-3′
STAT3	5′-TTTGAGACAGAGGTGTACCACCAAG-3′	5′-ACCACAGGATTGATGCCCAAG-3′
GAPDH	5′-GGCACAGTCAAGGCTGAGAATG-3′	5′-ATGGTGGTGAAGACGCCAGTA-3′

Foxp3, forkhead box p3; RORrt, retinoic acid-related orphan receptor; STAT3, signal transducer and activator of transcription 3.
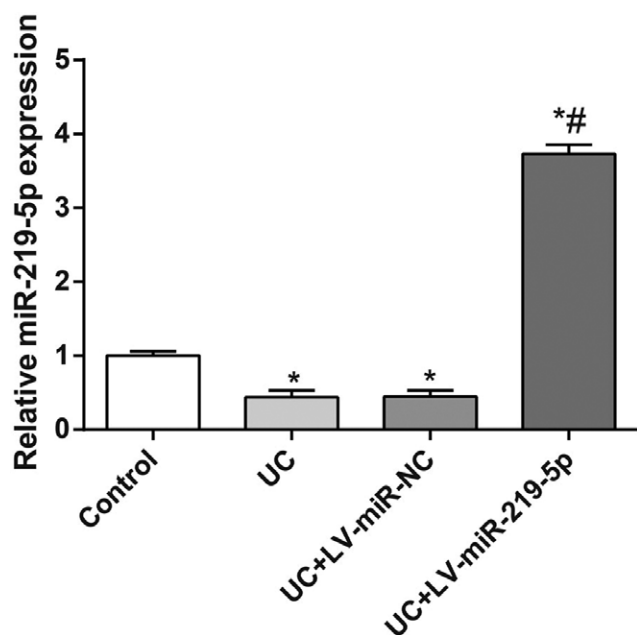

Fig. 1. The expression of miR-219-5p in colonic mucosal tissues of ulcerative colitis (UC) mice at the mRNA level. Control, normal mice; UC, UC mice; UC + LV-miR-NC, mice injected with lentivirus-miR-219-5p-negative control; UC + LV-miR-219-5p, mice intravenous injected with lentivirus-miR-219-5p. $*P < 0.05$ vs. Control; $\#P < 0.05$ vs. UC and UC + LV-miR-NC.

Statistical analyses

All data were expressed as mean ± SD. Statistical analysis was performed by SPSS version 18.0 (SPSS Inc., Chicago, Illinois, USA). Comparison between different groups was determined by LSD-*t*-test (two groups) or one-way analysis of variance (more than two groups). A *P*-value of less than 0.05 represented significantly different.

Results

MiR-219-5p was downregulated in colonic mucosal tissues of ulcerative colitis mice

The expression of miR-219-5p was detected in colonic mucosal tissues of UC mice. qRT-PCR showed that the expression of miR-219-5p in colonic mucosal tissues was significantly lower in UC mice than in the control ($P < 0.05$). The expression of miR-219-5p in colonic mucosal tissues of UC mice was significantly increased by the injection of LV-miR-219-5p ($P < 0.05$), but was not significantly influenced by the injection of LV-miR-NC (Fig. 1).

Upregulation of miR-219-5p relieved colonic damage in ulcerative colitis mice

The histopathological changes of colon tissues in UC mice were observed by hematoxylin-eosin staining. As shown in Fig. 2a, obvious intestinal wall thickening, inflammatory cell infiltration, indistinct mucosal and muscular layers, as well as intestinal gland disappearance were observed in the colon tissues of UC mice. These histopathological changes in UC mice were significantly relieved by the injection of LV-miR-219-5p (Fig. 2a). In addition, significantly shorter colon length, and higher DAI were observed in UC mice than in the control ($P < 0.05$). The injection of LV-miR-219-5p significantly increased the colon length and decreased the DAI in UC mice ($P < 0.05$) (Fig. 2b and c).

Upregulation of miR-219-5p inhibited the inflammatory response of ulcerative colitis mice

The inflammatory response of UC mice was evaluated by four proinflammatory cytokines. As shown in Fig. 3a–d, UC mice exhibited significantly higher serum levels of IL-6, -17A, -21, and -23 than the control ($P < 0.05$). The serum levels of IL-6, -17A, -21, and -23 in UC mice were significantly decreased by the injection of LV-miR-219-5p ($P < 0.05$), but were not significantly influenced by the injection of LV-miR-NC (Fig. 3a–d).

Upregulation of miR-219-5p balanced the differentiation of Treg/Th17 cells

The differentiation of Treg/Th17 cells was detected by Flow cytometry. As shown in Fig. 4a and b, significantly lower percentage of Treg cells and higher percentage of Th17 cells were observed in UC mice than in the control ($P < 0.05$). The injection of LV-miR-219-5p significantly increased the percentage of Treg cells and decreased the percentage of Th17 cells in UC mice ($P < 0.05$). The differentiation of Treg/Th17 cells in UC mice was not significantly influenced by the injection of LV-miR-NC (Fig. 4a and b).

Upregulation of miR-219-5p downregulated retinoic acid-related orphan receptor and signal transducer and activator of transcription 3, and upregulated forkhead box p3

In order to reveal the regulatory mechanisms of miR-219-5p on the differentiation of Treg/Th17 cells, the expression of three important transcription factors (TFs) involved in Treg/Th17 cell balance was detected. As shown in Fig. 5a–c, the expression of retinoic acid-related orphan
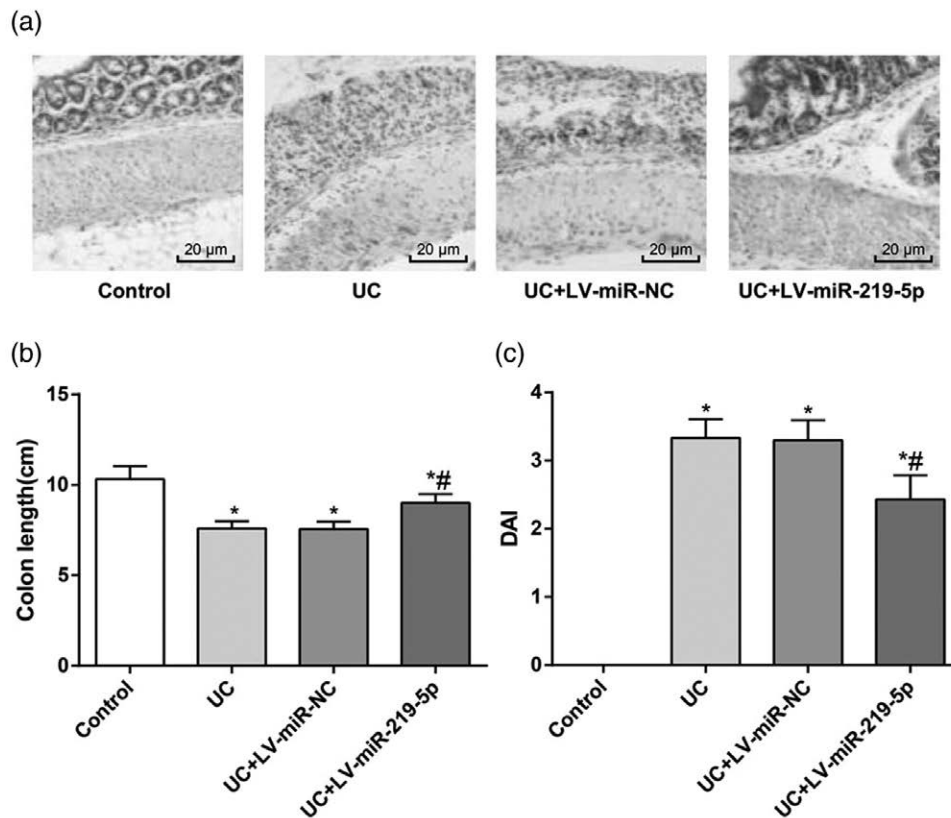
Fig. 2. The colon tissue damage in ulcerative colitis (UC) mice. (a) Hematoxylin-eosin staining of colon tissues; (b) colon length; (c) disease activity index (DAI). Control, normal mice; UC, UC mice; UC + LV-miR-NC, mice injected with lentivirus-miR-219-5p-negative control; UC + LV-miR-219-5p, mice injected with lentivirus-miR-219-5p. *$P < 0.05$ vs. Control; #$P < 0.05$ vs. UC and UC + LV-miR-NC.

receptor (RORrt) and STAT3 was significantly higher, and the expression of Foxp3 was significantly lower in colonic mucosal tissues of UC mice than those of the control at both the mRNA and protein level ($P < 0.05$). The injection of LV-miR-219-5p significantly decreased the expression of RORrt and STAT3 in colonic mucosal tissues of UC mice ($P < 0.05$) (Fig. 5a and b). In addition, the downregulated Foxp3 in UC mice was also reversed by the injection of LV-miR-219-5p ($P < 0.05$) (Fig. 5c). The expression of these above TFs in colonic mucosal tissues of UC mice was not significantly influenced by the injection of LV-miR-NC (Fig. 5a–c).

Discussion

UC is a severe chronic inflammatory disease of the colon that associated with high risk of colorectal carcinoma [28]. UC can be induced by diverse factors, such as genetic susceptibility variation, inappropriate immunoresponse, undetermined environmental factors, as well as intestinal dysbiosis [16,29]. In this study, the mouse model of chronic UC was established by oral administration of 3% DSD for three cycles. Fifty days later, obvious histopathological changes on colon tissues were observed, including intestinal wall thickening, inflammatory cell infiltration, indistinct mucosal and muscular layers, and intestinal gland disappearance. In addition, significantly decreased colon length and increased DAI were observed in UC mice. These phenomena indicate that the chronic UC model is successfully induced in mice. Since the pathological characteristics of chronic UC are well simulated in the mouse

model, the specific regulatory roles of miR-219-5p on UC were further analyzed.

MiR-219-5p is known as a tumor suppressor that downregulated in diverse tumors, including colorectal cancer [30], glioblastoma [31], chordoma [32], and hepatocellular carcinoma [33]. In this study, we found that the expression of miR-219-5p was significantly decreased in the colonic mucosal tissues of UC mice. This result is consistent with previous studies on miR-124 [34], -192 [9], -214-3p [35], and -4284 [36]. A previous study indicates that miR-20b, -98, and -125b-1 are upregulated in UC patients, and these miRs may serve as diagnostic biomarkers in UC [37]. Since miR-219-5p was significantly downregulated in UC, we suspect that miR-219-5p may also be used as a potential biomarker for the diagnosis of UC.

In this study, miR-219-5p was upregulated in UC mice by the injection of LV-miR-219-5p. The injection of LV-miR-219-5p into UC mice significantly relieved the histopathological changes of colon tissues, increased the colon length, and decreased the DAI. These results indicate that the upregulation of miR-219-5p relieves the colonic damage of UC mice. Previous studies have proved that miRs are closely associated with the therapeutic effects of UC-targeting agents. For example, herb-partitioned moxibustion decreases the macroscopic colon injury scores and microscopic histopathology scores of UC mice by upregulating miR-184 and downregulating miR-490-5p [38]. MiR-214 inhibitor suppresses the inflammatory response in colonic explants from three UC patients, and reduces the DAI in UC mice [14]. Nicotine and thiopurine protects against UC via upregulating miR-124 and downregulating
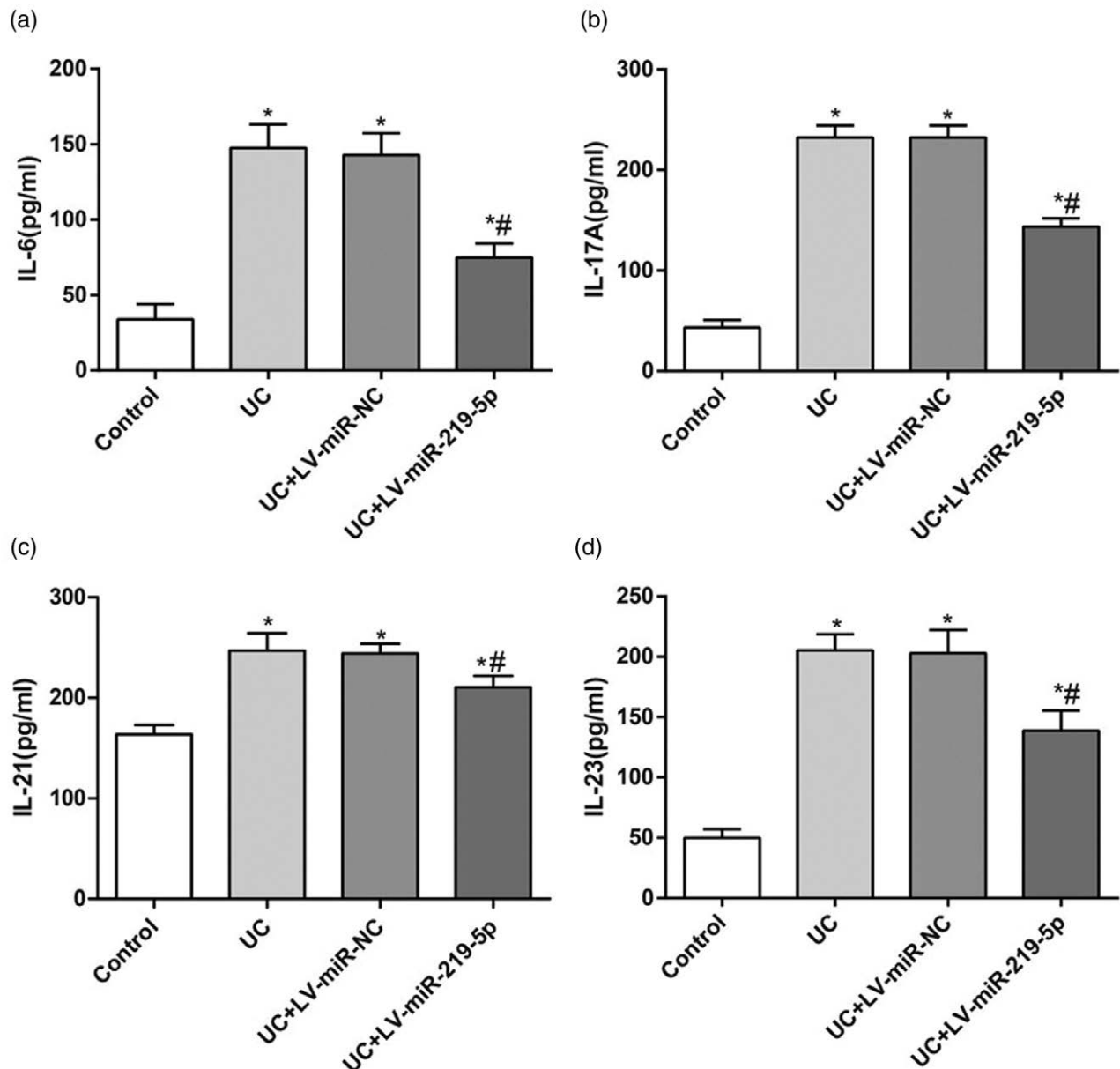
Fig. 3. The levels of proinflammatory cytokines in ulcerative colitis (UC) mice. (a) Interleukin (IL)-6; (b) IL-17A; (c) IL-21; (d) IL-23. Control, normal mice; UC, UC mice; UC + LV-miR-NC, mice injected with lentivirus-miR-219-5p-negative control; UC + LV-miR-219-5p, mice injected with lentivirus-miR-219-5p. *P < 0.05 vs. Control; #P < 0.05 vs. UC and UC + LV-miR-NC.

miR-31, respectively [39,40]. The upregulation of miR-219-5p may exert similar protective effects on UC with the upregulation of miR-184 and -124, as well as the downregulation of miR-490-5p, -214, and -31.

UC is an inflammatory disease that associated with the overproduction of diverse pro-inflammatory cytokines, including IL-1β, -6, -8, -17, -23, and TNF-α [41,42]. During acute flares of UC, there is a marked infiltration of macrophages into the inflamed mucosa, manifested by an increase in macrophage-derived inflammatory cytokines, such as IL-1β, IL-6, and TNF-α [43]. In this study, we found that the serum levels of IL-6, -17A, -21, and -23 were significantly increased in UC mice. These results illustrate that the inflammatory response is enhanced in UC mice. In addition, the injection of LV-miR-219-5p significantly decreased the serum levels of IL-6, -17A, -21, and -23 in UC mice. Our findings are just consistent with

a previous study that miR-219-5p mimics significantly inhibits the levels of TNF-α, IL-1β, and IL-6 in SCI mice [15]. We suspect that the upregulation of miR-219-5p may relieve the colonic damage of UC through inhibiting the inflammatory response.

The imbalance of Treg/Th17 cells plays a key regulatory role in the inflammatory response of UC [44]. In this study, we found that the percentage of Treg cells was significantly decreased, and the percentage of Th17 cells was significantly increased in UC mice. Since Th17 cells can initiate and amplify inflammatory response through secreting proinflammatory cytokines, the increased Th17 cells contribute to the promotion of inflammatory response in UC. In addition, we found that the injection of LV-miR-219-5p significantly increased Treg cells, and decreased Th17 cells in UC mice. These results indicate that the upregulation of miR-219-5p promotes the recovery of Treg/Th17 cell
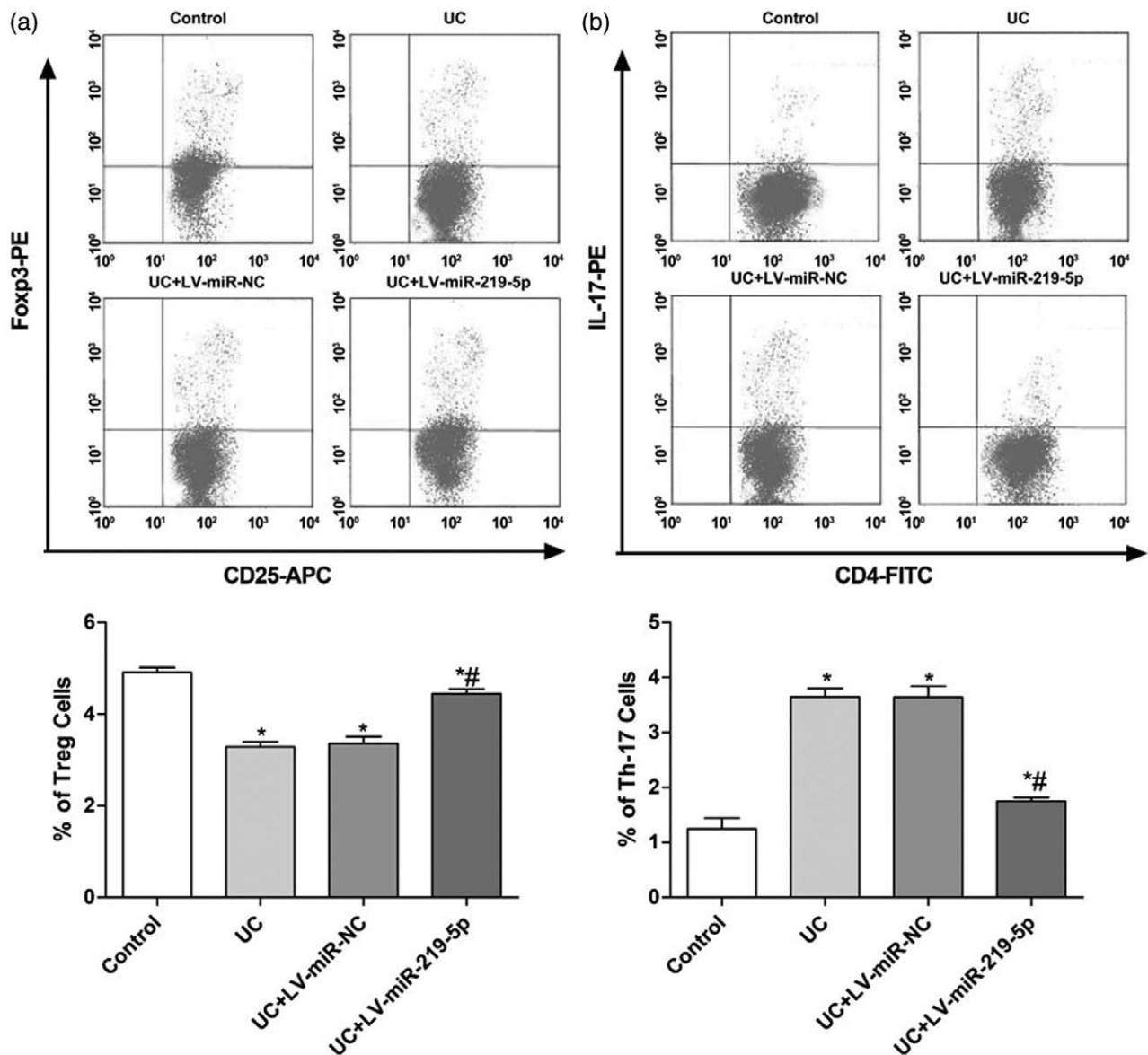
Fig. 4. The differentiation of Treg/Th17 cells in ulcerative colitis (UC) mice. (a) The percentage of Treg cells; (b) the percentage of Th17 cells. Control, normal mice; UC, UC mice; UC + LV-miR-NC, mice injected with lentivirus-miR-219-5p-negative control; UC + LV-miR-219-5p, mice injected with lentivirus-miR-219-5p. *$P < 0.05$ vs. Control; #$P < 0.05$ vs. UC and UC + LV-miR-NC.

balance. The upregulation of miR-219-5p may exert consistent functions on Treg/Th17 cell balance with some UC-targeting agents, such as resveratrol [16], mesalazine [18], norisoboldine [19], and heme oxygenase-1 [20]. Moreover, our further assays showed that the injection of LV-miR-219-5p significantly downregulated RORrt and STAT3, and upregulated Foxp3 in UC mice. Since RORγt and STAT3 are positively associated with Th17 cell differentiation, and Foxp3 is positively associated with Treg cell differentiation, our findings further illustrate that the upregulation of miR-219-5p contributes to the balance of Treg/Th17 cell differentiation. We suspect that the upregulation of miR-219-5p may inhibit the inflammatory response of UC through balancing the differentiation of Treg/Th17 cells. There is a massive infiltration of neutrophil granulocytes into the affected mucosa during acute flares of UC, which is manifested clinically by an increase in stool neutrophil granulocytes. The initial inflammatory cell influx consists predominantly of neutrophil

granulocytes, which in turn orchestrate the recruitment of monocytes and the activation of lymphocytes required for a mature inflammatory response [43]. We speculate that the miR-219-5p-mediated imbalance of Treg/Th17 cells is closely associated with neutrophil granulocytes. However, the changes of neutrophil granulocytes in UC mice, as well as the specific regulatory role of miR-219-5p on neutrophil granulocytes are not evaluated in this study. Further research on this field is still needed.

In conclusion, miR-219-5p was downregulated in colonic mucosal tissues of UC mice. The upregulation of miR-219-5p balances the differentiation of Treg/Th17 cells, inhibits the production of proinflammatory cytokines, thereby relieving the colonic damage in UC mice. The upregulation of miR-219-5p may be a promising therapeutic target for UC. However, this study is still limited in mouse model. Further researches on the efficacy and safety of downregulated miR-219-5p in the treatment of UC are still needed.
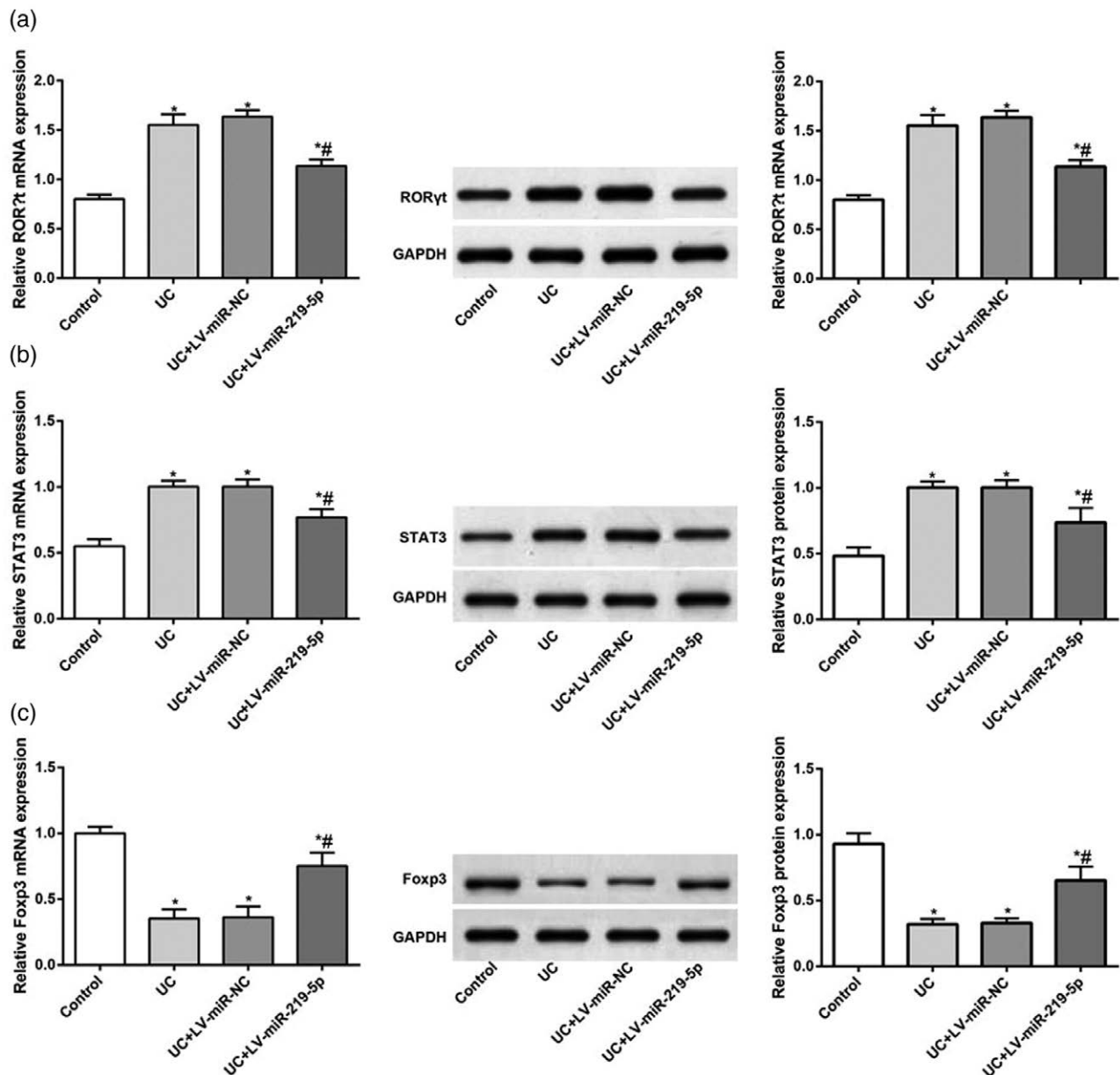
Fig. 5. The expression of three transcription factors (TFs) involved in the regulation of Treg/Th17 cell balance at both the mRNA and protein level. (a) Retinoic acid-related orphan receptor (RORrt); (b) signal transducer and activator of transcription 3 (STAT3); (c) forkhead box p3 (Foxp3). Control, normal mice; UC, UC mice; UC + LV-miR-NC, mice injected with lentivirus-miR-219-5p-negative control; UC + LV-miR-219-5p, mice injected with lentivirus-miR-219-5p. *$P < 0.05$ vs. Control; #$P < 0.05$ vs. UC and UC + LV-miR-NC.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

References

1 Panés J, Alfaro I. New treatment strategies for ulcerative colitis. *Expert Rev Clin Immunol* 2017; 13:963–973.

2 Feuerstein JD, Cheifetz AS. Ulcerative colitis: epidemiology, diagnosis, and management. *Mayo Clin Proc* 2014; 89:1553–1563.

3 Tan YG, Zhang YF, Guo CJ, Yang M, Chen MY. Screening of differentially expressed microrna in ulcerative colitis related colorectal cancer. *Asian Pac J Trop Med* 2013; 6:972–976.

4 Danese S, Colombel JF, Peyrin-Biroulet L, Rutgeerts P, Reinisch W. Review article: the role of anti-TNF in the management of ulcerative colitis – past, present and future. *Aliment Pharmacol Ther* 2013; 37:855–866.

5 Thomas AG, Akobeng AK. Tumour necrosis factor alpha blocking agents for induction of remission in ulcerative colitis. *Cochrane Database Syst Rev* 2006; 4:CD005112.

6 Stidham RW, Lee TC, Higgins PD, Deshpande AR, Sussman DA, Singal AG, *et al*. Systematic review with network meta-analysis: the

efficacy of anti-tumour necrosis factor-alpha agents for the treatment of ulcerative colitis. *Aliment Pharmacol Ther* 2014; 39:660–671.

7   Cheon JH. Understanding the complications of anti-TNF therapy in East Asian patients with inflammatory bowel disease. *J Gastroenterol Hepatol* 2016;32:769–777.

8   Schaefer JS, Attumi T, Opekun AR, Abraham B, Hou J, Shelby H, *et al*. Microrna signatures differentiate Crohn's disease from ulcerative colitis. *BMC Immunol* 2015; 16:5.

9   Feng WZ, Trindade A. MicroRNAs are differentially expressed in ulcerative colitis and alter expression of macrophage inflammatory peptide-2alpha. *Gastroenterology* 2008; 135:1624–1635.e24.

10 Ahmed FE, Jeffries CD, Vos PW, Flake G, Nuovo GJ, Sinar DR, *et al*. Diagnostic microrna markers for screening sporadic human colon cancer and active ulcerative colitis in stool and tissue. *Cancer Genomics Proteomics* 2009; 6:281–295.

11 Neudecker V, Yuan X, Bowser JL, Eltzschig HK. MicroRNAs in mucosal inflammation. *J Mol Med* 2017; 95:1–15.

12 Wu W, He Y, Feng X, Ye S, Wang H, Tan W, *et al*. MicroRNA-206 is involved in the pathogenesis of ulcerative colitis via regulation of adenosine A3 receptor. *Oncotarget* 2016; 8:705–721.

13 Georgios K, Christos P, Kaplan JL, Alessio MF, Beatriz GM, Efi K, *et al*. MicroRNA-124 regulates STAT3 expression and is down-regulated in colon tissues of pediatric patients with ulcerative colitis. *Gastroenterology* 2013; 145:842.

14 Polytarchou C, Hommes DW, Palumbo T, Hatziapostolou M, Koutsioumpa M, Koukos G, *et al*. Microrna214 is associated with progression of ulcerative colitis, and inhibition reduces development of colitis and colitis-associated cancer in mice. *Gastroenterology* 2015; 149:981–92.e11.

15 Fu X, Shen Y, Wang W, Li X. MiR-30a-5p ameliorates spinal cord injury-induced inflammatory responses and oxidative stress by targeting Neurod 1 through MAPK/ERK signaling. *Clin Exp Pharmacol Physiol* 2017; 45:68–74.

16 Wei C, Wang JY, Zhang R, Li YX, Wang LS. Effect of resveratrol on Treg/Th17 signaling and ulcerativecolitis treatment in mice. *World J Gastroenterol* 2015; 21:6572–6581.

17 Raza A, Yousaf W, Giannella R, Shata MT. Th17 cells: interactions with predisposing factors in the immunopathogenesis of inflammatory bowel disease. *Expert Rev Clin Immunol* 2012; 8:161–168.

18 Zhu B, Zhang HX, Zeng JC, Bo-Ran AN, Jun-Fa XU, Shu JC. Effect of mesalazine on Th1,Th17 and Treg cells in mice with DSS-induced ulcerative colitis. *Chinese Journal of Pathophysiology* 2014; 30:2219–2225.

19 Qi L, Si-Miao Q, Ying X, Can S, Yu-Feng X, Gui-Xin C, *et al*. Norisoboldine ameliorates DSS-induced ulcerative colitis in mice through induction of regulatory T cells in colons. *Int Immunopharmacol* 2015; 29:787–797.

20 Liya Z, Yanjie Z, Wenwei Z, Caixia D, Xiaoliang L, Zhenwei X. Heme oxygenase-1 ameliorates dextran sulfate sodium-induced acute murine colitis by regulating Th17/Treg cell balance. *J Biol Chem* 2014; 289:26847–26858.

21 Wu YH, Liu W, Xue B, Zhang L, Liu XY, Liu B, *et al*. Upregulated expression of microrna-16 correlates with th17/treg cell imbalance in patients with rheumatoid arthritis. *DNA Cell Biol* 2016; 35:853–860.

22 Dong L, Wang X, Tan J, Li H, Qian W, Chen J, *et al*. Decreased expression of microrna-21 correlates with the imbalance of th17 and Treg cells in patients with rheumatoid arthritis. *J Cell Mol Med* 2014; 18:2213–2224.

23 Yao R, Ma YL, Liang W, Li HH, Ma ZJ, Yu X, Liao YH. Microrna-155 modulates treg and th17 cells differentiation and th17 cell function by targeting SOCS1. *PLoS One* 2012; 7:e46082.

24 Naghavian R, Ghaedi K, Kiani-Esfahani A, Ganjalikhani-Hakemi M, Etemadifar M, Nasr-Esfahani MH. Mir-141 and mir-200a, revelation of new possible players in modulation of th17/treg differentiation and pathogenesis of multiple sclerosis. *PLoS One* 2015; 10: e0124555.

25 Qu X, Han J, Zhang Y, Wang Y, Zhou J, Fan H, *et al*. MiR-384 regulates the Th17/Treg ratio during experimental autoimmune encephalomyelitis pathogenesis. *Front Cell Neurosci* 2017; 11:88.

26 Hou J, Hu X, Chen B, Chen X, Zhao L, Chen Z, *et al*. Mir-155 targets est-1 and induces ulcerative colitis via the IL-23/17/6-mediated th17 pathway. *Pathol Res Pract* 2017; 213:1289–1295.

27 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.

28 Castaño-Milla C, Chaparro M, Gisbert JP. Systematic review with meta-analysis: the declining risk of colorectal cancer in ulcerative colitis. *Aliment Pharmacol Ther* 2014; 39:645–659.

29 Feng X, Wang H, Ye S, Guan J, Tan W, Cheng S, *et al*. Up-regulation of microrna-126 may contribute to pathogenesis of ulcerative colitis via regulating NF-kappab inhibitor iκbα. *PLoS One* 2012; 7:e52782.

30 Wang Q, Zhu L, Jiang Y, Xu J, Wang F, He Z. Mir-219-5p suppresses the proliferation and invasion of colorectal cancer cells by targeting calcyphosin. *Oncol Lett* 2017; 13:1319–1324.

31 Jiang Y, Yin L, Jing H, Zhang H. Microrna-219-5p exerts tumor suppressor function by targeting ROBO1 in glioblastoma. *Tumour Biol* 2015; 36:8943–8951.

32 Wei W, Zhang Q, Wang Z, Yan B, Feng Y, Li P. Mir-219-5p inhibits proliferation and clonogenicity in chordoma cells and is associated with tumor recurrence. *Oncol Lett* 2016; 12:4568–4576.

33 Huang N, Lin J, Ruan J, Su N, Qing R, Liu F, *et al*. Mir-219-5p inhibits hepatocellular carcinoma cell proliferation by targeting glypican-3. *FEBS Lett* 2012; 586:884–891.

34 Koukos G, Polytarchou C, Kaplan JL, Morley-Fletcher A, Gras-Miralles B, Kokkotou E, *et al*. Microrna-124 regulates STAT3 expression and is down-regulated in colon tissues of pediatric patients with ulcerative colitis. *Gastroenterology* 2013; 145:842–52.e2.

35 Li JA, Wang YD, Wang K, Wang ZL, Jia DY, Yang BY, Xiong CB. Downregulation of mir-214-3p may contribute to pathogenesis of ulcerative colitis via targeting STAT6. *Biomed Res Int* 2017; 2017:8524972.

36 Koukos G, Polytarchou C, Gras-Miralles B, Kokkotou E, Kaplan JL, Pothoulakis C, *et al*. Tu1197 MicroRNA-4284 Regulates CXCL5 expression and is down-regulated in colon tissues of pediatric patients with ulcerative colitis. *Gastroenterology* 2014; 146:S–781.

37 Mehmet C, Jacob Tveiten B, Jakob Benedict S, Jesper Thorvald TJ, Rgen O, Ole Haagen N. miR-20b, miR-98, miR-125b-1*, and let-7e* as new potential diagnostic biomarkers in ulcerative colitis. *World J Gastroenterol* 2013; 19:4289–4299.

38 Huang Y, Ma Z, Cui YH, Dong HS, Zhao JM, Dou CZ, *et al*. Effects of herb-partitioned moxibustion on the mirna expression profiles in colon from rats with DSS-induced ulcerative colitis. *Evid Based Complement Alternat Med* 2017; 2017:1767301.

39 Whiteoak SR, Sanchez-Elsner T, Cummings JF. Mo1727 Drug Therapies in Ulcerative Colitis Influence the Expression of MicroRNAs and Cytokines in the Sigmoid Mucosa in an Ex Vivo Model. *Gastroenterology* 2015; 148:S-695.

40 Qin Z, Wan JJ, Sun Y, Wu T, Wang PY, Du P, *et al*. Nicotine protects against DSS colitis through regulating microRNA-124 and STAT3. *J Mol Med* 2016; 95:221–233.

41 Ravi V, Nirmal V, Jaishree P. Expression of inflammatory genes in the colon of ulcerative colitis patients varies with activity both at the mRNA and protein level. *Eur Cytokine Netw* 2013; 24:130–138.

42 Biesiada G, Czepiel J, Ptak-Belowska A, Targosz A, Krzysiek-Maczka G, Strzalka M, *et al*. Expression and release of leptin and proinflammatory cytokines in patients with ulcerative colitis and infectious diarrhea. *J Physiol Pharmacol* 2012; 63:471–481.

43 Amanzada A, Malik IA, Blaschke M, Khan S, Rahman H, Ramadori G, Moriconi F. Identification of CD68(+) neutrophil granulocytes in *in vitro* model of acute inflammation and inflammatory bowel disease. *Int J Clin Exp Pathol* 2013; 6:561–570.

44 Gong Y, Lin Y, Zhao N, He X, Lu A, Wei W, Jiang M. The th17/treg immune imbalance in ulcerative colitis disease in a Chinese Han population. *Mediators Inflamm* 2016; 2016:7089137.