European Review for Medical and Pharmacological Sciences 2020; 24: 2640-2646

MiR-195 alleviates ulcerative colitis in rats *via* MAPK signaling pathway

X.-S. BAI, G. BAI, L.-D. TANG, Y. LI, Y. HUAN, H. WANG

Department of Gastroenterology, Hospital of Liaoning University of Traditional [illegible]ese Medici[illegible] Shenyang, China

Abstract. – OBJECTIVE: To study the effect of micro ribonucleic acid (miR)-195 on the inflammatory response of ulcerative colitis (UC) model rats and to explore its regulatory mechanism, thus providing a new scheme for the clinical treatment of UC.

MATERIALS AND MATERIALS: A rat model of UC was prepared by 2, 4, 6-trinitrobenzene-sulfonic acid (TNBS)/ethanol assay, and the rats were randomly divided into Control group, Model group, and miR-195 mimic (miR-195 agomir) group. The disease activity index (DAI) in each group was observed. Hematoxylin and eosin (H&E) s[illegible] was utilized to detect the pathological [illegible] in the rat colon tissues in each group. The [illegible]ls of interleukin-6 (IL-6) and IL-1β in the colo[illegible] sues of the rats in each group were detected enzyme-linked immunosorbent assay (ELISA). addition, the messenger RNA [illegible] and pro tein levels of p38 mitogen-a[illegible]tein ki-nase (p38 MAPK) and tumor [illegible]rosis f[illegible]r-alpha (TNF-α) in the colon tissu[illegible] each g[illegible]f rats were examined *via* Rev[illegible] Trans merase Chain Reacti[illegible] (R[illegible]d Western blotting, respectivel[illegible]

RESULTS: Com[illegible]ed with th[illegible] Control group, the rats i[illegible]l group had [illegible]reased DAI score, seve[illegible] p[illegible]gically dama[illegible]ed colon tissues, raise[illegible]vels o[illegible]nd IL-1β in the colon tissues and [illegible]ignificantly e[illegible]d mRNA and protein level[illegible]38 MAPK and [illegible]n comparison with th[illegible] Model group, the [illegible] score was decreas[illegible]he path[illegible]gical damage to the rat colon tiss[illegible]was im[illegible]ed, the levels of IL-6 and IL-1β in t[illegible] co[illegible] tissue[illegible]re reduced, and the mRNA [illegible]ein lev[illegible] p38 MAPK and TNF-α [illegible] nota[illegible]were [illegible]miR-195 agomir group.

[illegible]CLUS[illegible]-195 mimics can alleviate the [illegible]nologica[illegible]mage to the colon and inflam[illegible]ory respon[illegible]es in UC model rats, and its me[illegible]be related to the inhibition on t[illegible]signaling pathway.

Words:
[illegible]ative colitis, MiRNA, Inflammatory response, P38 [illegible].

[illegible]roduction

Inflammatory bo[illegible]isease (IBD) includes ulce[illegible]colitis (UC) a[illegible]rohn's disease, the f[illegible] of which is a chronic non-specific disease [illegible]lving colorec[illegible] mucosa and mucosa[1-3]. As ple's diet an[illegible]estyle change, the morbidity [illegible]of UC shows [illegible]increasing trend year by year, c[illegible] great p[illegible]cal pain and mental burden to patie[illegible]ase can occur in people at any age, mostly in those aged 20-40 years old. The can-[illegible]ration rates of UC in people aged 20-30 years old [illegible]7.0% and 16.0%, respectively[4,5].

As [illegible]olecular biology continuously advances, researchers have made some progress in the etiology of UC. Its pathogenesis primarily involves genetic factors, environmental factors, and immune factors. Cytokines released by abnormal inflammatory responses play a pivotal role in the pathogenesis of UC[6-9]. The p38 mitogen-activated protein kinase (p38 MAPK) signaling pathway exerts crucial effects during the development of UC[10]. It is one of the crucial MAPK signaling pathways discovered so far and plays important regulatory roles in inflammation and cell proliferation, differentiation, and apoptosis. Recent research results manifested that p38 MAPK signaling pathway is activated in the development of UC, which elevates the expression of tumor necrosis factor-alpha (TNF-α), a downstream target. This provides an idea for researchers that blocking the transmission of this signaling pathway can suppress abnormal inflammation responses. Therefore, it is expected to become a new approach for the treatment of UC[11,13].

Recent studies have confirmed that miR-195 has a close correlation with the pathogenesis of UC. Finding miRNAs that can adjust the differential expression of the p38 MAPK signaling pathway will further provide an experimental basis

Corresponding Author: Xuesong Bai, Ph.D; e-mail: xsbai@163.com

for revealing the pathological and physiological processes of UC.

This study, therefore, plans to explore the regulatory effect of miR-195 on the inflammatory response of UC rats and its regulatory mechanism by preparing a rat model of UC using 2,4,6-trinitrobenzenesulfonic acid (TNBS)/ethanol assay.

Materials and Methods

Reagents

MiR-195 mimics were purchased from Guangzhou Ribobio Co., Ltd. (Guangzhou, China; miR0017149-4-5); TNBS from Sigma-Aldrich (St. Louis, MO, USA), interleukin-6 (IL-6), and IL-1β enzyme-linked immunosorbent assay (ELISA) kits from R&D System (Minneapolis, MN, USA); the first strand complementary deoxyribonucleic acid (cDNA) synthesis kit and p38 MAPK and TNF-α primers from Invitrogen (Carlsbad, CA, USA); rabbit anti-p38 MAPK and TNF-α primary antibodies from Beijing ZSGB-BIO Co., Ltd. (Beijing, China), and horseradish peroxidase (HRP)-labeled secondary antibody from Beijing Bioss Co., Ltd (Beijing, China).

Instruments

A microplate reader was bought from Bio- (Biotek Winooski, VT, USA), electrophoresis apparatus and semi-dry film transfer apparatus from Bio-Rad (Hercules, CA, USA), thermostatic water bath pot from Shanghai Jinghong Scientific Instrument Co., Ltd (Shanghai, China), analytical balance from Sartorius Electronics Co., Ltd (Beijing, China).

Animals

Thirty Sprague Dawley (SD) rats weighing (220 ± 10) g were purchased from Jinan Jinfeng Laboratory Animal Co., Ltd. [License No.: SCXK (Shandong, China) 2014-0006]. This study was approved by the Animal Ethics Committee of Liaoning University of Traditional Chinese Medicine Animal Center.

Preparation of rat model of UC

A rat model of UC was prepared using the TNBS/ethanol assay. The rats were fasted for 12 h before experiment, anesthetized, and fixed. Then, a hose coated with Vaseline was inserted into about 8 cm of the anus intestinal tract of the rat, 0.25 mL TNBS/ethanol solution was injected, and the anus was clamped. Thereafter, the rats were hung upside down for about 1 min and then put into a cage for routine feeding. Finally, the disease activity index (DAI) of the rats was scored and counted.

Detection of the Pathological Damage to the Colon Tissues of the Rats in Each Group Via Hematoxylin and Eosin Staining

The colon tissues of the rats in each group were embedded with paraffin, cut into sections with a thickness of about 4 μm. Then, the sections were soaked in xylene for dewaxing for 5 min, put into 100%, 95%, 85% and 75% ethanol for 1 min, and washed with deionized water. Subsequently, the hematoxylin solution and eosin solution were added successively for staining for 5 min respectively. After hydration, the sections were soaked in xylene solution for transparentization for 10 min, followed by mounting with neutral resin and staining observation.

Measurement of the Levels of IL-6 and IL-1β in the Colon Tissues of Each Group of Rats by ELISA

After the last intervention with miR-195 agomir, rats were anesthetized with 10% chloral hydrate. Then, blood was taken from the abdominal aorta, let stand at room temperature, and centrifuged at 5000 rpm for 15 min after coagulation. After that, the upper serum was taken, added into a new Eppendorf (EP) tube, and marked. According to the instruction of the ELISA kit, the absorbance of IL-6 and IL-1β in the rat colon tissues in each group was detected and statistically analyzed.

Examination of the MRNA levels of p38 MAPK and TNF-α in the Rat Colon Tissues in Each Group Via reverse transcription-polymerase chain reaction (RT-PCR)

The total RNAs in the colon tissues of the rats in each group were extracted by TRIzol (Invitrogen, Carlsbad, CA, USA) lysis assay and reversely transcribed into complementary deoxyribose nucleic acids (cDNAs) according to the instructions of the first-strand cDNA kit (TaKaRa, Otsu, Shiga, Japan). Subsequently, PCR amplification was carried out on a PCR instrument. The sequences of the primers added are shown in Table I. Reaction system: annealing at 65°C and extension at 72°C for 30 cycles. The reaction product was subjected to gel electrophoresis, and the optical density value was analyzed under a gel instrument.

Table I. P38 MAPK and TNF-α primer sequences.

Gene	Sequences
P38 MAPK	GTCCTGAGCACCTGGTTTCT GAGATGACAGTTCCCATCGGC
TNF-α	CCTCTCTCTAATCAGCCCTCTG GAGGACCTGGGAGTAGATGAG
β-actin	GGCTGTATTCCCCTCCATCG CCAGTTGGTAACAATGCCATGT

Determination of the protein levels of p38 MAPK and TNF-α in the rat colon tissues in each group via Western blotting

The rat colon tissues in each group were collected and lysed by protein lysate, and the supernatant was collected. The protein concentration in each group was determined *via* Bradford assay, and loading dye was then added to boil and denature the proteins. Thereafter, 8% gel was prepared, and the proteins were transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA) under 25 V for 2 h after electrophoresis and blocked for 1 h. After that, rabbit antibodies against p38 MAPK and TNF-α were added [illegible] cubation overnight. On the next day, incuba[illegible] conducted again with the HRP-labeled sec[illegible]ry antibody, the color was developed using dia[illegible] benzidine (DAB) assay (Solarbio, Beijing, Chi[illegible] and the optical density of the bands [illegible] tested.

Statistical Analysis

Statistical Product a[illegible] Service[illegible]lutions (SPSS) 17.0 (SPSS, Chi[illegible]L, U[illegible] was adopted for data [illegible]alys[illegible]easurement data were expresse[illegible] mean [illegible]dard deviation. The *t*-test w[illegible]ed for analy[illegible]easurement data. Diff[illegible]etween two g[illegible]ps were analyzed by [illegible]g th[illegible]ent's *t*-test. Comparison betwe[illegible]multiple g[illegible] was done using One-way [illegible]OVA test follow[illegible] Post-Hoc Test (Least [illegible]ificant Difference). p<0.05 suggested that t[illegible]ifferen[illegible]as statistically significant.

[illegible]ults

Mi[illegible]5 Mim[illegible]ould Reduce the DAI Sco[illegible]f UC Rats

[illegible]n based on body weight changes, [illegible] characteristics and bloody stool scores showed [illegible]the rats in Control group had a normal diet, no d[illegible] no bloody stool, and no abnormal condition, [illegible]le anorexia, weight loss, and fecal occult blood or hematochezia occurred in the rats in Model group. After treatment with miR-195 [illegible] weight of the rats recovered, and the [illegible]atoche[illegible] was relieved. Compared with Cont[illegible]roup, Model group had a notably increased D[illegible]re ($^{*}p$<0.05). In comparison with that in Model [illegible] the DAI score of the rats in miR-195 [illegible]omir g[illegible]s de[illegible]creased significantly ($^{\#}p$<0[illegible] (Figure 1).

MiR-195 Mimics C[illegible]d Imp[illegible]e Colon Pathological Inju[illegible]UC[illegible]ts

H&E staining [illegible]sul[illegible]e 2) re[illegible]ed that the rats in Con[illegible]group ha[illegible]t co[illegible]c mucosa epithelium, [illegible] cell structu[illegible]goblet cells, better cry[illegible]logy, and no in[illegible]mmatory cell infiltrati[illegible]where[illegible]e in Model group suffered from edema and hype[illegible]in the colonic mucosa, dis[illegible]cells, and muc[illegible]issue hyperplasia in s[illegible]cases. Compared with that in Model group, [illegible]pathological d[illegible]mage to the colon tissues in [illegible]-195 agomir [illegible]p was remarkably improved.

M[illegible]5 Min[illegible]Was Able to Reduce IL-[illegible]Levels in the Colon Tissues of UC Rats

According to the results of ELISA kit (Figure [illegible]istical analysis manifested that in com[illegible]ris[illegible]with those in Control group, the levels of IL-6 and IL-1β in the colon tissues of the rats in Model group were raised ($^{*}p$<0.05). Besides, compared with those in Model group, these levels in the colon tissues of the rats in miR-195 agomir group were reduced ($^{\#}p$<0.05).

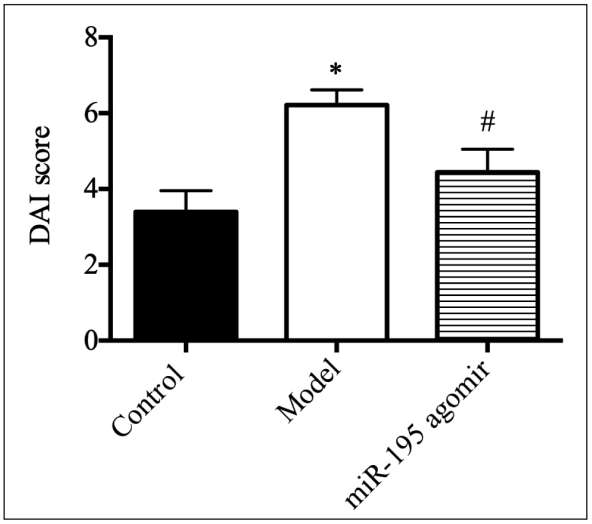


Figure 1. Comparison of the DAI score of the rats in each group ($^{*}p$<0.05: Control group vs. Model group, $^{\#}p$<0.05: Model group vs. miR-195 agomir group).

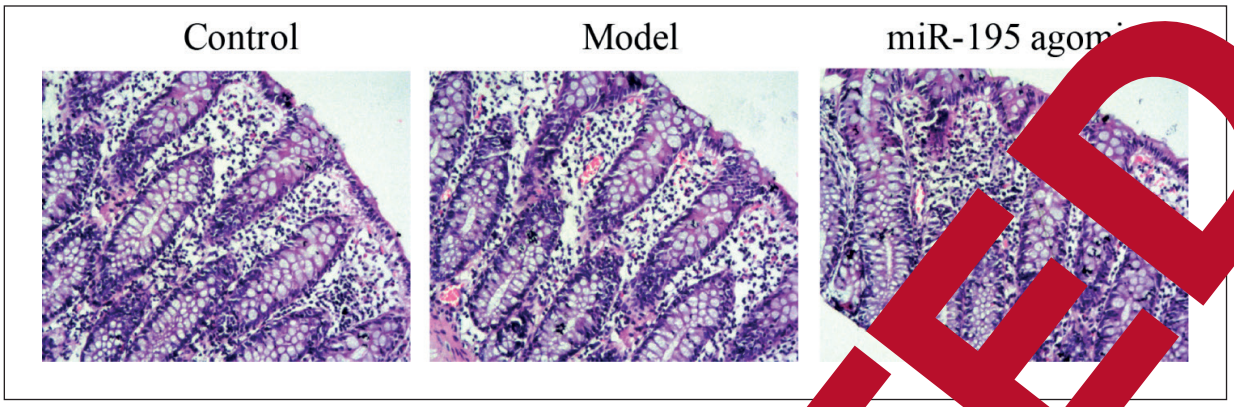


Figure 2. Pathological damage to the colon tissues of the ... each group (20...

MiR-195 Mimics Were Capable of Lowering the mRNA levels of p38 MAPK and TNF-α in the Colon Tissues of UC Rats

It was found from RT-PCR bands (Figure 5) that the rats in Model group had elevated mRNA levels of p38 MAPK and TNF-α in the colon tissues compared with those in Control group ($^{*}p<0.05$), while the rats in miR-195 agomir group exhibited decreased mRNA levels of p38 M...PK and TNF-α in the colon tissues in con... with those in Model group ($^{\#}p<0.05$) (Figu...

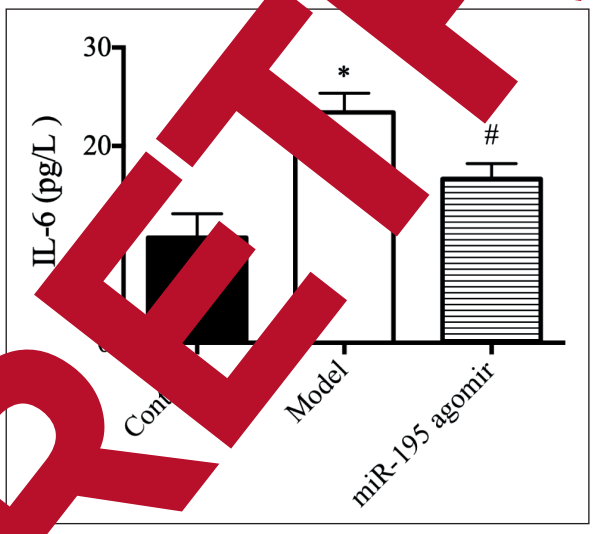
MiR-195 Mimics Could Decrease the Protein Levels of p38 MAPK and TNF-α in the Colon Tissue...ats

Western blotting bands (...re 7) ...strated that the rats in Model grou... raised ...ein lev-

els of p38 MAPK an... α in the colon tissues com...with those in ...ol group ($^{*}p<0.05$), w... the rats in miR-195 agomir group had de-...sed protein l...els of p38 MAPK and TNF-α ...he colon tiss...in comparison with those in ...el group ($^{\#}p$...5) (Figure 8).

Discussion

...ple's diet structure and lifestyle has ...ang... in recent years, the incidence rate of UC in China shows a year-by-year uptrend. UC is mainly clinically manifested as abdominal pain, diarrhea, hematochezia, tenesmus, and joint pain, and primarily occurs in the rectum and the colon mucosa and submucosa[14]. In severe



...3. Comparison of the IL-6 level ($^{*}p<0.05$: Control g... Model group, $^{\#}p<0.05$: Model group vs. miR-195 agom... oup).

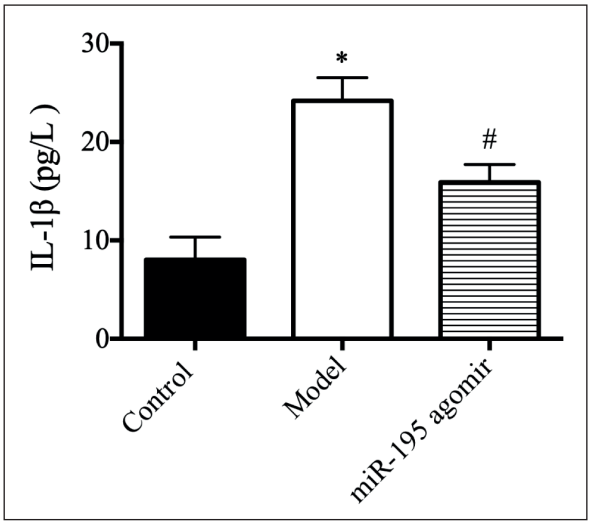


Figure 4. Comparison of the IL-1β level ($^{*}p<0.05$: Control group vs. Model group, $^{\#}p<0.05$: Model group vs. miR-195 agomir group).

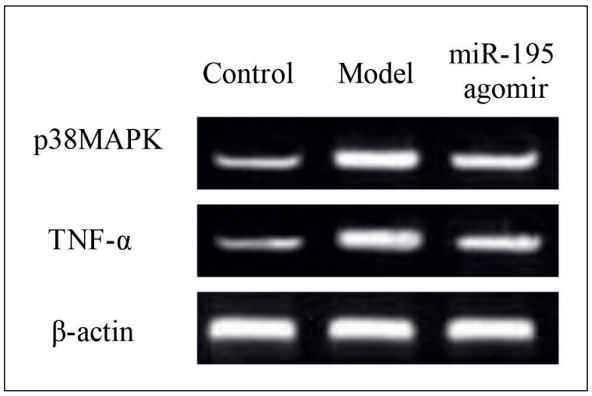


Figure 5. RT-PCR bands.

cases, it will cause systemic complications and even deteriorate to colon cancer[15]. Clinically, UC is majorly treated by glucocorticoids, aminosalicylic acids, and antibiotics. However, although these drugs can temporarily relieve the disease, long-term administration will cause adverse reactions. Therefore, finding new safe and effective therapeutic drugs is still a hot topic in research on UC[16].

A great number of literature has verifi[illegible] abnormal inflammatory responses exert[illegible] effects in the pathogenesis and developm[illegible] of UC. According to Salem and Wadie[17], th[illegible] pression level of pro-inflammatory factors in peripheral blood of UC patients is significantly increased, and the secretion [illegible] flammatory factors has a positive [illegible] ciati[illegible] with the inflammation degree. El[illegible] ed leve[illegible] of IL-6

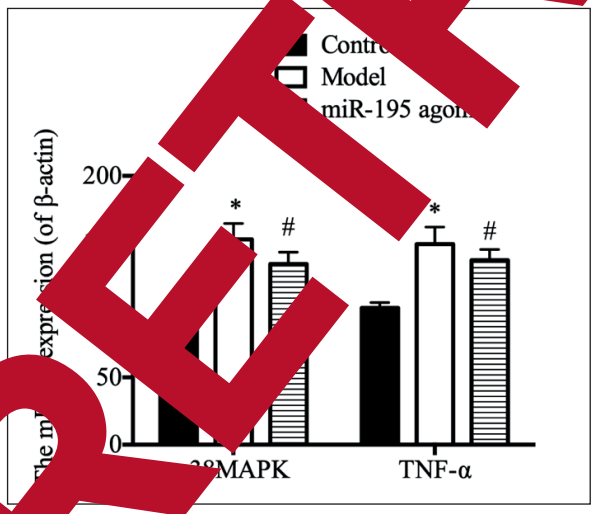


Figure 6. Comparisons of the mRNA levels of p38MAPK [illegible]NF-α in the colon tissues of the rats in each group (*p [illegible] Control group vs. Model group, #p<0.05: Model group [illegible] miR-195 agomir group).

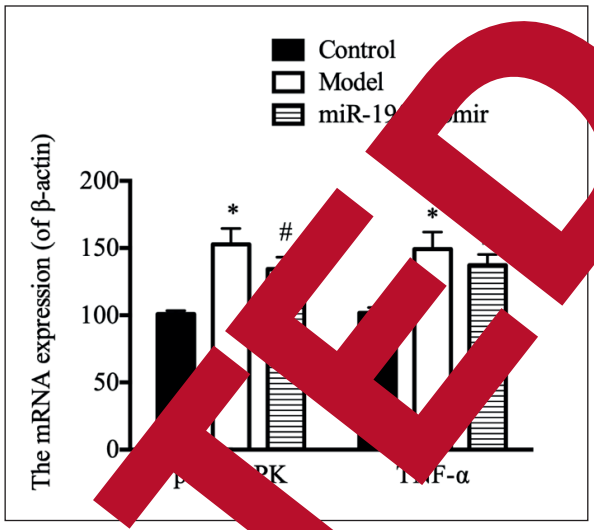


Figure 7. West[illegible]ting bands.

IL-1β can i[illegible]ence epithelial cell function e colon, cau[illegible]epithelial cell edema and increase permeability, thereby further resulting in i[illegible]ggregation, triggering UC, and aggravating its development. MAPK signaling pathway exerts a pivotal effect in the course of [illegible]cludes extracellular regulated kinase pathway, c-Jun amino-terminal kinase pathway, and p38 MAPK pathway, among which the p38 MAPK signaling pathway is able to stimulate the release of many factors, such as inflammatory factors, growth factors, and cell stress factors. Assi et al[18] used sodium dextran sulfate and

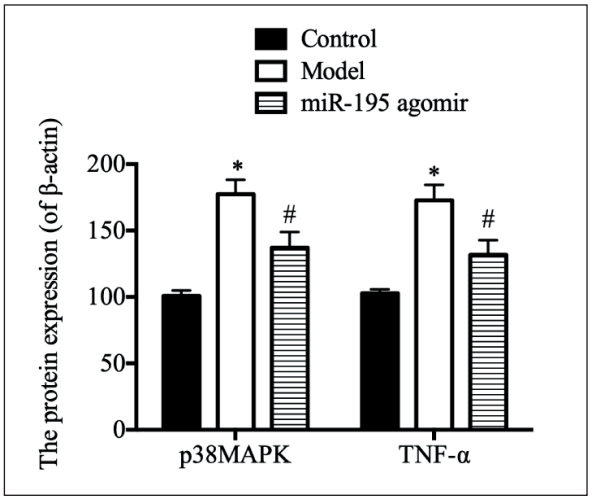


Figure 8. Comparisons of the protein levels of p38 MAPK and TNF-α in the colon tissues of the rats in each group (*p<0.05: Control group vs. Model group, #p<0.05: Model group vs. miR-195 agomir group).

TNBS to prepare the mice model of UC, respectively. After the application of p38 MAPK inhibitor (SB203580), macrophage infiltration in the mouse colon and intestinal mucosa was significantly alleviated, and the pathological score was elevated, suggesting that the p38 MAPK signaling pathway plays a vital role in UC. As the molecular biological technology continuously advances, the discovery of miRNAs has brought new hope for the treatment of UC. Valmiki et al[19] conducted biopsies in inflammatory and non-inflammatory areas in the colon of UC patients, and statistically analyzed the differentially expressed miRNAs using a microarray platform. The results manifested that specific changes appear in the expressions of miR-125, miR-155, miR-223, and miR-138 in the inflammation of patients with UC, thus providing a novel basis for research on miRNAs in UC.

In this investigation, the rat model of UC was first prepared by TNBS/ethanol assay. Literature has pointed out that ethanol can break the intestinal mucosal barrier. TNBS, as a hapten substance, can cause intestinal sensitization to autologous or allogenic proteins, attack the host immun tem, and lead to inflammatory cell inf and ulcer. The disease course of UC in rats m-ilar to that in patients, so rats are ideal mod The pathological damage to the colon tissue the rats in each group was exam*ined via* H& staining. It was discovered th agomi could relieve the pathologic amag the rat colon, hematochezia, and itration inflam-matory factors. Next, th se of ry factors in each gr w ined using the ELISA kit. The ts reve at miR-195 agomir was capa f repressing lease of inflammatory f -6 and IL-1β, dicating that miR-195 mir ts inflammatory responses. To fur her explo egulatory mechanism of m 95, the mRNA otein levels of the key ets, p38 MAPK and NF-α, in the p38 MAP gnalin thway were detected, respectiv was f that m -195 agomir could evidenti p38 M K signaling pathway.

nclusions

showed that miR-195 agomir able to reduce the pathological damage to the of UC model rats, inhibit inflammatory re-sp and reduce the release of inflammatory facto Also, its mechanism may be correlated with the inhibition on the p38 MAPK signaling pathway. Thus we provide new experi for the clinical treatment of UC wit NAs.

Conflict of Interests

The Authors declared that they h o confli rests

R ence

1) Ungaro R, M ru B, Pey oulet L, Colombel J erative c an 017; 389: 1756-177
2) Sun P Correlations 25-hydroxyvitami lev atients with ulcerative colitis with flammatio immunity and disease activity. Eur Rev M rmacol Sci 2018; 22: 639.
3) un GS, Harris A, Lobo AJ. Ulcerative colitis: management in a lts, children and young people - concise gu ce. Clin Med (Lond) 2017; 17: 429-433.
4) mut J, Sc Xuereb RB. The lived experience lcerative colitis. J Clin Nurs 2015; 24: 57.
5) Vejzovic V, Bramhagen AC, Idvall E, Wennick A. Swedish children's lived experience of ulcerative Gastroenterol Nurs 2018; 41: 333-340.
6) Espaillat MP, Kew RR, Obeid LM. Sphingolipids in neutrophil function and inflammatory responses: mechanisms and implications for intestinal immunity and inflammation in ulcerative colitis. Adv Biol Regul 2017; 63: 140-155.
7) Boal Carvalho P, Cotter J. Mucosal healing in ulcerative colitis: a comprehensive review. Drugs 2017; 77: 159-173.
8) Scarpa M, Castagliuolo I, Castoro C, Pozza A, Scarpa M, Kotsafti A, Angriman I. Inflammatory colonic carcinogenesis: a review on pathogenesis and immunosurveillance mechanisms in ulcerative colitis. World J Gastroenterol 2014; 20: 6774-6785.
9) Fiorino G, Bonovas S, Cicerone C, Allocca M, Furfaro F, Correale C, Danese S. The safety of biological pharmacotherapy for the treatment of ulcerative colitis. Expert Opin Drug Saf 2017; 16: 437-443.
10) Li CP, Li JH, He SY, Chen O, Shi L. Effect of curcumin on p38MAPK expression in DSS-induced murine ulcerative colitis. Genet Mol Res 2015; 14: 3450-3458.
11) Ishiguro Y, Ohkawara T, Sakuraba H, Yamagata K, Hiraga H, Yamaguchi S, Fukuda S, Munakata A, Nakane A, Nishihira J. Macrophage migration inhibitory factor has a proinflammatory activity via the p38 pathway in glucocorticoid-resistant ulcerative colitis. Clin Immunol 2006; 120: 335-341.
12) Soubh AA, Abdallah DM, El-Abhar HS. Geraniol ameliorates TNBS-induced colitis: involvement

of Wnt/beta-catenin, p38MAPK, NFkappaB, and PPARgamma signaling pathways. Life Sci 2015; 136: 142-150.

13) Viennois E, Zhao Y, Han MK, Xiao B, Zhang M, Prasad M, Wang L, Merlin D. Serum miRNA signature diagnoses and discriminates murine colitis subtypes and predicts ulcerative colitis in humans. Sci Rep 2017; 7: 2520.

14) Adams SM, Bornemann PH. Ulcerative colitis. Am Fam Physician 2013; 87: 699-705.

15) Dudley-Brown S, Baker K. Ulcerative colitis from patients' viewpoint: a review of two Internet surveys. Gastroenterol Nurs 2012; 35: 54-63.

16) Sandborn WJ, Su C, Sands BE, D'Haens GR, Vermeire S, Schreiber S, Danese S, Feagan BG, Reinisch W, Niezychowski W, Friedman G, Lawendy N, Yu D, Woodworth D, Mukherjee A, Zhang H, Healey P, Panés J; OCTAVE Induction 1, OCTAVE Induction 2, and OCTAVE Sustain Investigators. Tofacitinib as induction and maintenance therapy for ulcerative colitis. N Engl J Med 2017; 376:

17) Salem HA, Wadie W. Effect of nia mation and angiogenesis in a e model ulcerative colitis. Sci Rep 201 139.

18) Assi K, Pillai R, Gomez-Munoz en D, Salh B. The specific JNK inhibitor SP targets tumour necrosis factor- produc ep ithelial cell apoptosis acute murin Immunology 2006; 1 12-121.

19) Valmiki S, Ahuja V, . Micro exhibit altered expression in t me onic m sa of ulcerative coli pa d J Ga enterol 2017; 23: 5 332.

20) Gu P, Zh iu Y, Zhang en H. Protective of paeoniflorin NBS-induced ulcera through inhib g NF-kappaB path and a is in mice. Int Immunopharmacol 2017; 50: