ACTA ABP BIOCHIMICA POLONICA

Vol. 66, No 2/2019
157–163
https://doi.org/10.18388/abp.2020_5152

Regular paper

MiR-98-5p expression inhibits polarization of macrophages to an M2 phenotype by targeting Trib1 in inflammatory bowel disease*

Yunhua Peng#, Qingyuan Wang#, Wei Yang, Qiqi Yang, Ynani Pei and Wei Zhang✉

Department of Anorectal Diseases, Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai, 200021, China

Herein, we unfolded miR-98-5p mechanism in inflammatory bowel disease (IBD). IBD mouse model was established. The severity of colitis was assessed daily using the disease activity index (DAI). Murine peritoneal macrophages were stimulated by lipopolysaccharide (LPS). MiR-98-5p, tribbles homolog 1 (Trib1), M1 and M2 macrophage marker genes mRNA expression was analyzed. The relationship between miR-98-5p and Trib1 was explored using a luciferase reporter assay. The strategy of loss-of-function was used to explore the mechanism of miR-98-5p in macrophage polarization, inflammation and IBD. The results revealed that IBD mice had higher DAI index and miR-98-5p expression when compared to the Sham group. MiR-98-5p and Trib1 displayed a targeted regulation relationship. Knockdown of miR-98-5p transformed LPS-induced M1 macrophage polarization into M2 macrophage polarization and inhibited inflammation *via* up-regulating Trib1. However, shTrib1 reversed the effects. *In vivo* experiment, silencing of miR-98-5p, diminished the DAI and promoted M2 macrophage polarization. In conclusion, knockdown of miR-98-5p changed macrophage polarization to the M2 phenotype by increasing Trib1 expression, thereby alleviating IBD symptoms.

Key words: IBD, macrophage polarization, miR-98-5p, Trib1

Received: 03 January, 2020; revised: 08 January, 2020; accepted: 20 january, 2020; available on-line: 03 April, 2020

✉e-mail: WeiZhangfkl@163.com
#These authors contributed equally to the work.
***Acknowledgements of Financial Support**: This work was supported by the 2019 Shanghai Clinical Key Specialty Program (Grant No. shslczdzk04302).
Abbreviations: DAI, disease activity index; DSS, dextran sulphate sodium; IBD, inflammatory bowel disease; LPS, lipopolysaccharide; miRNAs, microRNAs; MUT, mutant; qPCR, quantitative real-time RT-PCR; Trib1, tribbles homolog 1; UC, ulcerative colitis; 3'-UTR, 3'-untranslated region; WT, wild type

INTRODUCTION

Inflammatory bowel disease (IBD) is a general term for gastrointestinal diseases caused by inflammatory disorders, which is mainly divided into two categories: Crohn's disease and ulcerative colitis (UC) (Wehkamp *et al.*, 2016). As it is a type of chronic, non-specific intestinal inflammation of unknown etiology, the diagnosis and treatment of IBD are demanding, and it is difficult to completely cure it with existing treatments. The continuous attack of IBD not only seriously affects the quality of life of the patients, but also can cause cancer, which seriously threatens their life and health (Wang *et al.,* 2018). The pathogenesis of IBD is related to genetic, environmental, microbial, and immune factors, but the exact pathogenesis is not clear (Abraham *et al.,* 2017). Recently, the researchers paid extensive attention to the role of immune abnormalities in IBD pathogenesis.

Macrophages are an important class of innate immune cells and antigen-presenting cells, which play a crucial role in anti-infection immunity (Zhang & Wang, 2014). It was reported that macrophages can secrete a large number of cytokines and bioactive substances to participate in the inflammatory response (Grainger *et al.*, 2017). According to the functional characteristics, macrophages are mainly divided into pro-inflammatory macrophages (M1 polarized phenotype) and anti-inflammatory macrophages (M2 polarized phenotype). Previous studies found that macrophages are closely related to the development of IBD (Rubio & Schmidt, 2018; Leonardi *et al.*, 2018; Fries *et al.*, 2013), and it was confirmed that IBD can be alleviated by inhibiting M1 macrophage polarization and promoting M2 macrophage polarization (Daskalaki *et al.*, 2019). Therefore, investigating the molecules that can affect macrophage polarization may provide new targets for IBD therapy.

MicroRNAs (miRNAs) are a class of non-coding single-stranded small RNA molecules with a length of approximately 22 nucleotides (Hu *et al.*, 2019). Previous papers demonstrated the critical role of miRNAs in IBD. For example, miR-665 could promote the progression of IBD by inhibiting XBP1 and ORMDL3 (Li *et al.*, 2017). Besides that, miRNAs are also closely related to the inflammation. Overexpression of miR-146a can diminish M1 macrophage polarization and promote M2 macrophage polarization, and then alleviate the inflammatory response (Huang *et al.*, 2016). miR-98-5p is a type of miRNA which plays a critical role in the inflammatory response (Vergadi *et al.*, 2018). It was revealed that miR-98-5p could negatively regulate the production of IL-10, thereby exerting a pro-inflammatory effect (Liu *et al.*, 2011). In UC, miR-98-5p expression was increased and served as a potential diagnostic target (Coskun *et al.*, 2013). However, the underlying mechanisms of miR-98-5p action in IBD are unclear.

Generally, miRNAs exert their role through the modulation of the target genes expression *via* binding to their 3'-untranslated region (3'-UTR). Tribbles homolog 1 (Trib1) belongs to Tribbles family, and it is critical for macrophage polarization (Satoh *et al.*, 2013). Silencing of Trib1 can inhibit M2 polarization and induce inflammation (Arndt *et al.*, 2018). Bioinformatics online software predicted a miR-98-5p binding site on Trib1. Thus, we speculated that miR-98-5p might regulate macrophage

polarization and inflammation through Trib1, and then modulate IBD progression.

In this study, we unfolded the mechanism of miR-98-5p action in macrophage polarization and IBD, aiming to discover a novel target for the treatment of IBD.

METHODS

IBD mouse model. All experimental procedures were approved by the Laboratory Animal Welfare and Ethics Committee of Shanghai University of traditional Chinese medicine. C57BL/6 mice (8 weeks old) were purchased from Guangdong Medical Laboratory Animal Center and divided into 4 groups (n=6/group): Sham, IBD, IBD+NC antagomir, and IBD+miR-98-5p antagomir. To establish IBD mouse model mice were provided with 4% dextran sulphate sodium (DSS) dissolved in distilled water for one week. Mice in the Sham group were provided with regular water. MiR-98-5p antagomir or NC antagomir were injected into the caudal vein of each mouse (Hou *et al*., 2017). Weight loss, stool consistency, and bleeding of each mouse were monitored daily. The severity of colitis was assessed daily using disease activity index (DAI) scoring system as previously described (Hou *et al*., 2017). Seven days later, mice were sacrificed and the colonic tissues were collected for quantitative real-time RT-PCR (qPCR).

Cells. Brewer's thioglycollate medium (4%, 1 ml) was injected intraperitoneally into C57BL/6 mice to elicit mouse peritoneal macrophages (Zhu *et al*., 2016). In order to get adherent peritoneal exudates cells, ice-cold phosphate-buffered saline was used for peritoneal lavage after 4 days. Then, the cells were incubated on dishes for 6 hours. After removing non-adherent cells macrophages were collected.

MiR-98-5p mimic and inhibitor, shTrib1 and their corresponding controls (RiboBio, Guangzhou, China) were transfected into macrophages using Lipofectamine 2000 (Invitrogen). After 48 hours, lipopolysaccharide (LPS, 1 μg/ml) was used to treat the macrophages for 24 hours.

qPCR. TRIzol reagent was used to extract the total RNA from colonic tissues or macrophages following the manufacturer's instruction. Reverse transcription system was used for reverse transcription. qRT-PCR was carried out using SYBR Green PCR Master Mix (Takara, Otsu, Japan) with the ABI StepOnePlus Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). The relative mRNA level of Trib1, monocyte chemoattractant protein 1 (Mcp1), Nitric Oxide Synthase (Nos2), and tumor necrosis factor alpha (Tnfa), mannose receptor (Mrc1), chitinase 3-like 3 (Chi3l3, Ym1), and arginase-1 (Arg1) was normalized to GAPDH. The relative expression of miR-98-5p was normalized to U6. All primers sequences were listed in Table 1.

Luciferase reporter assay. Trib1 3'UTR sequence containing wild type (WT) or mutant (MUT) miR-98-5p putative binding region was amplified by RiboBio (Guangzhou, China), and inserted into pGL3-Trib1-3'UTR plasmid (Invitrogen). Then, Lipofectamine 2000 (Invitrogen) was used for co-transfecting the plasmids and miR-98-5p mimics. Dual luciferase reporter gene assay kit (Promega, USA) was used to measure the luciferase signal intensity 48 hours after transfection.

Western blot. RIPA lysis buffer (Beyotime, Beijing, China) was used to extract the total protein from colonic tissues or macrophages. BCA assay was performed to measure the protein concentration. Samples with an equal amount of protein were separated and transferred using 10% SDS-PAGE and PVDF membranes. Then, membranes were incubated with primary anti-Trib1 antibody (1:500, Abcam) at 4°C overnight after blocking in skimmed milk (5%) at room temperature for two hours. Subsequently, the membranes were incubated with secondary antibody (1:2000, Abcam) for one hour at room temperature. The enhanced chemiluminescence detection system (ECL, Roche Molecular Biochemicals) was used to measure the signal. β-actin was used as an internal loading control.

Enzyme-linked immunosorbent assay (ELISA). The concentration of TNF-α, interleukin-23 (IL-23), and IL10 in cell culture supernatants was measured by an enzyme-linked immunosorbent assay kit (R&D Systems, Minnesota, USA) following the manufacturer's protocol.

Statistical analyses. SPSS 19.0 (SPSS, Chicago, IL, USA) was used to perform the statistical analysis. The measurements data were presented using mean ± standard deviation. All assays were performed in triplicate at least. Student's *t*-test and ANOVA were used to compare the differences between two groups and between multiple groups, respectively. $P<0.05$ was considered statistically significant.

Table 1. The primer sequences

Name	Primer	Sequence (5′-3′)
miR-98-5p	Forward	5′-ACACTCCCUAUACAACUUAC-3′
	Reverse	5′-GGGAAAGUAGUGAGGCCTCAGA-3′
U6	Forward	5′-GCTTCGGCAGCACATATACTAAAAT-3′
	Reverse	5′-CGCTTCACGAATTTGCGTGTCAT-3′
Trib1	Forward	5′-TAGCTGAGCGCGAGCATGTGT-3′
	Reverse	5′-CGTTTTCGGCTCCGCACATAGGA-3′
Mcp1	Forward	5′-AGGTCCCTGTCATGCTTCTG-3′
	Reverse	5′-TCTGGACCCATTCCTTCTTG-3′
Nos2	Forward	5′-ACCTTGTTCAGCTACGCCTT-3′
	Reverse	5'-CATTCCCAAATGTGCTTGT-3′
Tnfa	Forward	5′-CCCACACCGTCAGCCGATTT-3′
	Reverse	5′-GTCTAAGTACTTGGGCAGATTGACC-3′
Mrc1	Forward	5′-CAGCGGTTGGCAGTGGA-3′
	Reverse	5′-CAGCTGATGGACTTCCTGGTAAC-3′
Ym1	Forward	5′-GGATGGCTACACTGGAGAAA-3′
	Reverse	5′-AGAAGGGTCACTCAGGATAA-3′
Arg1	Forward	5′-ACAAGACAGGGCTCCTTTCAG-3′
	Reverse	5′-GGCTTATGGTTACCCTCCCG-3′
GAPDH	Forward	5′-CGGAGTCAACGGATTTGGTCGTAT-3′
	Reverse	5′-AGCCTTCTCCATGGTGGTGAAGAC-3′

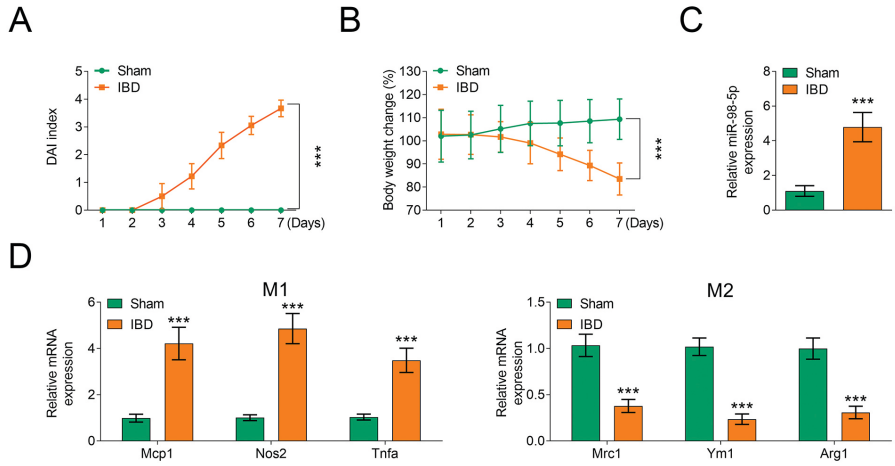

Figure 1. miR-98-5p expression in IBD mouse model.
IBD mouse model was established. (A) DAI assessment. (B) The weight change of mice. (C) miR-98-5p expression in the colonic tissues of mice was measured by qPCR. (D) The expression of M1 macrophage marker genes (Mcp1, Nos2, and Tnfa) and M2 macrophage marker genes (Mrc1, Ym1, and Arg1) was measured by qPCR. ***P< 0.01 *vs* Sham.

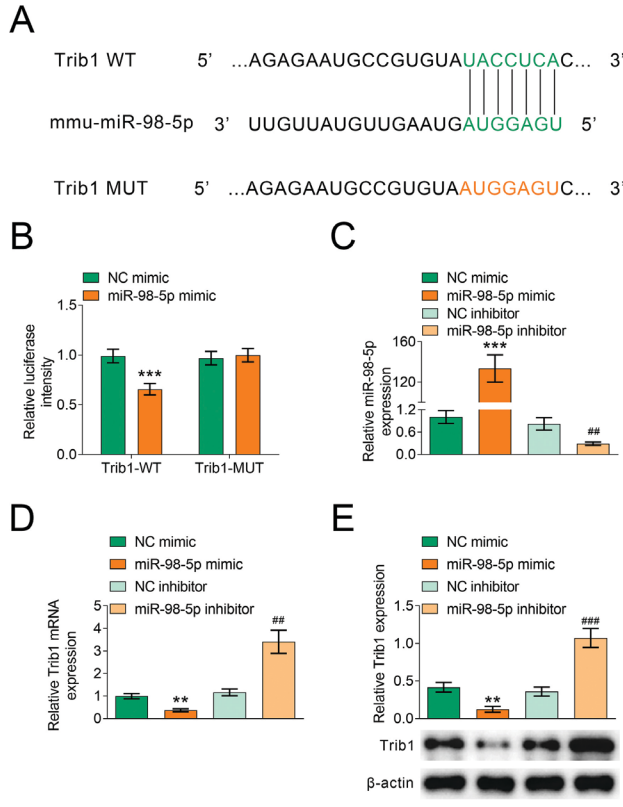

Figure 2. miR-98-5p regulated Trib1 expression.
(**A**) The miR-98-5p binding sites on Trib1. (**B**) The luciferase reporter assay was used to explore the relationship between miR-98-5p and Trib1. Macrophages were transfected with miR-98-5p mimic and miR-98-5p inhibitor. (**C**) miR-98-5p expression. (**D**) The mRNA level of Trib1. (**E**) The protein level of Trib1. ***P<0.01 *vs* NC mimic. **P<0.05 *vs* NC mimic. ##P<0.05 *vs* NC inhibitor. ###P<0.01 *vs* NC inhibitor.

RESULTS

MiR-98-5p expression was increased in IBD mouse model

Firstly, we constructed the IBD mouse model and measured the severity of intestinal inflammation by assessing the DAI score. As shown in Fig. 1A, DAI value was higher in the IBD group than in the Sham group, which indicated the IBD mouse model was successfully established. As expected, the weight of mice in the IBD group was lower than that in the Sham group (Fig. 1B). qPCR results revealed that miR-98-5p was highly expressed in the colonic tissues of IBD group compared to the Sham group (Fig. 1C). Moreover, the expression of M1 macrophage marker genes (Mcp1, Nos2, and Tnfa) was increased in IBD group, while the expression of M2 macrophage marker genes (Mrc1, Ym1, and Arg1) was

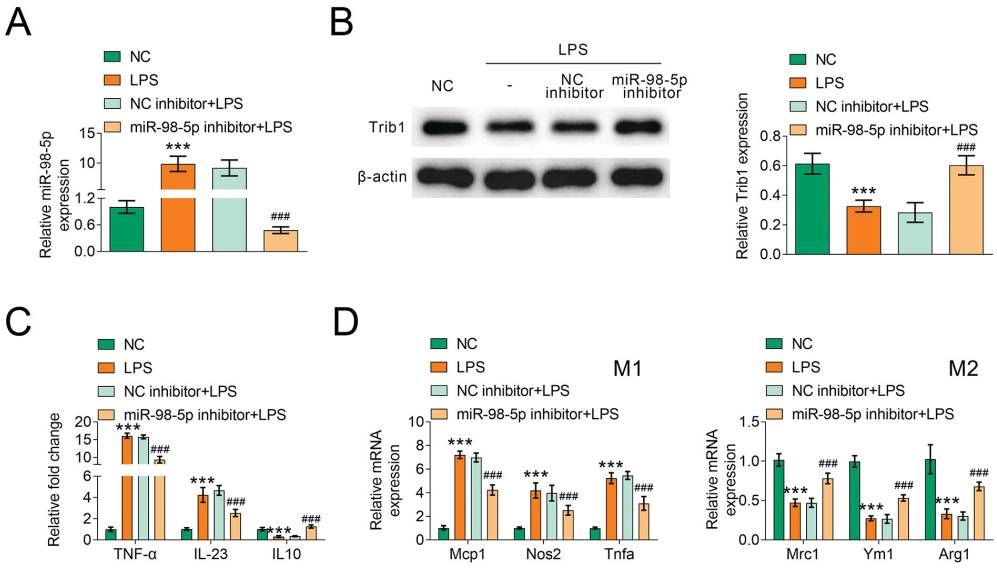

Figure 3. miR-98-5p regulated macrophage polarization and inflammation.
Macrophages were transfected with miR-98-5p inhibitor and treated with LPS. (**A**) miR-98-5p expression. (**B**) The protein level of Trib1. (**C**) The levels of TNF-α, IL-23, and IL10 were measured by ELISA. (**D**) The expression of M1 macrophage marker genes (Mcp1, Nos2, and Tnfa) and M2 macrophage marker genes (Mrc1, Ym1, and Arg1). ***P<0.01 *vs* NC. ###P<0.01 *vs* NC inhibitor+LPS.

decreased (Fig. 1D). These findings suggested that the aberrantly up-regulated miR-98-5p and M1 macrophage polarization might be associated with the development of IBD.

MiR-98-5p regulated Trib1 expression by targeting its 3'UTR

Targetscan (http://www.targetscan.org/vert_72/) predicted miR-98-5p and Trib had binding sites (Fig. 2A). To investigate the relationship between the two molecules, luciferase reporter assay was performed. The results in Fig. 2B showed that co-transfection of miR-98-5p mimic and Trib1-WT diminished the luciferase intensity. Then, we transfected miR-98-5p mimic and inhibitor into macrophage to up-regulate and down-regulate miR-98-5p expression, respectively (Fig. 2C). It was shown that overexpression of miR-98-5p reduced the mRNA and protein level of Trib1 in macrophages, but miR-98-5p knockout brought the opposite effect (Fig. 2D and E). These data indicated that miR-98-5p could negatively regulate Trib1 expression *via* targeting its 3'UTR.

Knockdown of miR-98-5p promoted M2 macrophage polarization and inhibited the inflammatory response

Herein, we explored the effects of miR-98-5p on the inflammatory response. LPS was used to induce macrophage inflammatory response. As shown in Fig. 3A, miR-98-5p expression was up-regulated in LPS-induced macrophages, but miR-98-5p knockout changed the effect. Western blot results showed that the protein level of Trib1 was reduced in the LPS group, while it was enhanced in miR-98-5p inhibitor+LPS group (Fig. 3B). In addition, miR-98-5p inhibitor attenuated the promoting effects of LPS on the expression of inflammatory cytokines (TNF-α, IL-23), and the inhibition effect of LPS on the expression of IL10 (Fig. 3C). As expected, the expression of M1 macrophage marker genes was up-regulated in the LPS group, but down-regulated in miR-98-5p inhibitor+LPS group (Fig. 3D). However, the expression of M2 macrophage marker genes showed the opposite trend in the same group (Fig. 3D). These findings revealed that LPS-induced macrophage polarization and inflammation could be affected by miR-98-5p.

MiR-98-5p modulated macrophage polarization and inflammatory response through Trib1

To investigate the mechanism of miR-98-5p action on macrophage polarization and inflammatory molecules level, macrophages were transfected with miR-98-5p inhibitor and shTrib1 and then stimulated with LPS. The results revealed that Trib1 protein level was diminished in NC inhibitor+shTrib1 group, and enhanced in miR-98-5p inhibitor+shNC group (Fig. 4A). On the other hand, Trib1 protein expression was decreased in miR-98-5p inhibitor+shTrib1 group (Fig. 4A). ELISA assay showed that shTrib1 abolished the inhibition effect of the miR-98-5p inhibitor on the expression of TNF-α, IL-23 and the promoting effect of the miR-98-5p inhibitor on IL-10 production (Fig. 4B). qPCR revealed that knockdown of miR-98-5p suppressed the expression of M1 macrophage marker genes (Fig. 4C) and promoted the expression of M2 macrophage marker genes (Fig. 4D). However, shTrib1 diminished the effects (Fig. 4C and D). The above data suggested that the function of miR-98-5p on macrophage polarization and inflammation was related to Trib1.

Knockdown of miR-98-5p improved IBD symptoms in mice

Herein, we verified miR-98-5p effects on IBD *in vivo*. IBD mice were treated with miR-98-5p antagomir. As shown in Fig. 5A, miR-98-5p expression was increased in the colonic tissues of IBD+NC antagomir group but decreased in IBD+miR-98-5p antagomir group. Furthermore, the DAI value was increased in IBD+NC antagomir group (Fig. 5B) and the mice in this group lost weight (Fig. 5C). As expected, miR-98-5p antagomir changed this trend (Fig. 5B and C). In addition, in accordance with the cell experiment, knockout of miR-98-5p inhibited the expression of M1 macrophage marker

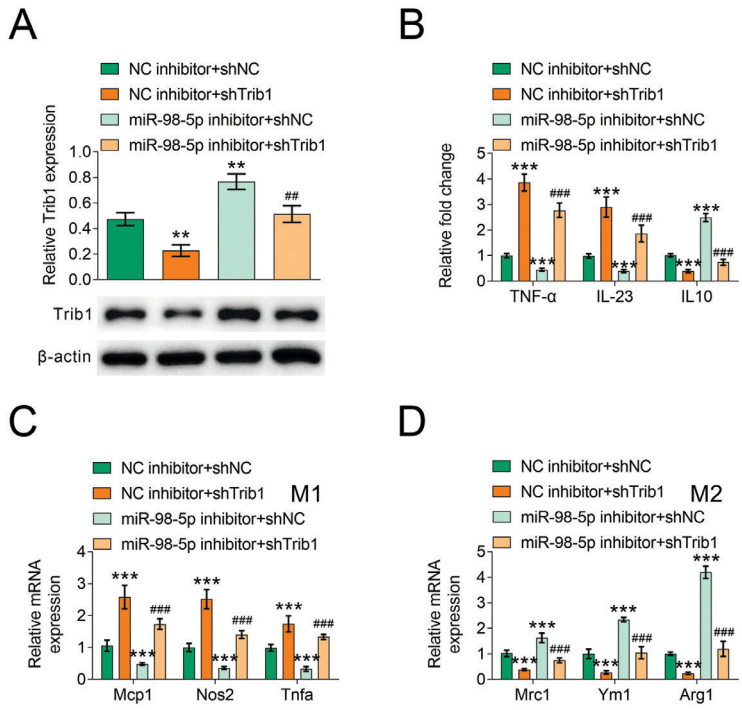

Figure 4. miR-98-5p modulated macrophage polarization and inflammation through Trib1.
Macrophages were transfected with miR-98-5p inhibitor and shTrib1 and treated with LPS. (**A**) The protein level of Trib1. (**B**) The levels of TNF-α, IL-23, and IL10 were measured by ELISA. (**C**) The expression of M1 macrophage marker genes (Mcp1, Nos2, and Tnfa). (**D**) The expression of M2 macrophage marker genes (Mrc1, Ym1, and Arg1). ***P*< 0.05 *vs* NC inhibitor+shNC or NC inhibitor+shTrib1. ##*P*<0.05 *vs* miR-98-5p inhibitor+shNC. ****P*<0.01 *vs* NC inhibitor+shNC or NC inhibitor+shTrib1. ###*P*<0.01 *vs* miR-98-5p inhibitor+shNC.

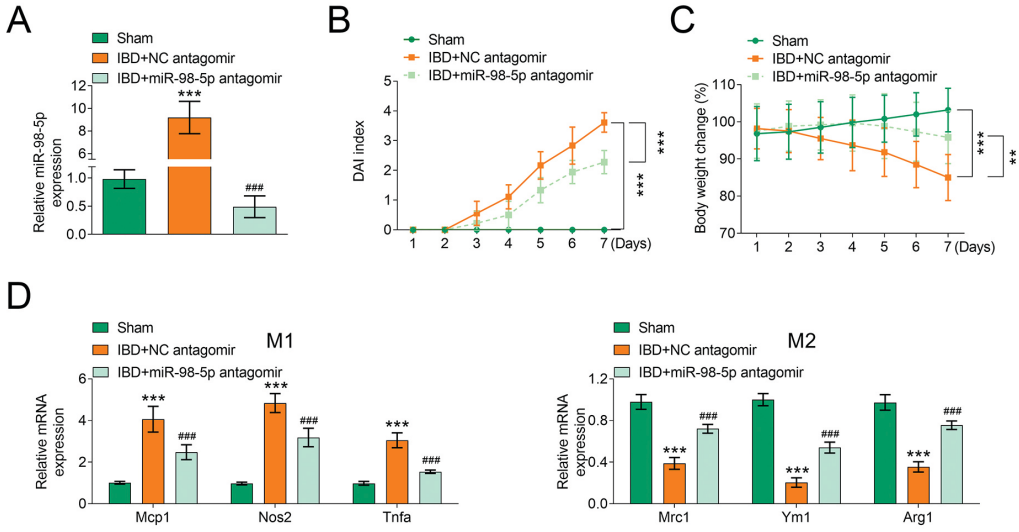

Figure 5. The function of miR-98-5p *in vivo*.
IBD mice were treated with miR-98-5p antagomir. (**A**) miR-98-5p expression in the colonic tissues of mice. (**B**) DAI assessment. (**C**) The weight change of mice. (**D**) The expression of M1 macrophage marker genes (Mcp1, Nos2, and Tnfa) and M2 macrophage marker genes (Mrc1, Ym1, and Arg1). ****P*<0.01 *vs* Sham. ###*P*<0.01 *vs* IBD+NC antagomir.

genes and enhanced the expression of M2 macrophage marker genes (Fig. 5D). Collectively, these results revealed that miR-98-5p could participate in regulating the development of IBD.

DISCUSSION

IBD is an unexplained colorectal inflammatory disease characterized by recurrent intestinal ulcers. Patients with IBD often suffer from diarrhea, mucous bloody stool, and abdominal pain (de Lange & Barrett, 2015). In Europe and the United States, IBD incidence has risen to more than 0.5% of the total population (Molodecky *et al.*, 2012). In China, the number of IBD cases tripled in the last 10 years (Ng *et al.*, 2018). It was pointed out that the risk of cancer increases with the course of IBD (Axelrad *et al.*, 2016). Therefore, it is urgent to find new strategies for the treatment of IBD.

One of the pathogenesis of IBD is the inflammation caused by the altered immune system (Wallace *et al.*, 2014, Rogler, 2017), thus, regulating this process has profound significance for IBD therapy. As immune cells, macrophages play an important role in many aspects of inflammation (Hamidzadeh *et al.*, 2017, Foss *et al.*, 2018). It is proved that the increase of M1 macrophages in the colon is related to the severity and progress of IBD (Leonardi *et al.*, 2018). In the present study, we similarly found that the expression of M1 macrophage marker genes was significantly increased in the colonic tissues of IBD mice, but the expression of M2 macrophage marker genes was reduced. These indicated that colonic M1 macrophage population expanded while the M2 macrophage population shrank during IBD. Thus, exploring the molecules involved in the process of macrophage polarization is expected to provide new ideas for IBD therapy.

It was confirmed that miRNAs are key regulators of macrophage polarization, and miRNAs-mediated macrophage polarization is associated with inflammation-related diseases (Essandoh *et al.*, 2016). For example, overexpression of miR-127 could promote M1 macrophage polarization but diminish M2 macrophage polarization through activating JNK pathway, and then aggravated lung inflammation and injury (Ying *et al.*, 2015). In IBD, 1,25(OH)2D3 could induce a transition from M1 macrophage polarization to M2 polarization by down-regulating miR-125b expression and ameliorated the symptoms of the disease (Zhu *et al.*, 2019).

As a member of let-7 family, miR-98-5p is considered a regulation factor in a variety of tumors. Jiang and others (Jiang *et al.*, 2017) suggested that miR-98-5p overexpression inhibited hepatocellular carcinoma cell growth *via* targeting IGF2BP1. Wang and others (Wang *et al.*, 2018) demonstrated that cisplatin resistance in epithelial ovarian cancer could be promoted by miR-98-5p regulating Dicer1. Moreover, miR-98-5p is also involved in other diseases, such as bronchial asthma (Du *et al.*, 2019), Alzheimer's disease (Li *et al.*, 2016), and cerebral ischemia/reperfusion injury (Sun *et al.*, 2018). In IBD, miR microarray analysis revealed that miR-98-5p was deregulated (Coskun *et al.*, 2013), but miR-98-5p function in IBD remains unclear. In the current study, miR-98-5p was found to be strongly expressed in the colonic tissues of IBD mice. Similarly, LPS treatment increased miR-98-5p expression in macrophages. Moreover, miR-98-5p knockdown could attenuate LPS promoting effect on M1 macrophage polarization and inflammation, as well as the inhibition effect of LPS on M2 macrophage polarization. These finding suggested that miR-98-5p had a critical role in the polarization of macrophages. To clarify this effect, we further studied the mechanism.

Based on the bioinformatics analysis, we found that miR-98-5p and Trib1 had a targeted regulation relationship. The previous study revealed that Trib1 participates in regulating macrophage polarization. For instance, Trib1 induced differentiation of macrophages into M2 in prostate cancer immune microenvironment (Liu *et al.*, 2019); in mice lacking Trib1, the production of proinflammatory cytokine was increased and M2-like macrophages differentiation was reduced (Akira *et al.*, 2013). In this study, we discovered that Trib1 was negatively regulated by miR-98-5p. Knockdown of miR-98-5p could promote M2 macrophage polarization and inhibited M1 macrophage polarization and inflammatory response. miR-98-5p function could be diminished by silencing Trib1. This regulatory mechanism was also confirmed in *in vivo* experiments.

In conclusion, this is the first time to reveal the mechanism of miR-98-5p action in macrophage polarization and IBD, and that the function was related to Trib1. This provides a more theoretical basis for miR-98-5p as a novel therapeutic target for IBD.

Acknowledgements

Not applicable.

Competing interests

The authors state that there are no conflicts of interest to disclose.

Ethics approval

All experimental procedures were approved by the Laboratory Animal Welfare and Ethics Committee of Shanghai University of traditional Chinese medicine.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

WZ conceived and designed the experiments, YHP and QYW analyzed and interpreted the results of the experiments, WY, QQY and YNP performed the experiments

Patient consent for publication

Not Applicable

REFERENCES

Abraham BP, Ahmed T, Ali T (2017) Inflammatory bowel disease: pathophysiology and current therapeutic approaches. *Handb Exp Pharmacol* **239**: 115–146. https://doi.org/10.1007/164_2016_122

Akira S, Misawa T, Satoh T, Saitoh T (2013) Macrophages control innate inflammation. *Diabetes Obes Metab* **15** (Suppl 3): 10–18. https://doi.org/10.1111/dom.12151

Arndt L, Dokas J, Gericke M, Kutzner CE, Muller S, Jeromin F, Thiery J, Burkhardt R (2018) Tribbles homolog 1 deficiency modulates function and polarization of murine bone marrow-derived macrophages. *J Biol Chem* **293**: 11527–11536. https://doi.org/10.1074/jbc.RA117.000703

Axelrad JE, Lichtiger S, Yajnik V (2016) Inflammatory bowel disease and cancer: The role of inflammation immunosuppression and cancer treatment. *World J Gastroenterol* **22**: 4794–4801. https://doi.org/10.3748/wjg.v22.i20.4794

Coskun M, Bjerrum JT, Seidelin JB, Troelsen JT, Olsen J, Nielsen OH (2013) miR-20b miR-98 miR-125b-1* and let-7e* as new potential diagnostic biomarkers in ulcerative colitis. *World J Gastroenterol* **19**: 4289–4299. https://doi.org/10.3748/wjg.v19.i27.4289

Daskalaki MG, Vyrla D, Harizani M, Doxaki C, Eliopoulos AG, Roussis V, Ioannou E, Tsatsanis C, Kampranis SC (2019) Neorogioltriol and related diterpenes from the red alga laurencia inhibit inflammatory bowel disease in mice by suppressing M1 and promoting M2-like macrophage responses. *Mar Drugs* **17**: 2. https://doi.org/10.3390/md17020097

de Lange KM, Barrett JC (2015) Understanding inflammatory bowel disease *via* immunogenetics. *J Autoimmun* **64**: 91–100. https://doi.org/10.1016/j.jaut.2015.07.013

Du J, Wu H, Wu Y (2019) MiR-98-5p may be a biomarker for screening bronchial asthma in children by targeting IL-13. *Clin Lab* **65**: 3. https://doi.org/10.7754/Clin.Lab.2018.180834

Essandoh K, Li Y, Huo J, Fan GC (2016) MiRNA-mediated macrophage polarization and its potential role in the regulation of inflammatory response. *Shock* **46**: 122–131. https://doi.org/10.1097/SHK.0000000000000604

Foss CA, Sanchez-Bautista J, Jain SK (2018) Imaging macrophage-associated inflammation. *Semin Nucl Med* **48**: 242–245. https://doi.org/10.1053/j.semnuclmed.2017.12.002

Fries W, Cottone M, Cascio A (2013) Systematic review: macrophage activation syndrome in inflammatory bowel disease. *Aliment Pharmacol Ther* **37**: https://doi.org/1033–1045. 10.1111/apt.12305

Grainger JR, Konkel JE, Zangerle-Murray T, Shaw TN (2017) Macrophages in gastrointestinal homeostasis and inflammation. *Pflugers Arch* **469**: 527–539. https://doi.org/10.1007/s00424-017-1958-2

Hamidzadeh K, Christensen SM, Dalby E, Chandrasekaran P, Mosser DM (2017) Macrophages and the recovery from acute and chronic inflammation. *Annu Rev Physiol* **79**: 567–592. https://doi.org/10.1146/annurev-physiol-022516-034348

Hou J, Hu X, Chen B, Chen X, Zhao L, Chen Z, Liu F, Liu Z (2017) miR-155 targets Est-1 and induces ulcerative colitis *via* the IL-23/17/6-mediated Th17 pathway. *Pathol Res Pract* **213**: 1289–1295. https://doi.org/10.1016/j.prp.2017.08.001

Hu Y, Liu Q, Zhang M, Yan Y, Yu H, Ge L (2019) MicroRNA-362-3p attenuates motor deficit following spinal cord injury *via* targeting paired box gene 2. *J Integr Neurosci* **18**: 57–64. https://doi.org/10.31083/j.jin.2019.01.12

Huang C, Liu XJ, Zhou Q, Xie J, Ma TT, Meng XM, Li J (2016) MiR-146a modulates macrophage polarization by inhibiting Notch1 pathway in RAW264.7 macrophages. *Int Immunopharmacol* **32**: 46–54. https://doi.org/10.1016/j.intimp.2016.01.009

Jiang T, Li M, Li Q, Guo Z, Sun X, Zhang X, Liu Y, Yao W, Xiao P (2017) MicroRNA-98-5p inhibits cell proliferation and induces cell apoptosis in hepatocellular carcinoma *via* targeting IGF2BP1. *Oncol Res* **25**: 1117–1127. https://doi.org/10.3727/096504016X14821952695683

Leonardi I, Li X, Iliev ID (2018) Macrophage interactions with fungi and bacteria in inflammatory bowel disease. *Curr Opin Gastroenterol* **34**: 392–397. https://doi.org/10.1097/MOG.0000000000000479

Li M, Zhang S, Qiu Y, He Y, Chen B, Mao R, Cui Y, Zeng Z, Chen M (2017) Upregulation of miR-665 promotes apoptosis and colitis in inflammatory bowel disease by repressing the endoplasmic reticulum stress components XBP1 and ORMDL3. *Cell Death Dis* **8**: e2699. https://doi.org/10.1038/cddis.2017.76

Li Q, Li X, Wang L, ZhangY, Chen L (2016) miR-98-5p Acts as a Target for Alzheimer's Disease by Regulating Abeta Production Through Modulating SNX6 Expression. *J Mol Neurosci* **60**: 413–420. https://doi.org/10.1007/s12031-016-0815-7

Liu Y, Chen Q, Song Y, Lai L, Wang J, Yu H, Cao X, Wang Q (2011) MicroRNA-98 negatively regulates IL-10 production and endotoxin tolerance in macrophages after LPS stimulation. *FEBS Lett* **585**: 1963–1968. https://doi.org/10.1016/j.febslet.2011.05.029

Liu ZZ, Han ZD, Liang YK, Chen JX, Wan S, Zhuo YJ, Cai ZD, Deng YL, Lin ZY, Mo RJ, He HC, Zhong WD (2019) TRIB1 induces macrophages to M2 phenotype by inhibiting IKB-zeta in prostate cancer. *Cell Signal* **59**: 152–162. https://doi.org/10.1016/j.cellsig.2019.03.017

Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, Benchimol EI, Panaccione R, Ghosh S, Barkema HW, Kaplan GG (2012) Increasing incidence and prevalence of the inflammatory bowel diseases with time based on systematic review. *Gastroenterology* **142**: 46–54. https://doi.org/10.1053/j.gastro.2011.10.001

Ng SC, Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, Panaccione R, Ghosh S, Wu JCY, Chan FKL, Sung JJY, Kaplan GG (2018) Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet* **390**: 2769–2778. https://doi.org/10.1016/S0140-6736(17)32448-0

Rogler G (2017) Resolution of inflammation in inflammatory bowel disease. *Lancet Gastroenterol Hepatol* **2**: 521–530. https://doi.org/10.1016/S2468-1253(17)30031-6

Rubio CA, Schmidt PT (2018) Severe defects in the macrophage barrier to gut microflora in inflammatory bowel disease and colon cancer. *Anticancer Res* **38**: 3811–3815. https://doi.org/10.21873/anticanres.12664

Satoh T, Kidoya H, Naito H, Yamamoto M, Takemura N, Nakagawa K, Yoshioka Y, Morii E, Takakura N, Takeuchi O, Akira S (2013) Critical role of Trib1 in differentiation of tissue-resident M2-like macrophages. *Nature* **495**: 524–528. https://doi.org/10.1038/nature11930

Sun X, Li X, Ma S, Guo Y, Li Y (2018) MicroRNA-98-5p ameliorates oxygen-glucose deprivation/reoxygenation (OGD/R)-induced neuronal injury by inhibiting Bach1 and promoting Nrf2/ARE signalling. *Biochem Biophys Res Commun* **507**: 114–121. https://doi.org/10.1016/j.bbrc.2018.10.182

Vergadi E, Vaporidi K, Tsatsanis C (2018) Regulation of endotoxin tolerance and compensatory anti-inflammatory response syndrome by non-coding RNAs. *Front Immunol* **9**: 2705. https://doi.org/10.3389/fimmu.2018.02705

Wallace KL, Zheng LB, Kanazawa Y, Shih DQ (2014) Immunopathology of inflammatory bowel disease. *World J Gastroenterol* **20**: 6–21. https://doi.org/10.3748/wjg.v20.i1.6

Wang S, Huang Y, Zhou C, Wu H, Zhao J, Wu L, Zhao M, Zhang F, Liu H (2018) The role of autophagy and related micrornas in inflammatory bowel disease. *Gastroenterol Res Pract* **2018**: 7565076. https://doi.org/10.1155/2018/7565076

Wang Y, Bao W, Liu Y, Wang S, Xu S, Li X, Li Y, Wu S (2018) miR-98-5p contributes to cisplatin resistance in epithelial ovarian cancer by suppressing miR-152 biogenesis *via* targeting Dicer1. *Cell Death Dis* **9**: 447. https://doi.org/10.1038/s41419-018-0390-7

Wehkamp J, Gotz M, Herrlinger K, Steurer W, Stange EF (2016) Inflammatory bowel disease. *Dtsch Arztebl Int* **113**: 72–82. https://doi.org/10.3238/arztebl.2016.0072

Ying H, Kang Y, Zhang H, Zhao D, Xia J, Lu Z, Wang H, Xu F, Shi L (2015) MiR-127 modulates macrophage polarization and promotes lung inflammation and injury by activating the JNK pathway. *J Immunol* **194**: 1239–1251. https://doi.org/10.4049/jimmunol.1402088

Zhang L, Wang CC (2014) Inflammatory response of macrophages in infection. *Hepatobiliary Pancreat Dis Int* **13**: 138–152. https://doi.org/10.1016/s1499-3872(14)60024-2

Zhu W, Jin Z, Yu J, Liang J, Yang Q, Li F, Shi X, Zhu X, Zhang X (2016) Baicalin ameliorates experimental inflammatory bowel disease through polarization of macrophages to an M2 phenotype. *Int Immunopharmacol* **35**: 119–126. https://doi.org/10.1016/j.intimp.2016.03.030

Zhu X, Zhu Y, Li C, Yu J, Ren D, Qiu S, Nie Y, Yu X, Xu X, Zhu W (2019) 1 25Dihydroxyvitamin D regulates macrophage polarization and ameliorates experimental inflammatory bowel disease by suppressing miR-125b. *Int Immunopharmacol* **67**: 106–118. https://doi.org/10.1016/j.intimp.2018.12.015