Original Article

MicroRNA-155-5p inhibition alleviates irritable bowel syndrome by increasing claudin-1 and ZO-1 expression

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Background: Irritable bowel syndrome (IBS) is a common gastrointestinal disease. Emerging studies have demonstrated that microRNAs (miRNAs) are commonly dysregulated in patients with IBS, and aberrant miRNAs are implicated in IBS occurrence. Although miR-155-5p participates in inflammatory bowel disease (IBD) and intestinal barrier dysfunction, the role of miR-155-5p in IBS is unclear.

Methods: In the present study, colon samples were obtained from IBS patients and IBS mice induced by trinitrobenzenesulfonic acid (TNBS), and the levels of miR-155-5p, claudin-1 (CLDN1), and zonula occludens-1 (ZO-1) were assessed using quantitative real-time polymerase chain reaction (qRT-PCR) and immunohistochemical analysis. The regulatory role of miR-155-5p in CLDN1 and ZO-1 expression was validated using dual luciferase reporter assay.

Results: We found that miR-155-5p levels were upregulated in colon samples of IBS patients and mice compared with healthy subjects and normal mice, respectively. Meanwhile, the levels of CLDN1 and ZO-1 were decreased in colon samples of IBS patients and mice. Importantly, forced expression of miR-155-5p inhibited CLDN1 and ZO-1 expression. In IBS mice, intraperitoneal injection with miR-155-5p inhibitor increased CLDN1 and ZO-1 expression in intestinal mucosal epithelium, enhanced visceral response thresholds, and decreased myeloperoxidase (MPO) activity.

Conclusions: In summary, these results suggested that miR-155-5p participated in the pathogenesis of IBS, at least in part by inhibiting CLDN1 and ZO-1 expression, indicating that miR-155-5p may be a potential therapeutic target for IBS.

Keywords: Irritable bowel syndrome (IBS); miR-155-5p; claudin-1; ZO-1; intestinal permeability

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Introduction

Irritable bowel syndrome (IBS), a common functional bowel disease, influences the life quality of 5–10% of the general population (1,2). The main symptoms of IBS include chronic recurrent abdominal pain, diarrhea, and a change in bowel habits (3,4). Early diagnosis of IBS is difficult due to the multiple types of symptoms which commonly occur at least 6 months before detection. It is important to note the distinction between IBS and inflammatory bowel disease (IBD), microscopic colitis, and intestinal infection (5). IBS is frequently associated with damaged function of the intestinal mucosal barrier, which, as a physical barrier,

exerts a crucial role in restraining the aggression of pathogenic bacteria and other harmful substances (6). IBS is a heterogeneous disorder, and is closely associated with environmental factors and gut microbiota (7). Intestinal dysbiosis and hyperpermeability lead to persistent low-grade mucosal inflammation by activating innate immune-related pathways, causing diarrhea and visceral hypersensitivity in IBS patients (8-10). Tight junctions are the main intestinal barrier for regulating paracellular permeability between epithelial cells (11). The destruction of tight j unctions in intestinal epithelial cells results in intestinal hyperpermeability and is thus involved in the pathogenesis of IBS (12-14). Zonula occludens-1 (ZO-1) and claudin-1 (CLDN1) are 2 main components of tight junctions (15). Resveratrol protects intestinal epithelial barrier function by increasing ZO-1 expression (16). CLDN1 regulates paracellular permeability by binding to the actin cytoskeleton (17). However, the upstream mechanisms that control ZO-1 and CLDN1 expression in IBS remain poorly understood.

MicroRNAs (miRNAs, miRs) are a class of small noncoding RNA transcripts that regulate gene expression (18,19). Mounting evidence has demonstrated that miRNAs play a crucial role in regulating a wide variety of biological processes, and aberrant miRNAs are implicated in many human diseases, including cancer (20), allergic inflammation (21), bowel dysfunction (22), and IBS (23). For instance, miR-29a level is increased and its inhibition improves intestinal barrier function through enhancing ZO-1 and CLDN1 expression (2). miR-16 is downregulated in serum of IBS patients and its overexpression sustain tight junction integrity through suppressing NF-κB signaling (24).

MiR-155-5p is an important miRNA involved in modulating intestinal inflammation and epithelial barrier. Wang *et al.* demonstrated that miR-155-5p plays a vital role in regulating allergic inflammation by increasing the expression of tight-junction proteins and thus modulating the epithelial barrier in atopic dermatitis (21). During corneal wound repair, miRNA-155-5p contributes to decreasing corneal epithelial permeability by regulating tight-junction protein expression (25). MiR-155-5p overexpression disturbs the intestinal epithelial barrier through the posttranscriptional repression of ZO-1 and Ras homolog family member A (RhoA) expression in experimental severe acute pancreatitis (26). Nevertheless, the role of miR-155-5p in IBS remains unclear. Here we explored the biological role of miR-155-5p in IBS and identified the direct and indirect target genes of miR-155-5p to evaluate the effect of miR-155-5p on tight junctions. We present the following article in accordance with the ARRIVE reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-4859/rc).

Highlight box

Key findings

- miR-155-5p participated in the pathogenesis of IBS, at least in part by inhibiting CLDN1 and ZO-1 expression.

What is known and what is new?

- miRNAs are commonly dysregulated in patients with IBS, and aberrant miRNAs are implicated in IBS occurrence.
- In IBS mice, intraperitoneal injection with miR-155-5p inhibitor increased CLDN1 and ZO-1 expression in intestinal mucosal epithelium, enhanced visceral response thresholds, and decreased MPO activity.

What is the implication, and what should change now?

- miR-155-5p participated in the pathogenesis of IBS, indicating that miR-155-5p may be a potential therapeutic target for IBS.

Methods

Participants

A total of 24 volunteers [14 IBS with diarrhea (IBS-D) patients, 10 healthy controls] undergoing a screening colonoscopy were recruited to participate in the study between January 2010 and March 2021. Samples collected from the 24 participants were stored in liquid nitrogen or at −80 ℃. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Clinical Research Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine (No. 2022-996). Individual consent for this retrospective analysis was waived.

Establishment of the murine model of IBS

Animal experiments were performed under a project license (No. 2018-547) granted by The Animal Care and Use Committee of Zhejiang University, in compliance with institutional guidelines for the care and use of animals. A protocol was prepared before the study without registration. Male BALB/c mice (approximately 6-week-old and 20 g) were obtained from the Laboratory Animal Center of the Chinese Academy of Sciences (Shanghai,

Table 1 The qRT-PCR primer sequences used in the study

Genes	Sense (5'-3')	Anti-sense (5'-3')
Hsa ZO-1	TAACAGAAGGAGTGAGAAGATTTGG	GTGTGACTTTAGTAGGTTTAGCAGGC
Mmu ZO-1	CCCACTTCCCCAAAAACTCTTAT	CCTCATCTTCATCTTCTTCCACAG
Hsa CLDN1	GCATGGTATGGCAATAGAATCG	CTCCCAGAAGGCAGAGAGAAG
Mmu CLDN1	CTGGGTTTCATCCTGGCTTCTC	CACTGTATCTGCCCGGTGCTT
Has-miR-155-5p	CGGCTTAATGCTAATCGTGATAG	GTGCAGGGTCCGAGGT
Mmu-miR-155-5p	CGGCTTAATGCTAATTGTGATAG	GTGCAGGGTCCGAGGT

qRT-PCR, quantitative real-time polymerase chain reaction.

China) and sustained under constant laboratory conditions (room temperature: 22–24 ℃; humidity: 55–70%; 12-hour dark/12-hour light cycle). All mice were anesthetized by 2% isoflurane, and a murine model of IBS was then established by administrating 1.5 mg trinitrobenzenesulfonic acid (TNBS) into the proximal colon as previously described (23,27). Mice were kept in a head-down position for 1.5 minutes following administration of TNBS. The control mice were administrated with an equal volume of 50% ethanol into the proximal colon. Visceral pain assay was carried out as previously described (28). Mice were euthanized and colon samples were collected.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated from colon tissues using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA). First-strand complementary DNA (cDNA) was synthesized from approximately 2 μg of total RNA with Moloney murine leukemia virus reverse transcriptase (Takara, Kyoto, Japan) and Oligo(dT)18 primers (Takara). qRT-PCR was performed using SuperScript™ III Platinum™ SYBR™ Green (Thermo Fisher Scientific) on a QuantStudio™ 5 Dx Real-Time PCR System. The temperature protocol was: 95 ℃ for 5 minutes, followed by 36 cycles of 95 ℃ for 15 seconds and 58 ℃ for 15 seconds, with a final 5-minute extension at 72 ℃. The primer sequences used in the study are shown in *Table 1*. qRT-PCR analysis was carried out at least 3 times. Relative RNA level was calculated using the $2^{-\Delta\Delta CT}$ method.

Western blot

Total protein was isolated from colon tissues using radioimmunoprecipitation assay (RIPA) buffer (Beyotime Biotechnology, Shanghai, China) and protein concentration was measured using a bicinchoninic acid (BCA)-protein quantification kit (Beyotime Biotechnology). Equal amounts of total proteins (approximately 50 μg) were subjected to a 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) to separate total proteins and then transferred onto polyvinylidene difluoride (PVDF) membranes (Thermo Fisher Scientific). Membranes were blocked using 3% bovine serum albumin (BSA) at room temperature (RT) for 60 minutes and then treated with the indicated primary antibody against ZO-1 (1:800, ab216880; Abcam, Cambridge, MA, USA), CLDN1 (1:2,000, ab15098, Abcam), and β-actin (1:6,500, ab6276, Abcam) for 60 minutes at RT. The membranes were washed 5 times with tris-buffered saline with Tween (TBST) and then incubated with the secondary antibody (1:10,000, G-21040, Thermo Fisher Scientific) at RT for 60 minutes. The immunoblots were visualized using chemiluminescence detection.

Immunohistochemistry (IHC)

Murine colon tissues were fixed in 4% paraformaldehyde (PFA), embedded in paraffin, and cut into sections of 5 μm. After deparaffinization and rehydration, sections were incubated with ZO-1 (1:150, ab216880) and CLDN1 (1:200, ab15098) primary antibody overnight at 4 ℃, followed by incubation with the secondary antibody (1:1000, G-21040), and the reaction products were visualized with 100 μL of 3,3'-diaminobenzidine (DAB) solution (Sigma-Aldrich, St Louis, MO, USA).

Dual-luciferase reporter assay

The recombinant plasmids of pGL3-CLDN1-3'-UTR-

wt and pGL3-ZO1-3'-UTR-wt and their mutants (pGL3-CLDN1-3'-UTR-mut and pGL3-ZO1-3'-UTR-mut) were constructed in our laboratory by inserting approximate 350 bp cDNA into pGL3 plasmid (Promega, Madison, WI, USA). Cells from human intestinal epithelial cell line NCM460 were seeded in a 48-well plate (0.1×10^5 cells per well) and then cotransfected with 20 ng of recombinant plasmids of pGL3-CLDN1-3'-UTR-wt or pGL3-ZO1-3'-UTR-wt (or their mutant), 2 ng of pRL-TK, and 40 nM of miR-155-5p (or miRNA control) with Lipofectamine™ 3000 (Thermo Fisher Scientific) according to the manufacturer's instructions. After transfection for 48 hours, NCM460 cells were collected and luciferase activities were assessed using the Dual-Luciferase Reporter Assay System (Promega).

Overexpression or inhibition of miR-155-5p

Has-miR-155-5p mimics (UUAAUGCUAAUCGUGAUAGGGGUU) were transfected into a human intestinal epithelial cell line (NCM460) to overexpress has-miR-155-5p. 2'-O-methyl modified mmu-miR-155-5p inhibitor (ACCCCUAUCACAAUUAGCAUUAA) or negative control (anti-miRcont) was administered through intraperitoneal injection (10 μg inhibitor in 100 μL PBS, n=7). After treatment with miR-155-5p inhibitor for 24 hours, the mice were euthanized.

Enzyme-linked immunosorbent assay (ELISA)

Blood samples were obtained from the murine abdominal aorta. Serum D-lactate (D-LA) and diamine oxidase (DAO) were assessed using D-LA (ab83429, Abcam) and DAO (ab241004, Abcam) ELISA kits in accordance with the manufacturer's instructions. Murine proximal colon tissues were obtained, cut into pieces, and homogenized in hexadecyltrimethylammonium bromide (HETAB) buffer. Myeloperoxidase (MPO) activity was assessed using the indicated ELISA kit (ab275109, Abcam) in accordance with manufacturer's instructions.

Statistical analysis

Data are shown as the mean ± standard error of mean and statistical analysis was carried out with SPSS 18.0 statistical software (IBM, Armonk, NY, USA). Differences between 2 groups were analyzed using Student's *t*-test or one-way ANOVA followed by the Scheffé test. The differences were deemed significant when P was less than 0.05.

Results

The expression of miR-155-5p, CLDN1, and ZO-1 in colon biopsy samples

To explore the effect of miR-155-5p on IBS progression and on regulating intestinal permeability, we first measured miR-155-5p, CLDN1, and ZO-1 expression in colon biopsy samples of IBS-D. *Figure 1A* shows that the miR-155-5p level was significantly upregulated in IBS-D patients (n=14) compared to controls (n=10). The miR-155-5p level was 3.16 times higher in the IBS-D patients than in the healthy controls. Meanwhile, the results from qRT-PCR and western blot analysis revealed that the expression of CLDN1 and ZO-1 in colon biopsy samples of IBS-D was significantly decreased in comparison with healthy controls (*Figure 1B-1D*).

The expression of miR-155-5p, CLDN1, and ZO-1 in IBS mice

To verify the dysregulated expression of miR-155-5p, CLDN1 and ZO-1 in IBS, a murine model of IBS was constructed. The mice with IBS exhibited a remarkable reduction of nociceptive threshold in the proximal colon (*Figure 2A*) and a marked enhancement of MPO activity (*Figure 2B*), suggesting that IBS mice were successfully constructed. Consistent with the above results in patients with IBS-D, the miR-155-5p level in IBS mice was significantly increased, whereas levels of CLDN1 and ZO-1 were concurrently decreased compared with control mice (*Figure 2C-2E*). IHC analysis also showed that the expression of CLDN1 and ZO-1 was downregulated in IBS mice (*Figure 2F,2G*). These results demonstrated that upregulated miR-155-5p might be involved in IBS progression by inhibiting CLDN1 and ZO-1.

MiR-155-5p repressed CLDN1 and ZO-1 expression in intestinal epithelial cells

To explore whether miR-155-5p directly inhibited CLDN1 and ZO-1 expression by targeting the 3'-untranslated region (UTR) of these genes, TargetScan 7.1 tool was used to predict the potential targets of miR-155-5p. Bioinformatics analysis results showed that *CLDN1* was a direct target gene of miR-155-5p (*Figure 3A*), whereas *ZO-1* was not the direct target of miR-155-5p. To prove this prediction, recombinant plasmids of pGL3-CLDN1-3'UTR containing a 3'-UTR fragment of the *CLDN1*

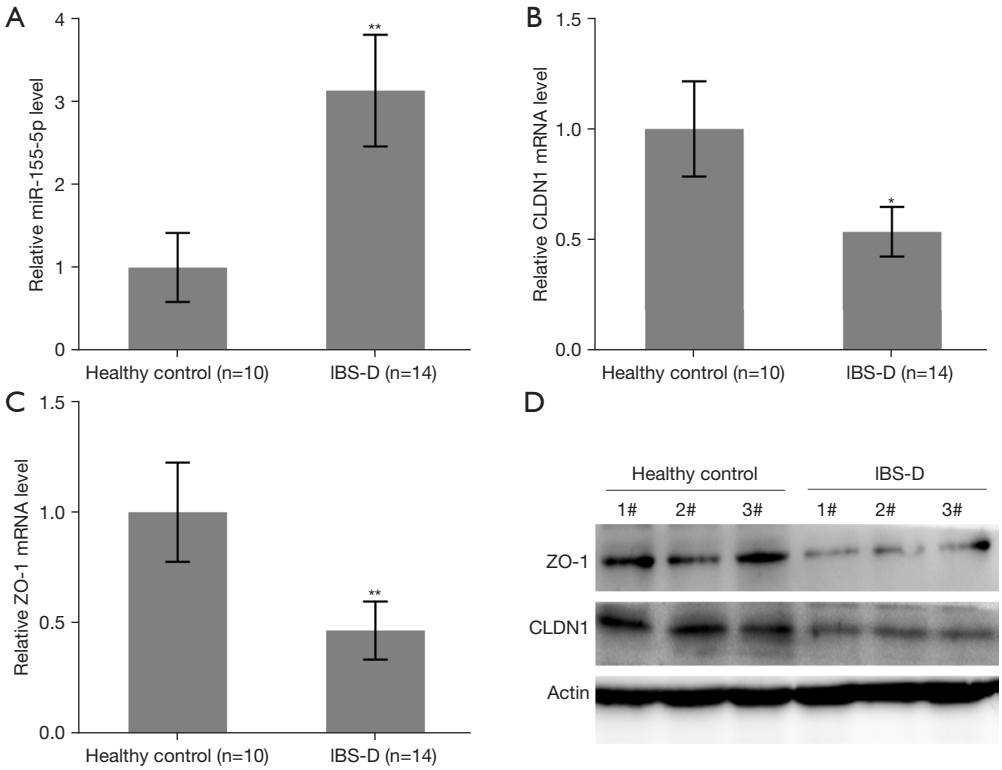

Figure 1 The expression of miR-155-5p, CLDN1, and ZO-1 in human colon biopsy samples. (A-C) qRT-PCR analysis of the miR-155-5p (A), CLDN1 (B), and ZO-1 (C) expression level in colon biopsy samples from normal controls (n=10) and patients with IBS-D (n=14). (D) Western blot analysis of CLDN1 and ZO-1 protein levels in colon biopsy samples from IBS-D patients (n=3) and controls (n=3). *P<0.05, **P<0.01. IBS-D, irritable bowel syndrome with diarrhea; CLDN1, claudin-1; ZO-1, zonula occludens-1; qRT-PCR, quantitative real-time polymerase chain reaction.

gene or its mutant (pGL3-CLDN1-3'UTR-mut) were constructed and cotransfected with miR-155-5p into human intestinal epithelial cell line NCM460. *Figure 3B,3C* show that the luciferase activity of pGL3-CLDN1-3'UTR was markedly suppressed after miR-155-5p overexpression, but the mutation of 4 nucleotides in CLDN1-3'UTR led to complete loss of the suppressive effect. MiR-155-5p did not affect the luciferase activity of pGL3-ZO1-3'UTR (*Figure 3D*). Interestingly, miR-155-5p overexpression in NCM460 cells markedly repressed the protein expression of CLDN1 and ZO-1 (*Figure 3E,3F*). These results demonstrated that miR-155-5p repressed CLDN1 expression by directly targeting 3'UTR of *CLDN1* and repressed ZO-1 expression via an indirect pathway.

MiR-155-5p inhibitor decreased intestinal permeability and inflammation and increased visceral response thresholds

Finally, we used the murine model of IBS to investigate whether miR-155-5p inhibition contributed to alleviating the symptoms of IBS. *Figure 4A,4B* show that miR-155-5p-inhibitor treatment resulted in a significant increase in CLDN1 and ZO-1 expression level in IBS mice. The levels of D-LA and DAO were significantly increased in IBS mice compared with control mice, whereas miR-155-5p inhibition effectively repressed the increase (*Figure 4C,4D*), indicating that miR-155-5p inhibition decreased intestinal permeability. MPO activity was increased in IBS mice, whereas miR-155-5p inhibition repressed the increase (*Figure 4E*), indicating that miR-155-5p inhibition decreased intestinal inflammation. MiR-155-5p inhibition also restored the values of visceral nociceptive thresholds (*Figure 4F*). These results suggested that miR-155-5p inhibition alleviated the symptoms of IBS.

Discussion

Despite significant progress in recent years, the

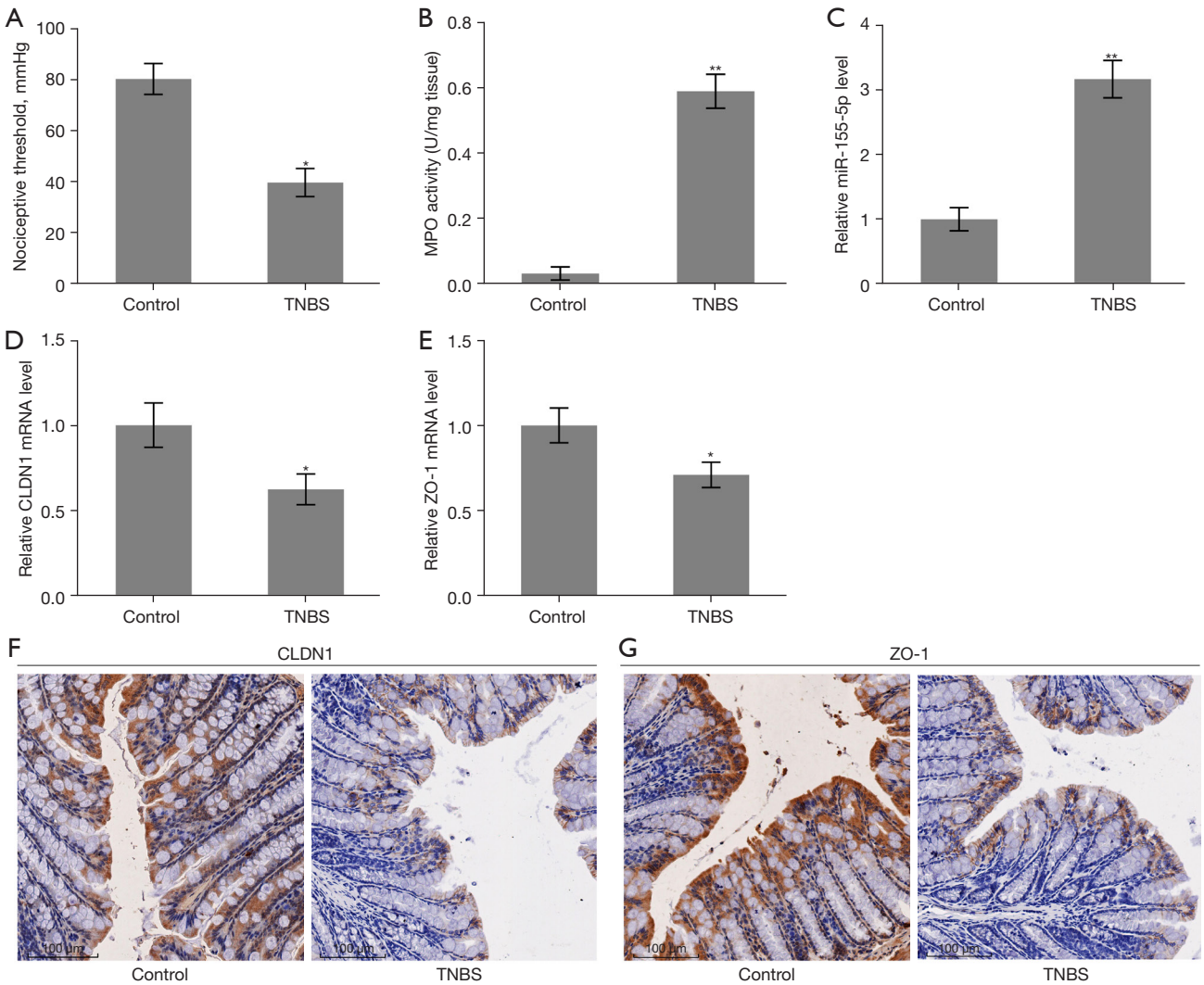

Figure 2 The expression of miR-155-5p, CLDN1, and ZO-1 in IBS mice. Murine model of IBS was established by administrating 1.5 mg of TNBS into the proximal colon. The level of nociceptive threshold (A) and MPO (Myeloperoxidase) activity (B) were then evaluated. (C-E) qRT-PCR analysis of the miR-155-5p (C), CLDN1 (D), and ZO-1 (E) expression level in murine colon tissues from controls (n=7) and IBS mice (n=7). IHC analysis of the (F) CLDN1 and (G) ZO-1 protein level in murine colon tissues from controls and IBS mice. Scale bar = 100 μm. *P<0.05, **P<0.01. MPO, myeloperoxidase; CLDN1, claudin-1; ZO-1, zonula occludens-1; IBS, irritable bowel syndrome; TNBS, trinitrobenzenesulfonic acid; qRT-PCR, quantitative real-time polymerase chain reaction.

pathophysiology of IBS remains poorly understood. Mounting studies have revealed several mechanisms involved in the pathogenesis of IBS, including intestinal barrier dysfunction (2,22,23), visceral hypersensitivity (29,30), intestinal inflammation (31,32), and imbalance of intestinal flora (33,34). In the current study, we demonstrated that: (I) miR-155-5p expression was increased and CLDN1 and ZO-1 expression was decreased in IBS patients and mice, (II) miR-155-5p suppressed CLDN1 and ZO-1 expression in intestinal epithelial cells, and (III) miR-155-5p inhibition reduced intestinal permeability and inflammation and increased visceral response thresholds. These results revealed the critical role of miR-155-5p in IBS and indicated that miR-155-5p might be a potential therapeutic target for IBS.

The intestinal mucosa contains the largest and most complex immune system (35,36). Emerging evidence has demonstrated that the level of proinflammatory cytokines

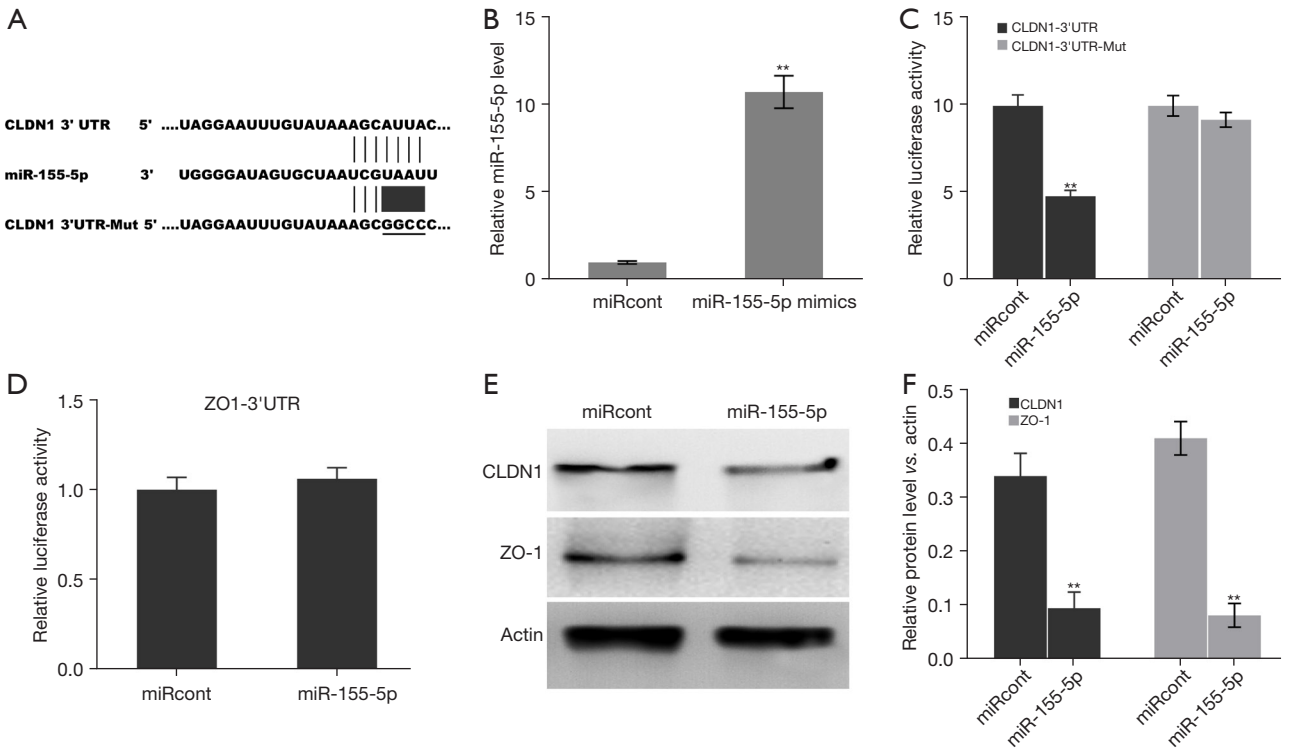

Figure 3 MiR-155-5p repressed CLDN1 and ZO-1 expression. miRcont as a miRNA control group. (A) Schematic diagram of miR-155-5p binding to CLDN1 3'-UTR. (B) qRT-PCR analysis of the miR-155-5p expression level in NCM460 cells following miR-155-5p overexpression. (C) Dual-luciferase reporter assay was performed in NCM460 cells cotransfected with miR-155-5p (40 nM) and 20 ng of pGL3-CLDN1-3'-UTR-WT (or pGL3-CLDN1-3'-UTR-Mutation) after transfection for 48 hours. (D) Dual-luciferase reporter assay was performed in NCM460 cells cotransfected with miR-155-5p (40 nM) and 20 ng of pGL3-ZO1-3'-UTR-WT after transfection for 48 hours. (E, F) Western blot analysis of CLDN1 and ZO-1 protein levels after miR-150-5p overexpression in NCM460 cells. **P<0.01. CLDN1, claudin-1; ZO-1, zonula occludens-1; qRT-PCR, quantitative real-time polymerase chain reaction.

is increased in the intestinal mucosa (37,38), while the level of anti-inflammatory cytokines is decreased (39). Nuclear factor kappa-B (NF-κB) signaling acts as a vital role in intestinal immune response (40). NF-κB activation facilitates the production of proinflammatory factors, resulting in subsequent abdominal pain and enhancing intestinal permeability (41,42). Yu *et al.* reported that berberine decreases intestinal inflammation and visceral hypersensitivity in IBS rats by repressing NF-κB-dependent intestinal mucosal inflammation (38).

Mounting evidence has demonstrated that miRNAs are commonly differentially expressed in IBS and may be potential theragnostic targets for IBS. For instance, the levels of miR-23a, miR-422b, and miR-375 are significantly increased, while the levels of miR-219a and miR-338 are decreased in colonic tissues from IBS patients compared with controls (43). Furthermore, several miRNAs (miR-663b, miR-342, etc.) could be used to precisely distinguish the different subtypes of IBS (44, 45). miRNAs exert a critical role in intestinal inflammation. Wu *et al.* revealed that miRNAs are dysregulated in chronic inflammatory bowel diseases, including ulcerative colitis, IBS, and infectious colitis (46). A previous study found that in IBS rats, miR-181c-5p expression was downregulated, while interleukin-1 alpha (IL-1α) expression was concurrently upregulated (47). Functionally, miR-181c-5p overexpression represses low-grade intestinal inflammation by inhibiting IL-1α expression in IBS rats (47). MiR-510 expression is decreased in the colon tissues of IBS patients, and downregulated miR-510 results in proinflammatory response and subsequent inflammatory injury by derepressing peroxiredoxin 1 (32). MiR-155 is frequently upregulated in inflammatory bowel disease. MiR-155 overexpression facilitates experimental colitis

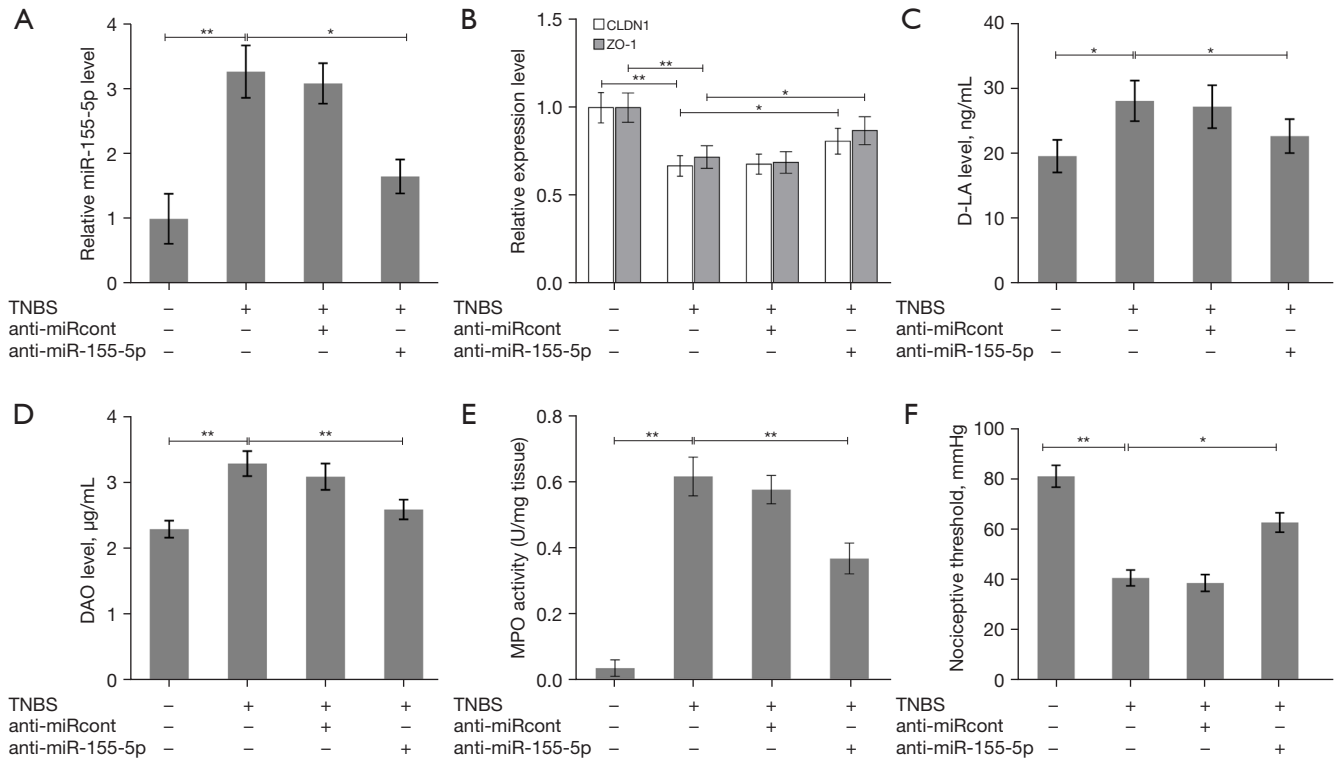

Figure 4 MiR-155-5p inhibitor decreased intestinal permeability and inflammation and increased visceral response thresholds. (A,B) qRT-PCR analysis was carried out to assess the expression levels of miR-155-5p (A), and CLDN1 and ZO-1 (B) in control mice, IBS mice, and miR-155-5p-treated IBS mice (n=7). The level of D-LA (C), DAO (D), MPO activity (E), and nociceptive threshold (F) were evaluated to assess the effect of miR-155-5p inhibition on intestinal permeability, inflammation, and visceral response threshold. *P<0.05, **P<0.01. TNBS, trinitrobenzenesulfonic acid; qRT-PCR, quantitative real-time polymerase chain reaction; CLDN1, claudin-1; ZO-1, zonula occludens-1; IBS, irritable bowel syndrome; D-LA, D-lactate; DAO, diamine oxidase; MPO, Myeloperoxidase.

progression, whereas loss of miR-155 protects against experimental colitis by regulating Src homology-2 domain-containing inositol 5'-phosphatase-1 expression (48), inactivating NF-κB signaling (49), T-helper type 1/type 17 responses (50), or forkhead box O3 (51). Loss of miR-155 also attenuates intestinal barrier dysfunction by inactivating NF-κB signaling and thus increasing occludin and ZO-1 expression (49). Given the similarly of IBS and IBD (chronic bowel inflammation, intestinal barrier dysfunction, etc.), we investigated whether miR-155 was correlated with IBS. We demonstrated that miR-155-5p expression was increased, and the expression of CLDN1 and ZO-1 was decreased in the colon samples of IBS patients and mice. Functionally, miR-155-5p overexpression repressed CLDN1 and ZO-1 expression, while miR-155-5p inhibition decreased intestinal permeability and inflammation and increased visceral response thresholds. Gut microbiota is dys-regulated in IBS patients, and gut microbiota dysbiosis results in pro-inflammatory response and injures intestinal barrier function (52,53). Based on this, probiotics have been developed into an effective treatment option in IBS (54). However, the correlation between miR-155-5p and gut microbiota dysbiosis remains unclear, and is worth exploring. Taken together, our results revealed that miR-155-5p inhibition may alleviate the symptoms of IBS by increasing claudin-1 and ZO-1 expression.

Conclusions

In summary, we comfirmed that miR-155-5p participated in the pathogenesis of IBS, at least in part by inhibiting CLDN1 and ZO-1 expression. Therefore, the miR-155-5p could be a potential therapeutic target for IBS, which warrant further investigation.

Acknowledgments

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Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-4859/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-4859/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-4859/coif). JJ is from HangZhou Dunen Medical Laboratory Co., Ltd. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Clinical Research Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine (No. 2022-996). Individual consent for this retrospective analysis was waived. Animal experiments were performed under a project license (No. 2018-547) granted by The Animal Care and Use Committee of Zhejiang University, in compliance with institutional guidelines for the care and use of animals.

References

1. Piche T, Barbara G, Aubert P, et al. Impaired intestinal barrier integrity in the colon of patients with irritable bowel syndrome: involvement of soluble mediators. Gut 2009;58:196-201.
2. Zhu H, Xiao X, Shi Y, et al. Inhibition of miRNA-29a regulates intestinal barrier function in diarrhea-predominant irritable bowel syndrome by upregulating ZO-1 and CLDN1. Exp Ther Med 2020;20:155.
3. Adriani A, Ribaldone DG, Astegiano M, et al. Irritable bowel syndrome: the clinical approach. Panminerva Med 2018;60:213-22.
4. Xing Y, Xue S, Wu J, et al. Serum Exosomes Derived from Irritable Bowel Syndrome Patient Increase Cell Permeability via Regulating miR-148b-5p/RGS2 Signaling in Human Colonic Epithelium Cells. Gastroenterol Res Pract 2021;2021:6655900.
5. Nakov R, Snegarova V, Dimitrova-Yurukova D, et al. Biomarkers in Irritable Bowel Syndrome: Biological Rationale and Diagnostic Value. Dig Dis 2022;40:23-32.
6. Quigley E. Gut permeability in irritable bowel syndrome: more leaks add to slightly inflamed bowel syndrome conspiracy theory. Gastroenterology 2009;137:728-30.
7. Yao C, Li Y, Luo L, et al. Significant Differences in Gut Microbiota Between Irritable Bowel Syndrome with Diarrhea and Healthy Controls in Southwest China. Dig Dis Sci 2022. [Epub ahead of print]. doi: 10.1007/s10620-022-07500-0.
8. Camilleri M, Lasch K, Zhou W. Irritable bowel syndrome: methods, mechanisms, and pathophysiology. The confluence of increased permeability, inflammation, and pain in irritable bowel syndrome. Am J Physiol Gastrointest Liver Physiol 2012;303:G775-85.
9. Barbara G, Zecchi L, Barbaro R, et al. Mucosal permeability and immune activation as potential therapeutic targets of probiotics in irritable bowel syndrome. J Clin Gastroenterol 2012;46 Suppl:S52-5.
10. Collins SM, Denou E, Verdu EF, et al. The putative role of the intestinal microbiota in the irritable bowel syndrome. Dig Liver Dis 2009;41:850-3.
11. Liu Z, Li N, Neu J. Tight junctions, leaky intestines, and pediatric diseases. Acta Paediatr 2005;94:386-93.
12. Utech M, Brüwer M, Nusrat A. Tight junctions and cell-cell interactions. Methods Mol Biol 2006;341:185-95.
13. Hou Q, Zhu S, Zhang C, et al. Berberine improves intestinal epithelial tight junctions by upregulating

A20 expression in IBS-D mice. Biomed Pharmacother 2019;118:109206.
14. Piche T. Tight junctions and IBS--the link between epithelial permeability, low-grade inflammation, and symptom generation? Neurogastroenterol Motil 2014;26:296-302.
15. Zhuang Y, Peng H, Mastej V, et al. MicroRNA Regulation of Endothelial Junction Proteins and Clinical Consequence. Mediators Inflamm 2016;2016:5078627.
16. Wang N, Han Q, Wang G, et al. Resveratrol Protects Oxidative Stress-Induced Intestinal Epithelial Barrier Dysfunction by Upregulating Heme Oxygenase-1 Expression. Dig Dis Sci 2016;61:2522-34.
17. Bertiaux-Vandaële N, Youmba SB, Belmonte L, et al. The expression and the cellular distribution of the tight junction proteins are altered in irritable bowel syndrome patients with differences according to the disease subtype. Am J Gastroenterol 2011;106:2165-73.
18. Ha M, Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol 2014;15:509-24.
19. Zhou Q, Costinean S, Croce CM, et al. MicroRNA 29 targets nuclear factor-κB-repressing factor and Claudin 1 to increase intestinal permeability. Gastroenterology 2015;148:158-169.e8.
20. Khan P, Ebenezer NS, Siddiqui JA, et al. MicroRNA-1: Diverse role of a small player in multiple cancers. Semin Cell Dev Biol 2022;124:114-26.
21. Wang X, Chen Y, Yuan W, et al. MicroRNA-155-5p is a key regulator of allergic inflammation, modulating the epithelial barrier by targeting PKIα. Cell Death Dis 2019;10:884.
22. Martínez C, Rodiño-Janeiro BK, Lobo B, et al. miR-16 and miR-125b are involved in barrier function dysregulation through the modulation of claudin-2 and cingulin expression in the jejunum in IBS with diarrhoea. Gut 2017;66:1537-8.
23. Liao XJ, Mao WM, Wang Q, et al. MicroRNA-24 inhibits serotonin reuptake transporter expression and aggravates irritable bowel syndrome. Biochem Biophys Res Commun 2016;469:288-93.
24. Xi M, Zhao P, Li F, et al. MicroRNA-16 inhibits the TLR4/NF-κB pathway and maintains tight junction integrity in irritable bowel syndrome with diarrhea. J Biol Chem 2022;298:102461.
25. Wang F, Wang D, Song M, et al. MiRNA-155-5p Reduces Corneal Epithelial Permeability by Remodeling Epithelial Tight Junctions during Corneal Wound Healing. Curr Eye Res 2020;45:904-13.
26. Tian R, Wang RL, Xie H, et al. Overexpressed miRNA-155 dysregulates intestinal epithelial apical junctional complex in severe acute pancreatitis. World J Gastroenterol 2013;19:8282-91.
27. Du C, Peng L, Kou G, et al. Assessment of Serum sTREM-1 as a Marker of Subclinical Inflammation in Diarrhea-Predominant Patients with Irritable Bowel Syndrome. Dig Dis Sci 2018;63:1182-91.
28. Zhou Q, Price DD, Dreher KL, et al. Localized colonic stem cell transplantation enhances tissue regeneration in murine colitis. J Cell Mol Med 2012;16:1900-15.
29. Jin X, Gharibani P, Yin J, et al. Neuro-Immune Modulation Effects of Sacral Nerve Stimulation for Visceral Hypersensitivity in Rats. Front Neurosci 2021;15:645393.
30. Camilleri M, Ford AC. Irritable Bowel Syndrome: Pathophysiology and Current Therapeutic Approaches. Handb Exp Pharmacol 2017;239:75-113.
31. Zhang L, Cheng J, Fan XM. MicroRNAs: New therapeutic targets for intestinal barrier dysfunction. World J Gastroenterol 2014;20:5818-25.
32. Zhang Y, Wu X, Wu J, et al. Decreased expression of microRNA-510 in intestinal tissue contributes to post-infectious irritable bowel syndrome via targeting PRDX1. Am J Transl Res 2019;11:7385-97.
33. Ivashkin V, Poluektov Y, Kogan E, et al. Disruption of the pro-inflammatory, anti-inflammatory cytokines and tight junction proteins expression, associated with changes of the composition of the gut microbiota in patients with irritable bowel syndrome. PLoS One 2021;16:e0252930.
34. Naseri K, Dabiri H, Rostami-Nejad M, et al. Influence of low FODMAP-gluten free diet on gut microbiota alterations and symptom severity in Iranian patients with irritable bowel syndrome. BMC Gastroenterol 2021;21:292.
35. Barbara G, Cremon C, Annese V, et al. Randomised controlled trial of mesalazine in IBS. Gut 2016;65:82-90.
36. Sinagra E, Pompei G, Tomasello G, et al. Inflammation in irritable bowel syndrome: Myth or new treatment target? World J Gastroenterol 2016;22:2242-55.
37. Gwee KA, Collins SM, Read NW, et al. Increased rectal mucosal expression of interleukin 1beta in recently acquired post-infectious irritable bowel syndrome. Gut 2003;52:523-6.
38. Yu ZC, Cen YX, Wu BH, et al. Berberine prevents stress-induced gut inflammation and visceral hypersensitivity and reduces intestinal motility in rats. World J Gastroenterol 2019;25:3956-71.

39. Bennet SM, Polster A, Törnblom H, et al. Global Cytokine Profiles and Association With Clinical Characteristics in Patients With Irritable Bowel Syndrome. Am J Gastroenterol 2016;111:1165-76.
40. Bradesi S, McRoberts JA, Anton PA, et al. Inflammatory bowel disease and irritable bowel syndrome: separate or unified? Curr Opin Gastroenterol 2003;19:336-42.
41. Hartung JE, Eskew O, Wong T, et al. Nuclear factor-kappa B regulates pain and COMT expression in a rodent model of inflammation. Brain Behav Immun 2015;50:196-202.
42. Hou Q, Huang Y, Zhu Z, et al. Tong-Xie-Yao-Fang improves intestinal permeability in diarrhoea-predominant irritable bowel syndrome rats by inhibiting the NF-κB and notch signalling pathways. BMC Complement Altern Med 2019;19:337.
43. Krishnachaitanya SS, Liu M, Fujise K, et al. MicroRNAs in Inflammatory Bowel Disease and Its Complications. Int J Mol Sci 2022;23:8751.
44. Fourie NH, Peace RM, Abey SK, et al. Perturbations of Circulating miRNAs in Irritable Bowel Syndrome Detected Using a Multiplexed High-throughput Gene Expression Platform. J Vis Exp 2016;(117):54693.
45. Bravo-Vázquez LA, Medina-Ríos I, Márquez-Gallardo LD, et al. Functional Implications and Clinical Potential of MicroRNAs in Irritable Bowel Syndrome: A Concise Review. Dig Dis Sci 2022. [Epub ahead of print]. doi: 10.1007/s10620-022-07516-6.
46. Wu F, Zikusoka M, Trindade A, et al. MicroRNAs are differentially expressed in ulcerative colitis and alter expression of macrophage inflammatory peptide-2 alpha. Gastroenterology 2008;135:1624-1635.e24.
47. Ji LJ, Li F, Zhao P, et al. Silencing interleukin 1α underlies a novel inhibitory role of miR-181c-5p in alleviating low-grade inflammation of rats with irritable bowel syndrome. J Cell Biochem 2019;120:15268-79.
48. Lu ZJ, Wu JJ, Jiang WL, et al. MicroRNA-155 promotes the pathogenesis of experimental colitis by repressing SHIP-1 expression. World J Gastroenterol 2017;23:976-85.
49. Cao YY, Wang Z, Wang ZH, et al. Inhibition of miR-155 alleviates sepsis-induced inflammation and intestinal barrier dysfunction by inactivating NF-κB signaling. Int Immunopharmacol 2021;90:107218.
50. Singh UP, Murphy AE, Enos RT, et al. miR-155 deficiency protects mice from experimental colitis by reducing T helper type 1/type 17 responses. Immunology 2014;143:478-89.
51. Min M, Peng L, Yang Y, et al. MicroRNA-155 is involved in the pathogenesis of ulcerative colitis by targeting FOXO3a. Inflamm Bowel Dis 2014;20:652-9.
52. Zhou Y, Zhang F, Mao L, et al. Bifico relieves irritable bowel syndrome by regulating gut microbiota dysbiosis and inflammatory cytokines. Eur J Nutr 2022. [Epub ahead of print]. doi: 10.1007/s00394-022-02958-0.
53. Bhattarai Y, Muniz Pedrogo DA, Kashyap PC. Irritable bowel syndrome: a gut microbiota-related disorder? Am J Physiol Gastrointest Liver Physiol 2017;312:G52-62.
54. Ford AC, Quigley EM, Lacy BE, et al. Efficacy of prebiotics, probiotics, and synbiotics in irritable bowel syndrome and chronic idiopathic constipation: systematic review and meta-analysis. Am J Gastroenterol 2014;109:1547-61; quiz 1546, 1562.

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