miR-146a-5p negatively regulates the IL-1β-stimulated inflammatory response via downregulation of the IRAK1/TRAF6 signaling pathway in human intestinal epithelial cells

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Abstract. The primary pathophysiological alteration caused by inflammatory bowel disease (IBD) is prolonged, excessive inflammatory response to stimulation factors, which leads to intestinal mucosal lesions. microRNA (miR)-146a-5p is broadly activated in the mucosal immune response. At present, the biogenesis, function and role of miR-146a-5p in intestinal epithelial cells (IECs) during the pathogenesis of IBD remain elusive. The human colon cancer epithelial Caco-2 cell line was cultured with 10 ng/ml recombinant human IL-1 β for 3 h to establish an in vitro IECs inflammatory model. Relative levels of miR-146a-5p and inflammatory factors (IL-6, IL-1β, TNF-α and IP-10) were measured by reverse transcription-quantitative PCR (RT-qPCR) and western blotting. Transfection of miR-146a-5p mimic or inhibitor into Caco-2 cells was performed to identify the influence of miR-146a-5p on Caco-2 cell inflammatory factors expression. The targeting relationship between miR-146a-5p and interleukin 1 receptor associated kinase 1 (IRAK1)/tumor necrosis factor receptor-associated factor 6 (TRAF6) was predicted by TargetScan 8.0. The present study demonstrated that miR-146a-5p and inflammatory factors (IL-6, IL-1β, TNF-α and IP-10) were upregulated in a dose- and time-dependent manner in IL-1β-stimulated Caco-2 cells. Moreover, upregulation of miR-146a-5p negatively regulated the expression of inflammatory factors, but the downregulation of miR-146a-5p increased their expression. The results of the present study demonstrated that miR-146a-5p decreased the expression of the inflammatory factors through targeted downregulation of IRAK1/TRAF6. These results suggest that miR-146a-5p negatively regulates the IL-1β-stimulated inflammatory response via downregulation of the IRAK1/TRAF6 signaling pathway in human IECs. Therefore, miR-146a-5p may act as an important diagnostic biomarker and treatment target of IBD.

Introduction

Inflammatory bowel disease (IBD) is a globally prevalent disease with increasing incidence in every continent, primarily in newly industrialized countries in Asia, South America and the Middle East (1). A number of potential risk factors, including environmental factors, imbalance of intestinal flora and immunological abnormalityintestinal infectious agents, have been linked to an increase in prevalence and incidence of IBD (2-4). IBD is associated with chronic relapsing inflammatory disorder of the gastrointestinal tract and is divided into two phenotypes: Ulcerative colitis (UC) and Crohn's disease (CD) (3). The etiology and pathogenesis of IBD are not fully understood. Intestinal mucosal barrier malfunction and immune response disorder in the intestinal mucosa are primary determinants in the activation of immunological and inflammation-associated signaling pathways in the gastrointestinal tract, which underpins the development of IBD (4-7).

In comparison with underlying cells, IECs lining the gastrointestinal tract serve as a physical barrier to pathogen incursion, therefore IECs are highly exposed to pathogens. IECs express multiple pattern-recognition receptors on their surface, which contribute to the intestinal inflammatory response by communicating with surrounding cells via synthesis and secretion of soluble, biologically active mediators (7,8). Among inflammatory mediators that serve a key role in the inflammatory response in the intestine, the proinflammatory mediator IL-1β, which is released by activated and infiltrating leucocytes, stimulates expression of a wide range of proinflammatory genes in IECs, including IL-6, IL-8 and TNF-α (9,10). These work with IL-1β to cause inflammation in patients with IBD (11) and high IL-1β levels are linked to higher disease severity (12). IL-1β causes tight junction dysfunction, which leads to impaired epithelial cell barrier function and increased immune activity, which in turn leads to the increase of inflammatory stimuli and further barrier dysfunction (7). The impaired intestinal barrier function of patients with IBD

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is closely related to the occurrence and development of intestinal inflammatory response. A large number of symbiotic bacteria in the intestinal gut enter lamina propria through the damaged intestinal mucosal barrier and activate a large number of pro-inflammatory cells, thus aggravating the intestinal mucosal inflammatory response. Inflammation leads to intestinal epithelial barrier injury, and the intestinal epithelial barrier injury further aggravates the inflammatory response, and the two are mutually causal, forming a closed loop interaction, ultimately leading to intestinal lesions. Recurrent attacks or long time treatment is not cured.

Several microRNAs (miRNAs or miRs) have been found to have a key role in modulating mucosal barrier function and mucosal inflammatory response (13). miRs are a family of endogenous non-coding RNAs that form the RNA-induced silencing complex with the Argonaute protein family and regulate various eukaryotic cellular activity by targeting the 3'-untranslated region of mRNAs for destruction or translational downregulation (14). As a result, miRs serve key regulatory roles in a variety of pathways and biological processes, including immune cellular differentiation, proliferation, apoptosis, cancer, autoimmunity and inflammation, as well as immunological homeostasis (15-17). Post-transcriptional regulation is an essential control mechanism for the expression of genes involved in inflammation, such as cytokines and chemokine-expressing genes (18,19). Several studies have found that miRs serve a key role in the pathological progression of IBD, particularly in maintaining homeostasis of the intracellular environment in the intestinal epithelium (20-22). For example, miR-19b alleviates intestinal inflammation by targeting downregulation of suppressor of cytokine signaling (SOCS)3 expression in patients with CD (20), whereas miR-29a promotes apoptosis of intestinal epithelial inflammatory cells in patients with UC by downregulating the expression of the induced myeloid leukemia cell differentiation protein (21). miR-155 targets SOCS1 gene and positively regulates the inflammatory phenotype of intestinal myofibroblasts in patients with UC (22). These miRs may serve as markers for predicting the course of IBD, as well as therapeutic targets.

miR-146a-5p has a key regulatory function in inflammatory disorders, such as acute lung injury, chronic gastritis and rheumatoid arthritis (23-25). Furthermore, studies have demonstrated that miR-146a-5p is highly expressed in the peripheral blood of patients with IBD and is involved in the progression of IBD (26,27). miR-146a-5p, one of the most important anti-inflammatory miRs (28), suppresses Toll-like receptor 4-induced NF-κB-regulated gene expression and serves as a negative feedback regulator of the innate immune response by targeting two adapter proteins, interleukin 1 receptor associated kinase 1 (IRAK1) and tumor necrosis factor receptor-associated factor 6 (TRAF6), which are key molecules downstream of TNF-α and IL-1β signaling (29). To the best of our knowledge, although miR-146a-5p is implicated in the regulation of IBD, there is little information about its expression and the mechanism by which it regulates inflammation-associated signaling in IECs and non-immune cells of the intestinal epithelial layer.

Therefore, the present study aimed to determine the regulatory role and mechanism of miR-146a-5p in IECs. IL-1β-induced IECs was used to construct an in vitro intestinal inflammation model, and the role of miR-146a-5p in IL-1β induced in vitro intestinal inflammation model and its regulatory mechanism were investigated, providing theoretical basis and experimental basis for the treatment of IBD.

Materials and methods

Cell line and culture. Human colon cancer epithelial Caco-2 cells (cat. no. CL-0050; Procell Life Science & Technology Co., Ltd.) were plated in 48-well plates at 37°C with DMEM (Gibco; cat. no. C11995500BT) supplemented with 1,000 mg/l glucose, 1,000 mg/l Penicillin-Streptomycin and 20% FBS (Gibco; cat. no. 10270-106) in a humidified incubator at 37°C with 5% CO₂.

Establishment of an in vitro inflammatory model in IECs. Recombinant human IL-1β was obtained from Enzo Life Sciences, Inc. (cat. no. ALX-520-001-C010). Cells at 85-90% confluence were incubated at 37°C with 10 ng/ml IL-1β for 3 h to establish an in vitro inflammatory model in IECs.

Transfection with miR-146a-5p mimics and inhibitors. miR-146a-5p mimics, inhibitors, mimic negative control (NC) and inhibitor NC were obtained from Sangon Biotech, Co., Ltd. The sequences of miR mimic, inhibitor and NC were as follows: miR-146a-5p mimics, 5'-UGAGAACUGAAU UCCAUUGGGUU-3'; miR-146a-5p inhibitors, 5'-AACCCCA UGGAAUUCGUUCUCA-3'; miRNA mimic NC, 5'-UGU UACUACACAAAGUAUCUG-3' and miRNA inhibitor NC, 5'-CAUACUUUGUGUAGUACAA-3'. A total of 500,000 Caco-2 cells were plated in 48-well plates with 200 µl/well DMEM supplemented with 1,000 mg/l glucose and 20% FBS. One day before transfection. Cells (50-70% confluence) were transfected with 10 pmol miR-146a-5p mimics, inhibitors, mimic NC or inhibitor NC using 0.5 µl Lipofectamine® 2000 (Invitrogen; cat. no. 11668-019; Thermo Fisher Scientific, Inc.) resuspended in 100 µl DMEM (Gibco; cat. no. C11995500BT). A volume of 200 µl transfection suspension was added to each well and incubated at 37°C for 5 h. Then we replaced the transfection suspension with 20% complete medium and continued culture for 24 h before subsequent experiments.

Quantification of mRNA and miR using reverse transcription-quantitative PCR (RT-qPCR). A total of 5x10⁵ cells/well was planted in 48 well cell culture plate, cultured at 37°C for 24 h, total RNA was extracted from Caco-2 cells (90-95%, confluence) according to the manufacturer's instructions using Tri Reagent® RNA/DNA/Protein Isolation Reagent (Molecular Research Center, Inc., Cat. No: TR118). miR and mRNA were transcribed into cDNA with miRcute Plus miR First-Strand cDNA kit (TIANGEN, KR211) and HiScript qPCR Master Mix (Vazyme, Q711). The thermocycling conditions were as follows: Pre-denaturation at 95°C for 15 min, followed by 5 cycles of 94°C for 20 sec, 60°C for 30 sec and 72°C for 34 sec, by 45 cycles of 94°C for 20 sec and 60°C for 34 sec.
34 sec. miR-146a-5p expression levels were normalized to U6. While, mRNA expression levels were normalized to GAPDH. Relative expression levels were calculated using the $2^{-\Delta\Delta C_q}$ method (30). The oligonucleotide primers of target genes were designed and synthesized by TsingKe Biological Technology (Table I).

**ELISA.** Cell-free supernatant was collected the precipitate was removed by centrifugation at 1,500 x g for 10 min at 4˚C. Serum samples were stored at -20˚C, the concentration of IL-6 (Cat. No. 555220), IL-1β (Cat. No. 557953), TNF-α (Cat. No. 555212) and IP-10 (Cat. No. 550926) was evaluated using ELISA kits (BD Pharmingen; BD Biosciences). According to the manufacturer' protocols. The optical density of each well was measured at 450 nm using a microplate reader.

**Western blotting.** RIPA buffer (Beyotime, P0013C) was used to extract total protein from Caco-2 cells. Enhanced BCA Protein Assay kit (Beyotime, P0010) was used for determination of protein lysate concentration. Protein lysate (20 µg/lane) was subjected to 10% SDS-PAGE and electroblotted onto an Immunobilon-P membrane. After blocking with 5% non-fat dry milk powder at room temperature for 1 h, wash PVDF membranes with PBS three times, 5 min each time, then the membranes were incubated overnight at 4˚C with the following primary antibodies: Rabbit anti-TRAF6 (cat. no. 67591S; 1:1,000; Cell Signaling Technology, Inc.), rabbit anti-IRAK1 (cat. no. ab180747; 1:1,000; Abcam,) and rabbit anti-GAPDH (cat. no. 21181; 1,000, Cell Signaling Technology, Inc.). Membranes were wash PVDF membranes with PBS three times, 5 min each time. Subsequently, cells were incubated at room temperature for 1 h with secondary antibody goat anti-rabbit IgG conjugated to HRP (cat. no. 7014; 1:1,000, Cell Signaling Technology, Inc.), GAPDH was used as the loading control. BeyoECL Moon (Beyotime, P0018FS) and chemiluminescence imaging systems (Cytiva, ImageQuant LAS 4000 mini) were used to capture the signal, and analyzed with Image J (version 1.8.0; National Institutes of Health).

**miRNA target protein prediction.** The potential association between miR-146a-5p and IRAK1 and TRAF6 was predicted by sequence complementarity according to TargetScan (targetscan.org/vert_80/v) database.

**Statistical analysis.** Data are presented as the mean ± standard deviation of three experimental replicates. Statistical comparisons were performed using Unpaired Student's t-test and one-way ANOVA for multiple comparisons followed by Tukey's test. All statistical analyses were performed using GraphPad Prism 8.0.2 software (GraphPad Software, Inc.). P<0.05 was considered to indicate a statistically significant difference.

**Results**

**Expression of inflammatory factors in IL-1β-treated inflammatory model model.** Caco-2 cells were incubated with 10 ng/ml IL-1β for 0.0, 1.5, 3.0, 6.0, 12.0 and 24.0 h to test if IL-1β induces expression of intracellular inflammatory cytokines and chemokines (Fig. 1A-H). ELISA was used to detect protein expression levels of inflammatory cytokines and chemokines. It was found that 10 ng/ml IL-1β promoted high expression levels of IL-6, IL-1β, TNF-α and IP-10. The expression of IL-6, TNF-α and IP-10 were increased from 1.5 h (Fig. 1A, C and D), while the expression of IL-1β was increased from 6 h (Fig. 1B). Moreover, as exogenous IL-1β is added into cell medium, it is difficult to detect exact endogenous IL-1β produced by cells. 10 ng/ml IL-1β was added to Caco-2 cells for different duration of time (1.5-24 h) to test if IL-1β induces expression of intracellular inflammatory cytokines and chemokines. It was found that 10 ng/ml IL-1β promoted high expression levels of IL-6, IL-1β, TNF-α and IP-10. The expression of IL-6, TNF-α and IP-10 increased from 1.5 h, while the expression of IL-1β increased from 6 h (Fig. 1A-D). Moreover, the expression of IL-6, TNF-α and IP-10 were increased from 1.5 h (Fig. 1A, C and D), while the expression of IL-1β was increased from 6 h (Fig. 1B). As exogenous IL-1β is added into cell medium, it is difficult to detect exact endogenous IL-1β produced by cells. 10 ng/ml IL-1β was added to Caco-2 cells for different duration of time (1.5-24 h) except for the group of 0 h. In Fig. 1B, the concentration of IL-1β in the group of 1.5 and 3 h was much higher than the group of 0 h. This could be attributed to the exogenous IL-1β added into the medium of 1.5-24 h group. Caco-2 cells increased significantly with the concentration of IL-1β (Fig. 1E-H). The expression of IL-6 and IL-1β were increased from 0.01 ng/ml (Fig. 1E and F), while the expression of TNF-α and IP-10 were increased from 0.10 ng/ml (Fig. 1G and H). When IL-1β was 10 ng/ml, the expression levels of IL-6, IL-1β, TNF-α and IP-10 were relatively high. Therefore, the optimal modeling condition was determined to be 10 ng/ml IL-1β treatment for 3.0 h.

Table I. Primer sequences.

| Gene   | Forward (5'-3')          | Reverse (5'-3')         |
|--------|--------------------------|-------------------------|
| U6     | CGCTTCGGCAGCACATACATAC   | CAGGGGCCCATGCTAATCTTT   |
| miR-146a-5p | UGAGAAUCGAUCCACUGGGUU   | CCCAUUGGAUCAGUUCUAUU   |
| GAPDH  | GGTGCTCTCCTCTGACTTCAACA | GTTGCTTAGCACCATTGTGTTG  |
| TRAF6  | TTTGCTCTTATGGATTGTCCTCCC | CATTGATGACACAGATTGTC    |
| IRAK1  | TGAGGAAACAGTTGTATGCTG    | GTTGGGTTAGCAAAACCTGGA   |
| IL-6   | TGGCTGAAAAAGATGGATGCT    | TCTGCACAGCTCTTGTTTGT    |
| IL-1β  | AAGCTGATGGCCCTAACACAG    | AGTTGCATCTGCTACACATTAG |
| TNF-α  | TGTAGCCCATGTGTTAGCAAACC  | GAGGACCTGGGAGTAGAGGTAAG |
| IP-10  | GTGCCATTCAAGGAGTACCCTC   | TGATGCGCTCTAGGATTCTGGA  |

IP-10, interferon γ-induced protein 10; miR, microRNA; IRAK1, interleukin 1 receptor associated kinase 1; TRAF6, tumor necrosis factor receptor-associated factor 6.
Expression of miR-146a-5p in IL-1β-treated intestinal epithelial mucositis model. RT-qPCR was performed to measure miR levels in Caco-2 cells following incubation with IL-1β. Following incubation with 10 ng/ml IL-1β for 3 h, the expression of miR-146a-5p increased over time (Fig. 2A) and with increasing IL-1β concentration compared with 0.00 or 0.01 ng/ml IL-1β (Fig. 2B).

miR-146a-5p negatively regulates inflammatory factors. RT-qPCR analysis of Caco-2 cells showed stable miR-146a-5p upregulation following transfection with miR-146a-5p mimics and downregulation after transfection with miR-146a-5p inhibitors (Fig. 3A and B). Following transfection, the cells were treated with 10 ng/ml IL-1β for 3 h and collected for RT-qPCR (Fig. 3C, E, G, I) and ELISA (Fig. 3D, F, H, J) analysis to assess the IL-6, TNF-α, IL-1β and IP-10 expression compared with untreated transfected control cells.

miR-146a-5p mimics significantly inhibited transcription and translation of IL-1β-induced inflammatory cytokines (IL-6, IL-1β and TNF-α) and chemokine IP-10 in intestinal epithelial cells, however, miR-146a-5p inhibitors significantly promoted the expression of IL-1β-induced inflammatory cytokines (IL-6, IL-1β and TNF-α) and chemokine IP-10 (Fig. 3C-J). miR-146a-5p negatively regulated expression of inflammatory factors expression in IL-1β-treated Caco-2 cells at the transcript and protein level.

IRA1 and TRAF6 are involved in expression of miR-146a-5p-suppressed inflammatory factors. TargetScan (targetscan.org/vert_80/v) database was used to predict targets of miR-146a-5p; IRAK1 and TRAF6 were identified (Fig. 4A and B). Furthermore, as aforementioned, IRAK1 and TRAF6 participated in the regulatory mechanism of miR-146a-5p in the inflammatory response. The protein
expression of IRAK1 and TRAF6 in miR-146a mimics + IL-1β and miR-146a-5p inhibitor + IL-1β groups were compared with the corresponding NC. According to western blot analysis, upregulation of miR-146a-5p inhibited expression of both IRAK1 and TRAF6, whereas miR-146a-5p downregulation increased expression of these target proteins (Fig. 4C-H).

Discussion

The precise etiology and pathogenesis of IBD is unknown. The primary pathophysiological hallmark of IBD is idiopathic disorder caused by chronic and excessive inflammation of the gastrointestinal tract; this is also involved in the pathogenesis of IBD (29,31). The intestinal mucosa is actively involved in the intestinal inflammatory response. IECs serve a key role in physical isolation of intestinal pathogens and control of intestinal mucosal inflammation due to their location and are surrounded by immune cells (32,33). The pathological alteration of IECs in the inflammation microenvironment needs to be investigated.

Due to the ability to produce and release multiple inflammatory cytokines, Caco-2 cells are routinely utilized as in vitro intestinal epithelial mucositis model of human intestinal inflammation following stimulation with proinflammatory factors, such as IL-1β (7). Intestinal inflammation is exacerbated by IL-1β, which increases granulocyte recruitment and formation and activation of innate lymphoid cells (12).

Aberrant miR expression in inflammatory intestinal mucosal tissue of patients with IBD is associated with intestinal inflammation, intestinal mucosal immune dysfunction and intestinal epithelial cell barrier degradation (34). miR-146a-5p has a strong regulatory function during the inflammatory response, which negatively regulates release of IL-5 and IL-13 from regulatory T cells by blockade of the NF-κB signal pathway (35). The upregulation of miR-146a-5p effectively improves symptoms of allergic conjunctivitis in a mouse model (36). miR-146a-5p inhibits expression of IL-1β-induced inflammatory cytokines IL-6 and L-1β in a cementoblast-like cell line (OCCM-30) via the IRAK1/TRAF6 pathway in the innate immune response (37). Upregulation of miR-146a-5p expression is observed in both active CD and UC mucosa compared with control mucosa (34). miR-146a-5p inhibits expression of IL-1β-induced inflammatory cytokines IL-6 and L-1β and alleviates periodontal inflammation (37).

In the present study, expression of miR-146a-5p was increased in the IL-1β-induced in vitro inflammation model.
The increased expression of miR-146a is in agreement with an earlier study in IL-1β-stimulated human lung alveolar epithelial cells (36). Moreover, upregulating miR-146a-5p decreased transcription and release of inflammatory factors, while downregulating miR-146a-5p enhanced inflammatory mediator expression in IL-1β-stimulated Caco-2 cells. The aforementioned results suggested that miR-146a-5p may serve a protective role in colonic inflammation. The dose- and time-dependent responses induced in Caco-2 cells due to exposure to exogenous IL-1β suggested that miR-146a-5p may be involved in regulation of inflammatory factor release (38). IL-1β induces IL-6, IL-8, TNF-α and IL-1β gene transcription in differentiated Caco-2 cells (39). In the present study, a 3-fold increase was observed in expression of miR-146a-5p in IL-1β-induced Caco-2 cells. Consistent with this observation, 24-fold miR-146a-5p increase has been reported in IL-1β-induced A549 cells (38). The present data indicated that secretion of inflammatory cytokines in IL-1β-induced Caco-2 cells was associated with the production of miR-146a-5p.

Dysregulated epithelial barrier function is associated with abnormal immunological response (40). IECs respond to IL-1β via their receptors and thus amplify the effects of IL-1β during the inflammatory response (41). IL-1β and TNF-α serve a key role in both the initial and expansion stages of intestinal injury by damaging tight junctions between Caco-2 cells, affecting the stability of tight junctions between cells, increasing permeability and promoting the occurrence and development of inflammation (11,42). In addition, TNF-α inhibits the expression of Na-H exchanger in the intestinal epithelium of patients with IBD and reduces its activity, which leads to dysfunctional secretion of inflammatory factors from IECs and diarrhea (43). Another key regulator of the inflammatory response, IL-6, is a central cytokine in IBD, the level of IL-6 in serum from patients with IBD is associated with disease activity and histological severity (44).
The protein levels of IL-1β, TNF-α and IL-6 were upregulated. T lymphocytes, macrophages, neutrophils and plasma cells penetrate the intestine of patients with IBD. These immune cells release chemokines that serve a role in inflammatory processes, such as IP-10, which recruits leukocytes in addition to acting as a chemokine, IP-10 is a T helper 1 pro-inflammatory chemokine that is generated by IFN-γ and interacts with C-X-C motif chemokine receptor 3. IP-10 recruits leukocytes to inflammatory sites (3). The vital proinflammatory role in immune-mediated signaling is at the core of intestinal inflammation (45). More specifically, miR-146a-5p decreased IL-1β-induced transcription and release of inflammatory factors (IL-6, IL-1β, TNF-α and IP-10). These findings suggest that the aforementioned inflammatory factors may govern immune cells in patients with IBD, affecting local intestinal inflammation and tissue damage; inhibiting these inflammatory mediators may ameliorate symptoms in patients with IBD. Similar effects have been reported in lung alveolar (36), human retinal (46), gastric mucosa (47) and human nasal epithelial cells (40) and are associated with resistance to IL-1β-induced epithelial cell inflammation. The anti-inflammatory effect of miR-146a-5p in epithelial cells is also supported by the results of the present study. High miR-146a-5p expression inhibited the inflammatory response and decreased production of inflammatory factors by IL-1β. By contrast, decreased miR-146a-5p increased production of inflammatory factors induced by IL-1β, suggesting that miR-146a-5p inhibited the inflammatory response in IL-1β-induced Caco-2 cells. IRAK1 and TRAF6, were identified using TargetScan Human database (targetscan.org/vert_80/) as involved in IL-1β signaling. miR-146a-5p directly targets TRAF6 and IRAK1 genes to decrease NF-κB activation and inflammation in human umbilical vein endothelial (48) and bronchial (BEAS-2B) (49) and primary lung epithelial cells (50). In the present study, expression of IRAK1 and TRAF6 was downregulated following incubation with IL-1β. Upregulation of miR-146a-5p further downregulated expression of IRAK1 and TRAF6. According to a prior study, decreased IRAK1 levels may be associated with proteasomal destruction following phosphorylation and subsequent ubiquitination (51). However, other studies have shown that miR-146a-5p regulates protein expression of IRAK1 and TRAF6 via post-transcriptional mechanisms and does not affect protein expression by regulating the stability of these proteins (37). The adverse effect of miR-146a-5p in the inflammatory response may be associated with the IRAK1/TRAF6 pathway.

In addition to regulation of miR-146a-5p in the inflammation of IECs, its regulation mechanism in IECs barrier function dysfunction is also worthy of exploration. The anti-inflammatory mechanism in IL-1β-stimulated Caco-2 cells has been linked to miR-146a-5p. The mechanism may involve regulation of IRAK1 and TRAF6. Therefore, altered miR-146a-5p expression in IECs may serve as a novel monitoring indicator for the prevention of IBD. High expression of miR-146a-5p inhibits overactivation of IECs inflammation and inappropriate production of pro-inflammatory cytokines, which leads to irreversible intestinal tissue damage. Future studies are key for developing miR-146a-5p-based interventions to control the occurrence and development of IBD.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions
LG, YL andYS conceived and designed the study. YL and ST performed the experiments. YL performed data analysis and wrote the article. LG and YS reviewed and edited the manuscript. All authors have read and approved the final manuscript. LG and YS confirm the authenticity of all the raw data.

Ethics approval and consent to participate
Not applicable.

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Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References
1. Kaplan GG: The global burden of IBD: From 2015 to 2025. Nat Rev Gastroenterol Hepatol 12: 720-727, 2015.
2. Schreiner P, Martinho-Grueber M, Studerus D, Vavricka SR, Tilg H and Biedermann L; on behalf of Swiss IBDnet, an official working group of the Swiss Society of Gastroenterology: Nutrition in inflammatory bowel disease. Digestion 101 (Suppl 1): S120-S135, 2020.
3. Guan Q: A comprehensive review and update on the pathogenesis of inflammatory bowel disease. J Immunol Res 2019: 7247238, 2019.
4. Ananthakrishnan AN, Bernstein CN, Iliopoulos D, Macpherson A, Neurath MF, Ali RAR, Vavricka SR and Fiocchi C: Environmental triggers in IBD: A review of progress and evidence. Nat Rev Gastroenterol Hepatol 15: 39-49, 2018.
5. Forbes JD, Bernstein CN, Tremlett H, Van Domselaar G and Knox NC: A fungal world: Could the gut mycobiome Be involved in neurological disease? Front Microbiol 9: 3249, 2019.
6. Wang H, Gu J, Hou X, Chen J, Yang N, Liu Y, Wang G, Du M, Qiu H, Luo Y, et al: Anti-inflammatory effect of miltirone on inflammatory bowel disease via TLR4/NF-kB/IKGAP2 signaling pathway. Biomed Pharmacother 85: 531-540, 2017.
7. Van De Walle J, Hendrickx A, Romier B, Larondelle Y and Schneider YF: Inflammatory parameters in Caco-2 cells: Effect of stimuli nature, concentration, combination and cell differentiation. Toxicol In Vitro 24: 1441-1449, 2010.
Böcker U, Yezerksy O, Feick P, Manifold T, Panja A, Kalina U, Herweck F, Rossol S and Singer MV: Responsiveness of intestinal epithelial cell lines to lipo polysaccharide is correlated with Toll-like receptor expression and Toll-like-receptor 2 or CD14 expression. Int J Colorectal Dis 18: 25-32, 1993.

Reinecker HC, Steffen M, Witthoeft T, Pfleuger I, Schreiber S, MacDermott RP and Raedler A: Enhanced secretion of tumour necrosis factor-alpha, IL-6, and IL-1 beta by isolated lamina propria mononuclear cells from patients with ulcerative colitis and Crohn's disease. Clin Exp Immunol 94: 174-181, 1993.

Ghorpade DS, Sinha AY, Holla S, Singh V and Balaji KN: NOD2-nitric oxide-responsive microRNA-146A activates Sonic hedgehog signaling to orchestrate inflammatory responses in mucosal inflammatory bowel disease. J Biol Chem 288: 33037-33048, 2013.

Mao L, Kitani A, Strober W and Fuss IJ: The role of NLRP3 and IL-1β in the pathogenesis of inflammatory bowel disease. Front Immunol 9: 2566, 2018.

Coccia M, Harrison OJ, Schiering C, Asquith MJ, Becker B, Powrie F and Maloy KJ: IL-1β mediates chronic intestinal inflammation by promoting the accumulation of IL-17A secreting innate lymphoid cells and CD4(+) Th17 cells. J Exp Med 209: 1595-1609, 2012.

Liu Z, Wang D, Hu Y, Zhou G, Zou H, Cui Q, Chi Y, Cao Y, Jia C and Zou Q: MicroRNA-146A negatively regulates the IL-1β-mediated inflammatory response in a cementoblast-derived cell line. Oral Dis 26: 1160-1165, 2020.

Ishmael FT, Fang X, Galdiero MR, Atasoy U, Rigby WF, Gorospe M, Cheadle A and Stellato C: Role of the RNA-binding protein tristetraprolin in glucocorticoid-mediated gene regulation. Nat Rev Immunol 10: 111-122, 2010.

McCurdy DK, Sharma S, Wong D, Hahn BH and Rehimi H: MicroRNA-146 is involved in the inflammatory phenotype of intestinal myofibroblasts by down-regulating Mcl-1. Int J Clin Exp Pathol 7: 33037-33048, 2014.

Schmutz C, Cenk E and Marko D: The alternaria mycotoxin alternariol triggers the immune response of IL-1β-stimulated, differentiated Caco-2 cells. Mol Nutr Food Res 63: e1900341, 2019.

Ghorpade DS, Sinha AY, Holla S, Singh V and Balaji KN: The effects of mycotoxins, fungal food contaminants, on the intestinal epithelial cell-derived innate immune response. Dtsch Med Wochenschr 113: 921, 2018 (In German).

Bouhet S and Oswald IP: The effects of mycotoxins, fungal food contaminants, on the intestinal epithelial cell-derived innate immune response. Vet Immunol Immunopathol 108: 199-209, 2005.

Peck BC, Mah AT, Pitman WA, Ding S, Lund PK and Sethupathy P: Functional transcriptomics in diverse intestinal epithelial cell types reveals robust MicroRNA sensitivity in intestinal stem cells to microbial stimuli. J Biol Chem 292: 25866-25870, 2017.

Sethupathy P: Functional transcriptomics in diverse intestinal epithelial cell types reveals robust MicroRNA sensitivity in intestinal stem cells to microbial stimuli. J Biol Chem 292: 25866-25870, 2017.

Poly(I:C) induced microRNA-146a regulates epithelial barrier function and upregulates miR-146a expression. Eur J Immunol 388: 111823, 2020.

Liang H, Chen X, Shi R, Jonas S and Izaurralde E: Towards a molecular understanding of MicroRNA biology. Trends Cell Biol 20: 737-750, 2010.

Peck BC, Mah AT, Pitman WA, Ding S, Lund PK and Sethupathy P: Functional transcriptomics in diverse intestinal epithelial cell types reveals robust MicroRNA sensitivity in intestinal stem cells to microbial stimuli. J Biol Chem 292: 25866-25870, 2017.

Lyu B, Wei Z, Jiang L, Yang G and Han S: MicroRNA-146A negatively regulates IL-33 in activated group 2 innate lymphoid cells by inhibiting IRAK1 and TRAF6. Genes Immun 21: 37-44, 2020.

Liu Z, Wang D, Hu Y, Zhou G, Zou H, Cui Q, Chi Y, Cao Y, Jia C and Zou Q: MicroRNA-146A negatively regulates the PTGS2 expression in a cementoblast-derived cell line. J Periodontal Res 55: 5689-5698, 2008.

Schmutz C, Cenk E and Marko D: The alternaria mycotoxin alternariol triggers the immune response of IL-1β-stimulated, differentiated Caco-2 cells. Mol Nutr Food Res 63: e1900341, 2019.

Miyata R, Kakuki T, Nomura K, Ohtani K, Ogasa Wara N, Takatoku K, Konno Y, Kurosawa S, Inazawa J, Setsuko S, Mihara K and Kameda T: Poly(I:C) induced microRNA-146a regulates epithelial barrier and secretion of proinflammatory cytokines in human nasal epithelial cells. Eur J Pharmacol 761: 375-382, 2015.

McGee DW, Vitkus SJ and Lee P: The effect of cytokine stimulation on IL-1 receptor mRNA expression by intestinal epithelial cells. Cell Immunol 168: 276-280, 1996.

Capaldo CT, Farkas AE, Hilgarth RS, Krug SM, Wolf MF, Benedik JK, Fromm M, Koval M, Parkos C and Nusrat A: Proinflammatory cytokine-induced tight junction remodeling through dynamic self-assembly of claudins. Mol Biol Cell 25: 2710-2719, 2014.

Jones GW, McLoughlin RM, Hammond VJ, Parker CR, Williams JD, Malhotra R, Scheller J, Williams AS, Rose-John S, Topley N and Jones SA: Loss of CD4+ T cell IL-6R expression is critical to host resistance following genital herpes simplex virus type 2 (HSV-2) infection. Open Immunol J 1: 33-41, 2008.

Thapa M and Carr DJ: Chemokines and chemokine receptors critical to host resistance following genital herpes simplex virus type 2 (HSV-2) infection. Open Immunol J 1: 33-41, 2008.

Kutty RK, Nagineni CN, Samuel W, Vijayasarithar C, Jaworski C, Duncan T, Cameron JE, Flemington EK, Hoeks JJ and Redmond TM: Differential regulation of microRNA-146a and microRNA-146b-5p in human retinal pigment epithelial cells by interleukin-β, tumor necrosis factor-α and interferon-γ. Mol Vis 19: 737-750, 2013.
47. Liu Z, Xiao B, Tang B, Li B, Li N, Zhu E, Guo G, Gu J, Zhuang Y, Liu X, *et al*: Up-regulated microRNA-146a negatively modulate Helicobacter pylori-induced inflammatory response in human gastric epithelial cells. Microbes Infect 12: 854-863, 2010.

48. Hou J, Deng Q, Deng X, Zhong W, Liu S and Zhong Z: MicroRNA-146a-5p alleviates lipopolysaccharide-induced NLRP3 inflammasome injury and pro-inflammatory cytokine production via the regulation of TRAF6 and IRAK1 in human umbilical vein endothelial cells (HUVECs). Ann Transl Med 9: 1433, 2021.

49. Liu LM, Wan C, Zhang W, Guan L, Tian G, Zhang F and Ding W: MiR-146a regulates PM2.5-induced inflammation via NF-κB signaling pathway in BEAS-2B cells. Environ Toxicol 33: 742-751, 2018.

50. Huang Y, Crawford M, Higuita-Castro N, Nana-Sinkam P and Ghadiali SN: miR-146a regulates mechanotransduction and pressure-induced inflammation in small airway epithelium. FASEBJ 26: 3351-3364, 2012.

51. Yamin TT and Miller DK: The interleukin-1 receptor-associated kinase is degraded by proteasomes following its phosphorylation. J Biol Chem 272: 21540-21547, 1997.