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

YANLI LI[1,2], SHILIAN TAN[1], YUANYING SHEN[1] and LE GUO[1]

[1]Department of Medical Microbiology and Immunology, College of Basic Medicine, Dali University, Dali, Yunnan 671003; [2]Clinical Laboratory of Zhumadian Traditional Chinese Medicine Hospital, Zhumadian, Henan 463002, P.R. China

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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-1β 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.

Correspondence to: Mr. Le Guo, Department of Medical Microbiology and Immunology, College of Basic Medicine, Dali University, 22 Wanhua Road, Dali Bai, Dali, Yunnan 671003, P.R. China
E-mail: le.guo@dali.edu.cn

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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

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 are 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% CO_2.

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 UCCAUGGGUU-3'; miR-146a-5p inhibitors, 5'-AACCCA UGGAAUUCAGUUCUCA-3'; miRNA mimic NC, 5'-UUG UACUACACAAAAGUACUG-3' and miRNA inhibitor NC, 5'-CAGUACUUUUGUGUAGUACAA-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 $5x10^5$ 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®III 1st Strand cDNA Synthesis kit (+gDNA wiper; Vazyme, R312-02) respectively, according to the manufacturer's protocol. RT-qPCR analysis was performed using the miRcute Plus miRNA qPCR kit (SYBR Green; TIANGEN, FP411-02) and ChamQ Universal SYBR 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

Table I. Primer sequences.

Gene	Forward (5'-3')	Reverse (5'-3')
U6	CGCTTCGGCAGCACATATAC	CAGGGGCCATGCTAATCTT
miR-146a-5p	UGAGAACUGAAUUCCAUGGGUU	CCCAUGGAAUUCAGUUCUCAUU
GAPDH	GGTGGTCTCCTCTGACTTCAACA	GTTGCTGTAGCCAAATTCGTTGT
TRAF6	TTTGCTCTTATGGATTGTCCCC	CATTGATGCAGCACAGTTGTC
IRAKI	TGAGGAACACGGTGTATGCTG	GTTTGGGTGACGAAACCTGGA
IL-6	TGGCTGAAAAAGATGGATGCT	TCTGCACAGCTCTGGCTTGT
IL-1β	AAGCTGATGGCCCTAAACAG	AGGTGCATCGTGCACATAAG
TNF-α	TGTAGCCCATGTTGTAGCAAACC	GAGGACCTGGGAGTAGATGAGGTA
IP-10	GTGGCATTCAAGGAGTACCTC	TGATGGCCTTCGATTCTGGATT

IP-10, interferon γ-induced protein 10; miR, microRNA; IRAK1, interleukin 1 receptor associated kinase 1; TRAF6, tumor necrosis factor receptor-associated factor 6.

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 $2^{-\Delta\Delta Cq}$ 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. 5509 26) was evaluated using ELISA kits (BD Pharmingen; BD Biosciences). According to the manufacturer' protocals. 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) for determination of protein lysate concentration. Protein lysate (20 μg/lane) was subjected to 10% SDS-PAGE and electroblotted onto an Immunobiolon-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 protien 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 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-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.

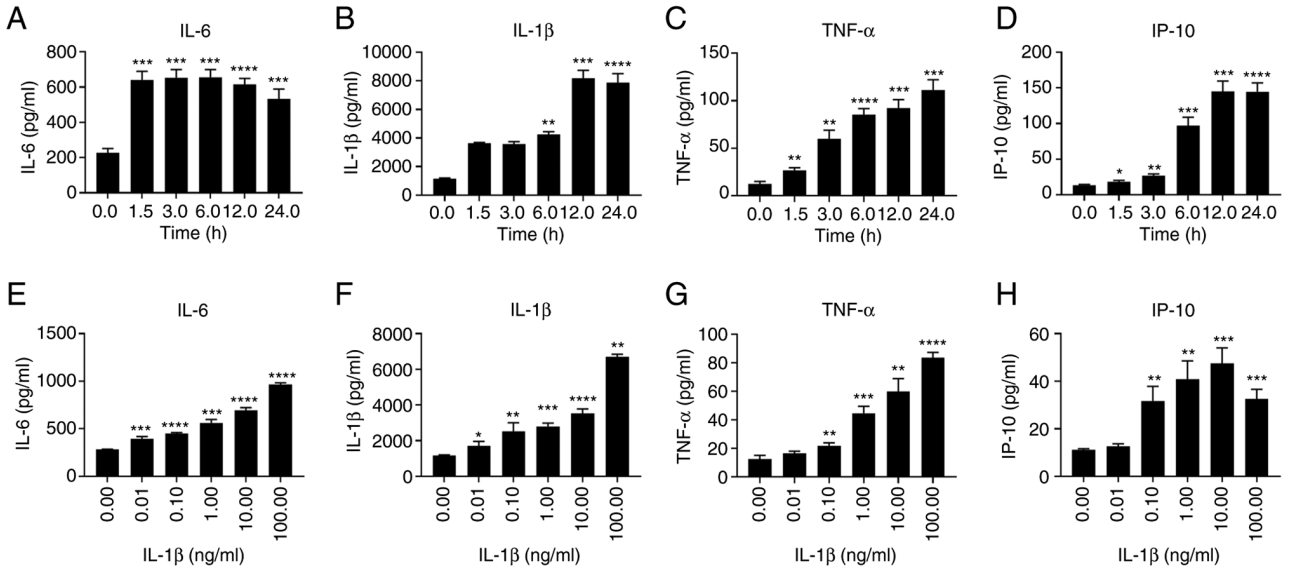

Figure 1. Time- and dose-dependent expression of inflammatory factors in Caco-2 cells induced by IL-1β. ELISA was used to detect protein expression levels of supernatant inflammatory cytokines (A) IL-6, (B) IL-1β, (C) TNF-α and chemokines (D) IP-10 in Caco-2 cells incubated with 10 ng/ml IL-1β for different time. The protein expression of (E) IL-6, (F) IL-1β, (G) TNF-α and (H) IP-10 in Caco-2 cells incubated with different concentration IL-1β for 3 h via ELISA. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 vs. 0 h or 0 ng/ml.

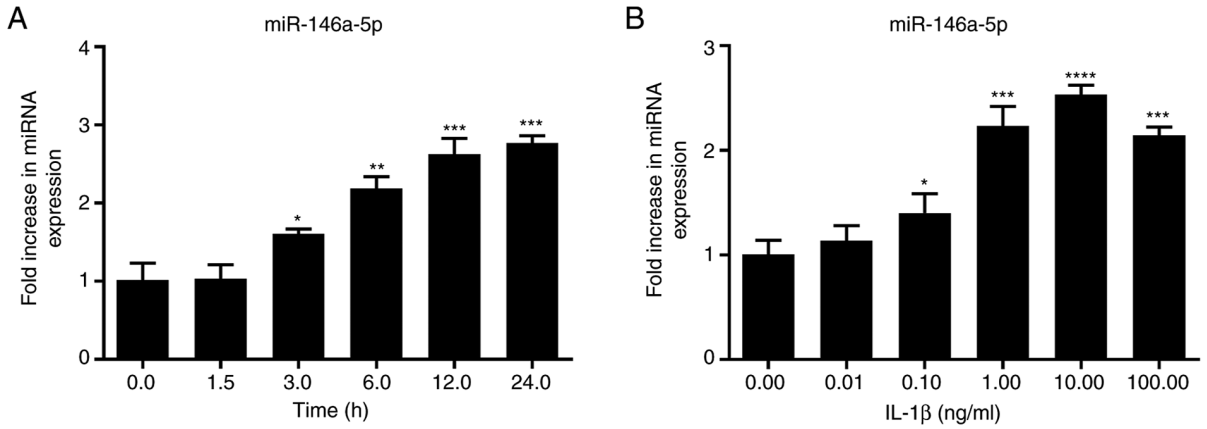

Figure 2. Expression of miR-146a-5p is elevated in IL-1β-stimulated Caco-2 cells. Caco-2 cells were incubated with (A) 10 ng/ml IL-1β or (B) IL-1β for 3 h at the indicated concentrations and relative expression of miR-146a-5p was determined by reverse transcription-quantitative PCR. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 vs. 0 h or 0 ng/ml. miR, microRNA.

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.

IRAK1 and TRAF6 are involved in expresion 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

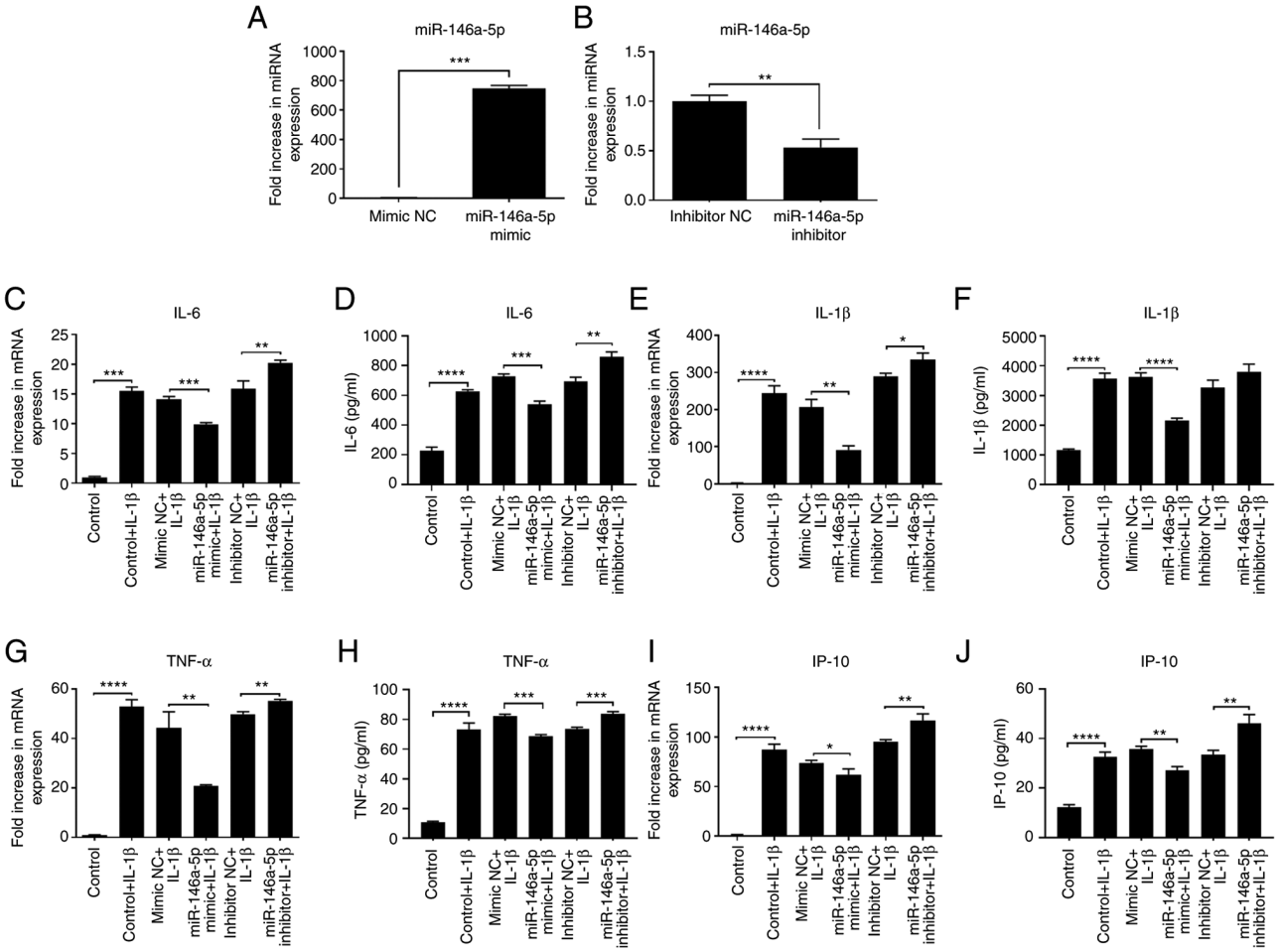

Figure 3. miR-146a-5p negatively regulates inflammatory factor expression. Caco-2 cells were transiently transfected with 10 pmol miR-146a-5p (A) mimic or (B) inhibitor or corresponding NC. IL-6 mRNA expression via (C) RT-qPCR and (D) ELISA. IL-1β mRNA expression level was measured using (E) RT-qPCR, and (F) ELISA. TNF-α mRNA expression level was measured using (G) RT-qPCR, and (H) ELISA. IP-10 mRNA expression level was measured using (I) RT-qPCR, and (J) ELISA. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. Reverse transcription-quantitative; miR, microRNA; IP-10, interferon γ-induced protein 10; NC, negative control.

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 mucosa 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.

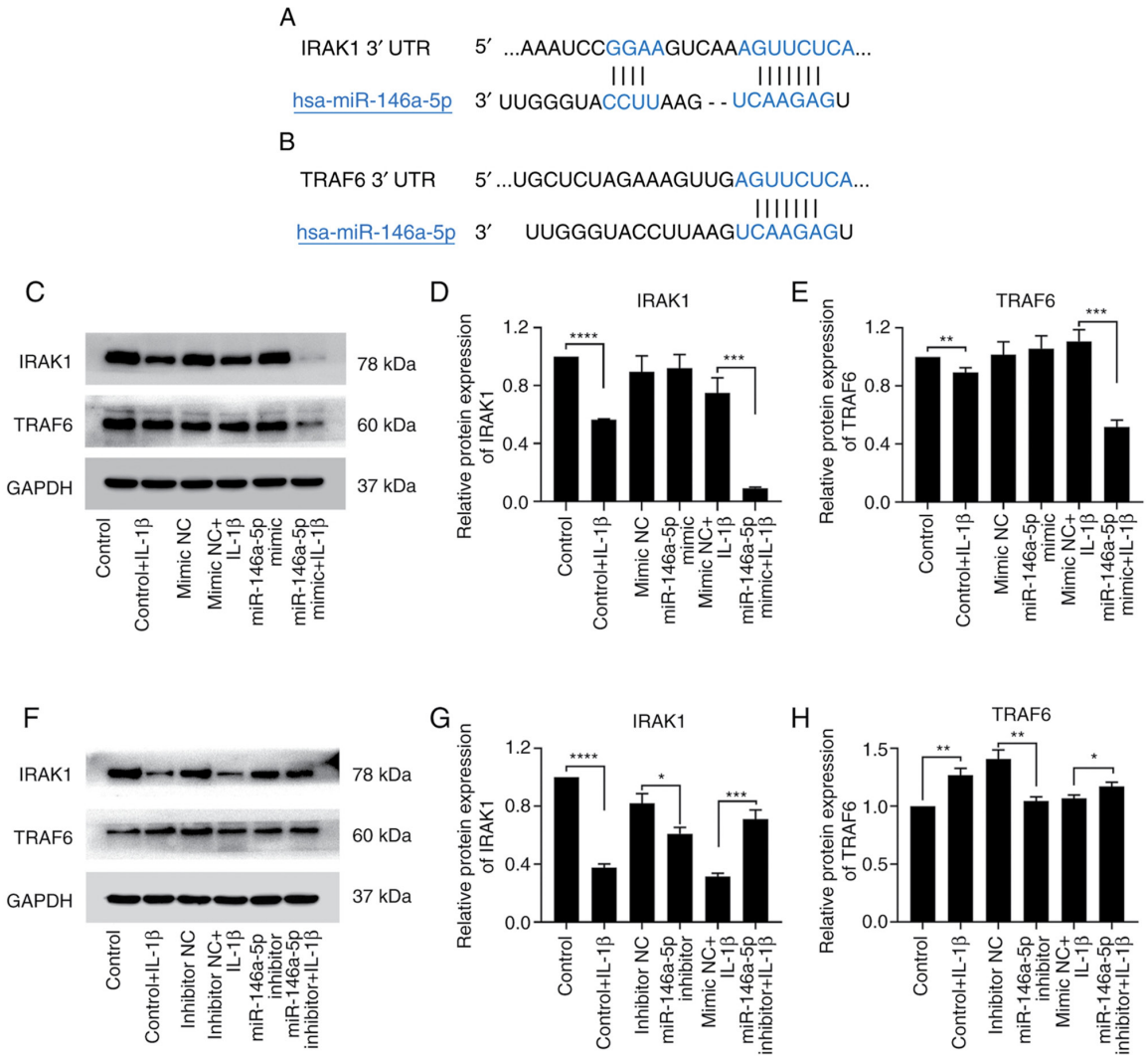

Figure 4. IRAK1 and TRAF6 are involved in expression of miR-146a-5p-suppressed inflammatory factors. TargetScan Human predicted (A) IRAK1 and (B) TRAF6 as target proteins of miR-146a-5p. Following transfection with miR-146a-5p mimics or inhibitor, Caco-2 cells were incubated with 10 ng/ml IL-1β for 24 h and collected. IRAK1 and TRAF6 protein was extracted, separated and analyzed by western blotting. (C) Relative protein expression of (D) IRAK1 and (E) TRAF6 in control, IL-1β, mimic NC, mimic NC + IL-1β, miR-146a-5p mimic and miR-146a-5p mimic + IL-1β groups were analyzed with Image J. (F) Relative protein expression of (G) IRAK1 and (H) TRAF6 in control, IL-1β, inhibitor NC, inhibitor NC + IL-1β, miR-146a-5p inhibitor and miR-146a-5p inhibitor + IL-1β groups were analyzed with Image J. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. IRAK1, interleukin 1 receptor associated kinase 1; TRAF6, tumor necrosis factor receptor-associated factor 6; UTR, untranslated region; miR, microRNA; NC, negative control.

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 mechisms 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 and YS 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.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Authors' information

ORCID ID Le Guo, orcid.org/0000-0001-9031-1328.

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