European Review for Medical and Pharmacological Sciences 2019; 23: 7543-7549

Hsa-miR-375 promotes the progression of inflammatory bowel disease by upregulating TLR4

C.-P. WU[1], Y.-J. BI[2], D.-M. LIU[3], L.-Y. WANG[1]

[1]Department of Gastroenterology, Shandong Provincial Qianfoshan Hospital, the First Hospital Affiliated with Shandong First Medical University, Jinan, China
[2]Department of Gastroenterology, Shandong Provincial PKUCare Luzhong Hospital, Zibo, China
[3]Department of Hepatology, Shandong Provincial Traditional Chinese Hospital of Zaozhuang, Zaozhuang, China

Abstract. – OBJECTIVE: To elucidate the biological function of hsa-miR-375 in the progression of inflammatory bowel disease (IBD) and the potential mechanism.

PATIENTS AND METHODS: Intestinal mucosa tissues of 26 IBD patients and 30 healthy volunteers who underwent colonoscopy were harvested for determining hsa-miR-375 level by quantitative Real-time polymerase chain reaction (qRT-PCR). Binding of hsa-miR-375 to toll-like receptor 4 (TLR4) was verified by the dual-luciferase reporter gene assay. Changes in the viability and apoptosis in Caco-2 cells influenced by hsa-miR-375 were examined by cell counting kit-8 (CCK-8) assay and flow cytometry, respectively. The regulatory effect of hsa-miR-375 on the intestinal epithelial barrier was examined by detecting transepithelial electrical resistance (TEER) and lucifer yellow flux. Relative levels of TLR4, nuclear factor-kappa B (NF-κB), zonula occludens-1 (ZO-1), occludin and inflammatory factors in Caco-2 cells were detected by qRT-PCR, Western blot and enzyme-linked immunosorbent assay (ELISA).

RESULTS: Hsa-miR-375 was downregulated in intestinal mucosa tissues of patients with Crohn's disease (CD) and ulcerative colitis (UC). Knockdown of hsa-miR-375 decreased viability and TEER, but elevated apoptotic rate and lucifer yellow flux. Overexpression of hsa-miR-375 achieved the opposite trends. TLR4 was the direct downstream of hsa-miR-375, and its level was negatively mediated by hsa-miR-375. In addition, TLR4 level in Caco-2 cells was upregulated after LPS induction, while hsa-miR-375 level was unchangeable. Knockdown of hsa-miR-375 upregulated NF-κB and pro-inflammatory factors TNF-α, IL-1β, IL-6 and IL-8, and downregulated ZO-1, occludin and anti-inflammatory factor IL-10.

CONCLUSIONS: Hsa-miR-375 is involved in the pathogenesis of IBD by upregulating TLR4 and inducing NF-κB activation.

Key Words
Hsa-miR-375, IBD, TLR4, NF-κB.

Introduction

Inflammatory bowel disease (IBD) is a group of chronic, recurrent and non-specific inflammatory diseases involving the gastrointestinal tract, including Crohn's disease (CD) and ulcerative colitis (UC)[1]. The etiology and pathogenesis of IBD remain unclear. Current studies indicated that alteration of intestinal flora, intestinal mucosal barrier dysfunction, immune response disorder in the intestinal mucosa, environmental factors and genetic susceptibility are all factors influencing the development of IBD[2,3]. In recent years, the incidence of IBD has increased year by year. Protracted course of disease, high rate of recurrence and carcinogenic risks of IBD seriously affect life quality and pose an economic burden on affected patients[3,4]. It is of great significance to elucidate the pathogenesis of IBD and search for novel therapeutic targets.

MicroRNAs (miRNAs) are a class of endogenous, small, non-coding RNAs with 19-25 bases in length. They promote the degradation or inhibit translation of target genes by complementary base pairing to mRNA 3'Untranslated Region (3'UTR), thereby mediating cellular behaviors[5,6]. At present, many miRNAs have been found to be abnormally expressed in the intestinal mucosa of IBD patients. Dysregulated IBD-related miRNAs cause immune dysfunction in the intestinal mucosa, impair intestinal epithelial barrier function, and ultimately lead to disordered inflammatory immune responses in the intestinal mucosa[6,7]. MiRNA exerts a strong regulatory ability on gene expressions at cellular level, which is capable of affecting the development of intestinal inflammation. It has a promising potential as a drug target for clinical treatment of IBD. A relevant study demonstrated

Corresponding Author: Liyun Wang, MD; e-mail: wly1984123@126.com

that miR-19b is downregulated in the intestinal mucosa of active CD patients. Enema treatment of miR-19b precursor in TNBS-induced colitis mice markedly alleviates inflammatory response in mouse intestinal mucosa[8]. In addition, miR-301a is highly expressed in the intestinal mucosa of active IBD patients than those same IBD patients in the remission phase and healthy controls. No significant difference in miR-301a level is identified between IBD patients in the remission phase and healthy controls. Application of miR-301a inhibitor in TNBS-induced mice with acute colitis reduces the ratio of Th17 cells and IL-17a level in the lamina propria of the intestinal mucosa[9]. It is suggested that miR-301a may serve as a biological hallmark for determining the disease activity and therapeutic target for IBD.

Toll like receptor 4 (TLR4) activates nuclear factor-kappa B (NF-κB) through MyD88, thus stimulating the secretion of downstream inflammatory cytokines[10]. TLR4 is upregulated in the colon and terminal ileal epithelium of UC and CD patients, whereas it is rarely expressed in the intestinal epithelial cells of healthy people. Dysregulated TLR4 may lead to local immune abnormalities in the intestinal mucosa, triggering the occurrence of IBD[11,12]. Heinsbroek et al[13] found out that miR-511-3p influences the progression of enteritis by upregulating TLR4. In this study, the potential regulatory effect of TLR4 on IBD and its interaction with has-miR-375 were specifically explored.

Patients and Methods

Sample Collection

Intestinal mucosa tissues were harvested from 26 IBD patients treated in Shandong Provincial Qianfoshan Hospital from 2017 to 2018. There were 14 UC patients (8 males and 6 females, aged 26-66 years, disease duration of 12-46 months) and 12 CD patients (6 males and 6 females, aged 23-50 years, disease duration of 10-45 months). 30 healthy volunteers who underwent colonoscopy in the same period were selected as controls. This study was approved by the Ethics Committee of Shandong Provincial Qianfoshan Hospital. Signed written informed consent was obtained from all participants before the study.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from tissues and cells by TRIzol method (Invitrogen, Carlsbad, CA, USA). After concentration determination, RNA was subjected to reverse transcription according to the instructions of the miScript II RT Kit. The obtained complementary deoxyribose nucleic acid (cDNA), primers, mixture buffer and diethyl pyrocarbonate (DEPC) water (Beyotime, Shanghai, China) were mixed for preparing the qRT-PCR system. Amplification was conducted on the ABI 7900 Fast PCR instrument (Applied Biosystems, Foster City, CA, USA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was selected as the internal reference and the relative level of target gene was calculated by $2^{-\Delta\Delta CT}$ method. Primer sequences were depicted in Table I.

Table I. Primer sequences of genes used in this study.

TLR4	F: 5'-ATATAAGCTTGATGCCAGGATGGTGT-3' R: 5'-TATGGAATCCCATTCCT -3'
TNF-α	F: 5'-CCTCTCTCTAATCAGCCCTCTG-3' R: 5'-GAGGACCTGGGAGTAGATGAG-3'
IL-1β	F: 5'- CGCTCGGAGTTGGAACTGAC-3' R: 5'- TGGGTACTGCGAATCACCAAG-3'
IL-6	F: 5'- CAACGATGATGCACTTGCAGA-3' R: 5'- CTCCAGGTAGCTATGGTACTCCAGA-3'
IL-8	F: 5'- GGCAAGAACACTGTGTCCAAAGA-3' R: 5'-GTGGATGACTGTCCATGCAGAA-3'
IL-10	F: 5'-GCCAGAGCCACATGCTCCTA-3' R: 5'-GATAAGGCTTGGCAACCCAAGTAA-3'
GAPDH	F: 5'-GCACCGTCAAGGCTGAGAAC-3' R: 5'-GGATCTCGCTCCTGGAAGATG-3'
hsa-miR-375	F: 5'-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGGUGUUUGG -3' R: 5'- ACACTCCAGCTGGGGCGACGAGCCCCUCGCA -3'
U6	F: 5'- CTCGCTTCGGCAGCACA-3' R: 5'-AACGCTTCACGAATTTGCGT -3'

Cell Culture and Transfection

Colorectal cancer cell line Caco-2 was cultured in Dulbecco's modified eagle's medium (DMEM) (Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA) and 1% penicillin-streptomycin, and incubated in a 5% CO_2 incubator at 37°C. Cells seeded in the 6-well plate were subjected to transfection at 50-70% of confluence. Transfection plasmids and Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) diluted in 250 μL of serum-free DMEM, respectively, were mixed and applied in each well with 1.5 mL of serum-free DMEM. 4-6 hours later, complete DMEM was replaced. Transfected cells for 24-48 h were collected for the following experiments.

Establishment of in Vitro Intestinal Mucosal Barrier

2×10^4 cells suspended in 200 μL of DMEM were applied on the upper side of the chamber (0.33 cm^2 in size and 4.0 μm of membrane pore size) placed in a 24-well plate, and 1.2 mL of DMEM was applied to the bottom side. Medium was replaced every other day. Polar cell monolayer was developed at 21 days.

TEER Examination

The integrity of the intestinal epithelial barrier was determined by measuring TEER using the Millicell-ERS instructor. Before the measurement, the electrode of the ERS resistance meter was immersed in 75% ethanol for 15 min, washed with Hank's buffer and equilibrated for 5 min. Two blank control wells were set. The measurement process should be carried out at a constant temperature for three times. TEER (Ω cm^2) = (TEER of sample - TEER of blank well) × effective membrane area of the cell chamber.

Determination of Lucifer Yellow Flux

The permeability of the intestinal epithelial barrier was determined by measuring lucifer yellow flux. The lucifer yellow dye was dissolved in Hank's solution at the final concentration of 100 μg/mL. Culture medium in the 24-well pate and the chamber was removed. Cells were washed with Hank's buffer for three times and incubated at 37°C for 0.5 h. 100 μL of Hank's buffer containing fluorescent yellow dye was applied on the apical chamber and incubated at 37°C for 4 h. Two blank control wells were set. Medium at ventral chamber was collected for determining the absorbance at 427 nm of excitation wavelength and 536 nm of the emission wavelength. Lucifer yellow flux rate (%) = lucifer yellow concentration in the basal chamber / lucifer yellow concentration in the apical chamber. Intestinal mucosal barrier establishment was considered to be successful if the lucifer yellow flux was lower than 10%.

Cell Proliferation Assay

Cells were seeded in the 96-well plate with 1×10^4 cells/mL. Optical density (OD) at 450 nm was recorded at the appointed time points using the cell counting kit-8 (CCK-8) kit (Dojindo Laboratories, Kumamoto, Japan) for depicting the viability curve.

Cell Apoptosis Detection

Cells were seeded in the 6-well plate for 48-h incubation, washed with pre-cooled PBS (phosphate-buffered saline) twice and reacted with fluorescein isothiocyanate (FITC)-Annexin V and 250 μg/mL Propidium Iodide (PI) in dark for 10 min. Cells were washed with PBS twice, followed by flow cytometry detection (FACSCalibur; BD Biosciences, Franklin Lakes, NJ, USA).

Dual-Luciferase Reporter Gene Assay

Wild-type and mutant-type pGL3-TLR4 plasmids were constructed. Caco-2 cells were co-transfected with pGL3-TLR4 wild-type/mutant-type and hsa-miR-375 mimics/negative control, respectively. 48 hours later, luciferase activity was detected using the dual-luciferase reporter gene assay kit (Promega, Madison, WI, USA).

Western Blot

Total protein from cells or tissues was extracted using radioimmunoprecipitation assay (RIPA) and loaded for electrophoresis. After transferring on a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA), it was blocked in 5% skim milk for 2 h, incubated with primary antibodies at 4°C overnight and secondary antibodies for 2 h. Bands were exposed by electrochemiluminescence (ECL) and analyzed by Image Software.

Enzyme-Linked Immunosorbent Assay (ELISA)

Standard curve was established according to the experimental instructions. Test samples were added to each well and incubated at 37°C for 2 h. Samples were incubated with the primary antibody at 37°C for 1 h and the luminescent substrate for 5-10 min. The reaction solution was finally added to each well. The optical density (OD value) of each well was measured using a microplate reader.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 (IBM, Armonk, NY, USA) was used for all statistical analysis. Data were represented as mean ± SD (standard deviation). The *t*-test and chi-square test were used for analyzing measurement data and categorical data, respectively. $p<0.05$ indicated the significant difference.

Results

Expression Patterns of hsa-miR-375 and TLR4 in IBD

Hsa-miR-375 was downregulated in the intestinal mucosa of 26 IBD patients (14 UC patients and 12 CD patients) relative to that of 30 normal controls ($p<0.05$, Figure 1A). In addition, the mRNA level of TLR4 was upregulated in the intestinal mucosa of IBD patients ($p<0.05$, Figure 1B). A negative correlation was identified between mRNA levels of hsa-miR-375 and TLR4 in IBD ($R^2=0.7849$, $p<0.001$, Figure 1C).

Binding Relationship between hsa-miR-375 and TLR4

Transfection of hsa-miR-375 mimics markedly downregulated the mRNA level of TLR4, and conversely, transfection of hsa-miR-375 inhibitor upregulated TLR4 level, indicating a negative regulation between them (Figure 2A). To verify the binding relationship between hsa-miR-375 and TLR4, Caco-2 cells were co-transfected with pGL3-TLR4 wild-type plasmid/mutant-type plasmid and hsa-miR-375 mimics/negative control, respectively. Luciferase activity was reduced in cells co-transfected with hsa-miR-375 mimics and pGL3-TLR4 wild-type plasmid, suggesting that hsa-miR-375 directly bound to TLR4 (Figure 2B, 2C).

Hsa-miR-375 Influenced Viability, Apoptosis and Intestinal Epithelial Barrier Function

Biological function of hsa-miR-375 in IBD was further investigated. As CCK-8 assay illustrated, knockdown of hsa-miR-375 decreased the viability of Caco-2 (Figure 3A). Apoptotic rate was elevated after transfection of hsa-miR-375 inhibitor in Caco-2 cells (Figure 3B). Moreover, TEER and lucifer yellow flux were determined to reflect the integrity and permeability of intestinal epithelial cell barrier. Transfection of hsa-miR-375 inhibitor markedly decreased TEER at 48 h and 72 h (Figure 3C), and increased lucifer yellow flux at 24 h, 48 h and 72 h (Figure 3D). These results demonstrated that hsa-miR-375 deficiency impaired the integrity and elevated the permeability of intestinal epithelial barrier.

Hsa-miR-375 Mediated TLR4/NF-κB Pathway and Inflammatory Factors

TLR4 was upregulated in LPS-induced Caco-2 cells relative to controls. Moreover, the relative level of TLR4 was downregulated in LPS-induced Caco-2 cells overexpressing hsa-miR-375, whereas hsa-miR-375 knockdown elevated TLR4 level in LPS-induced cells (Figure 4A). No significant difference was observed in hsa-miR-375 level after LPS induction in Caco-2 cells, indicating that the upregulated TLR4 in IBD was directly related to the low-level hsa-miR-375 ($p=0.768$, Figure 4B). Protein level of NF-κB was upregulated after LPS induction in Caco-2 cells, which was further

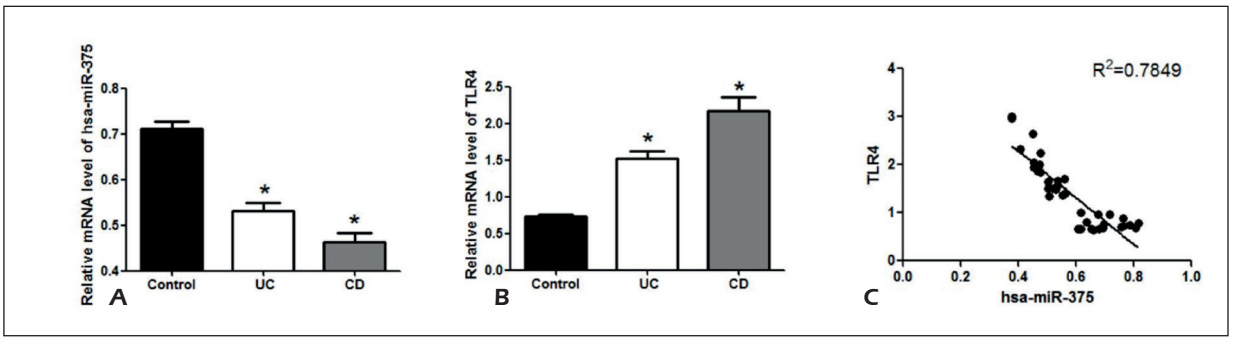

Figure 1. Expression patterns of hsa-miR-375 and TLR4 in IBD. ***A,*** Hsa-miR-375 was downregulated in the intestinal mucosa of 26 IBD patients (14 UC patients and 12 CD patients) relative to that of 30 normal controls. ***B,*** TLR4 was upregulated in the intestinal mucosa of 26 IBD patients (14 UC patients and 12 CD patients) relative to that of 30 normal controls. ***C,*** A negative correlation between mRNA levels of hsa-miR-375 and TLR4 in IBD (R2=0.7849, $p<0.001$).

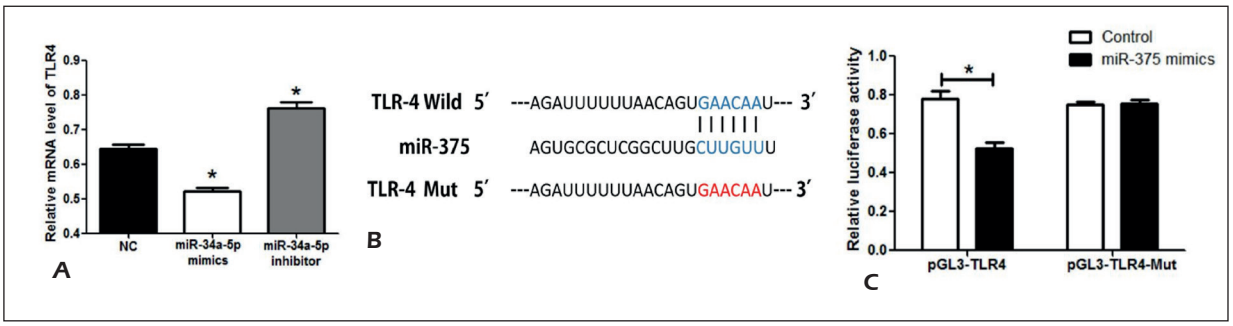

Figure 2. Binding relationship between hsa-miR-375 and TLR4. ***A,*** Transfection of hsa-miR-375 mimics downregulated the mRNA level of TLR4, and transfection of hsa-miR-375 inhibitor upregulated TLR4 level. ***B,*** Binding sequences between TLR4 hsa-miR-375. ***C,*** Luciferase activity in Caco-2 cells co-transfected with pGL3-TLR4 wild-type plasmid/mutant-type plasmid and hsa-miR-375 mimics/negative control.

elevated in LPS-induced cells transfected with hsa-miR-375 inhibitor (Figure 4C). We believed that hsa-miR-375 was involved in the pathogenesis of IBD by activating the TLR4/NF-κB pathway. Meanwhile, ELISA data showed that relative levels of ZO-1 and Occludin were downregulated after hsa-miR-375 knockdown, showing a protective role of hsa-miR-375 in the intestinal epithelial barrier function (Figure 4D, 4E). QRT-PCR data presented that hsa-miR-375 knockdown upregulated levels of TNF-α, IL-1β, IL-6 and IL-8, and downregulated IL-10 levels in LPS-induced Caco-2 cells (Figure 4F). Overexpression of hsa-miR-375 obtained the opposite trends at mRNA levels of these inflammatory factors.

Discussion

Chronic prolongation and recurrent episodes of IBD, accompanied by the insufficient monitoring and preventive methods in the early stage, altogether influence life quality and clinical outcome of IBD patients. Currently, the pathogenesis of IBD remains unclear. It is generally believed that genetic susceptibility, infection, environmental factors, intestinal flora imbalance, and immune dysfunction are associated with the intestinal mucosal damage caused by excessive intestinal mucosal immune response in IBD patients. MiRNAs are endogenous, non-coding, small RNAs that participate in the

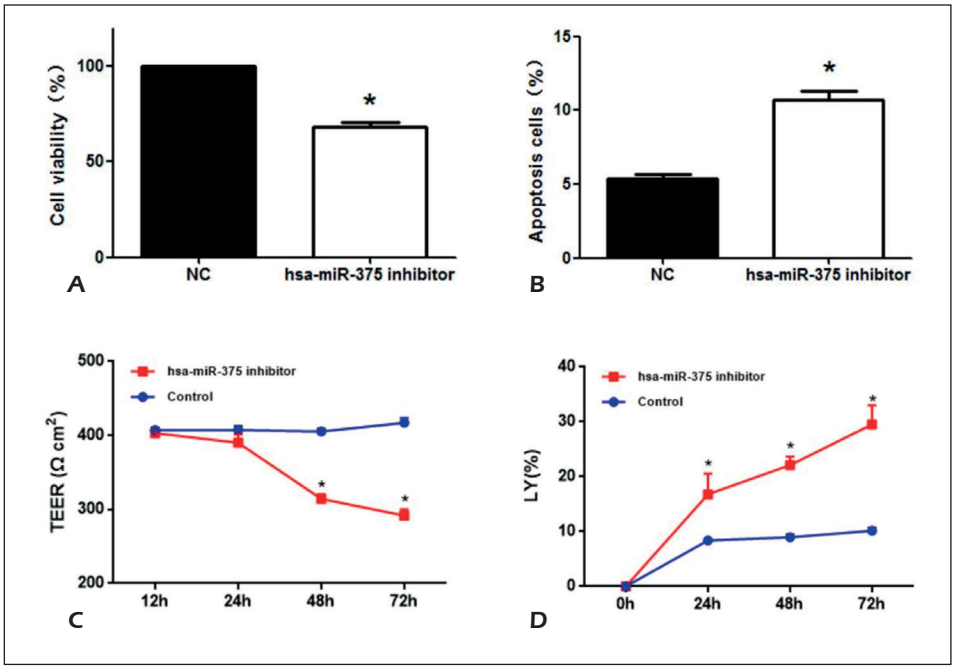

Figure 3. Hsa-miR-375 influenced viability, apoptosis and intestinal epithelial cell barrier function. ***A,*** CCK-8 assay revealed that transfection of hsa-miR-375 inhibitor decreased the viability of Caco-2 cells. ***B,*** Flow cytometry revealed that transfection of hsa-miR-375 inhibitor increased the apoptotic rate of Caco-2 cells. ***C,*** TEER decreased 48 h and 72 h in Caco-2 cells transfected with hsa-miR-375 inhibitor. ***D,*** Lucifer yellow flux increased at 24 h, 48 h and 72 h in Caco-2 cells transfected with hsa-miR-375 inhibitor.

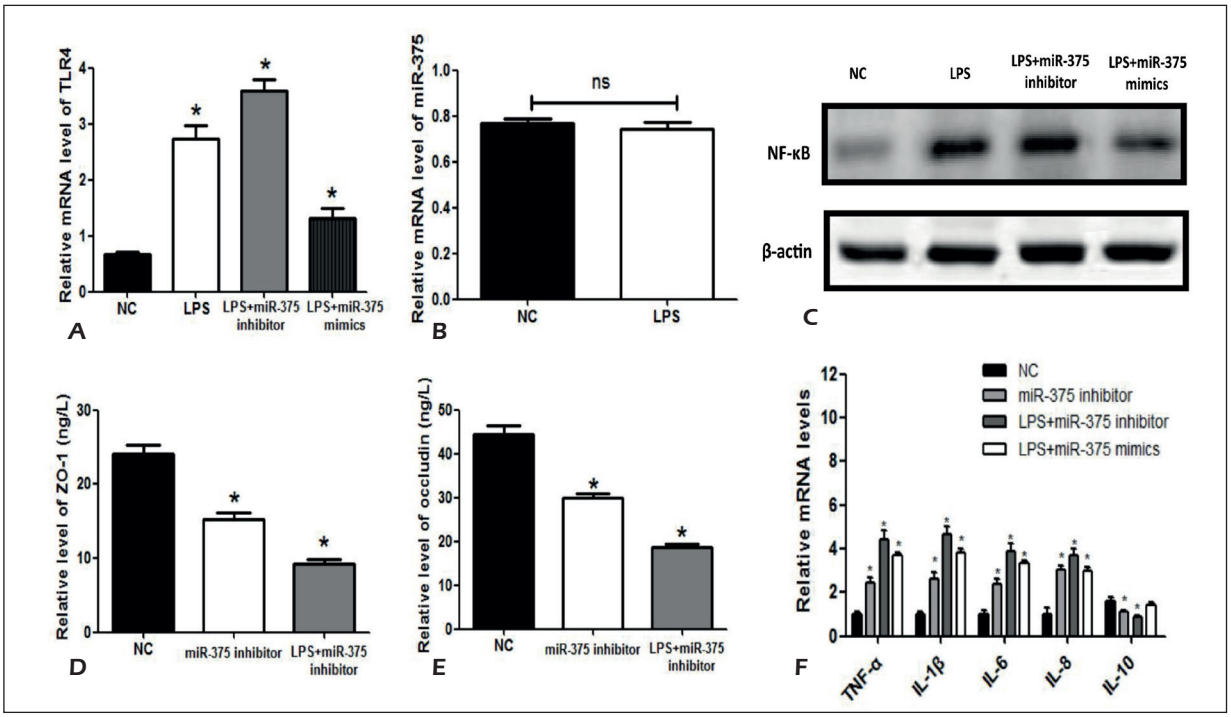

Figure 4. Hsa-miR-375 mediated TLR4/NF-κB pathway and inflammatory factors. ***A,*** The mRNA level of TLR4 in Caco-2 cells with no specific treatment, LPS induction, LPS induction+transfection of hsa-miR-375 inhibitor and LPS induction+transfection of hsa-miR-375 mimics. ***B,*** No significant difference in hsa-miR-375 level after LPS induction in Caco-2 cells. ***C,*** Protein level of NF-κB in Caco-2 cells with no specific treatment, LPS induction, LPS induction+transfection of hsa-miR-375 inhibitor and LPS induction+transfection of hsa-miR-375 mimics. ***D,*** Relative level of ZO-1 in culture medium of Caco-2 cells with no specific treatment, transfection of hsa-miR-375 inhibitor and LPS induction+transfection of hsa-miR-375 inhibitor. ***E,*** Relative level of Occludin in culture medium of Caco-2 cells with no specific treatment, transfection of hsa-miR-375 inhibitor and LPS induction+transfection of hsa-miR-375 inhibitor. ***F,*** The mRNA levels of TNF-α, IL-1β, IL-6, IL-8 and IL-10 in Caco-2 cells with no specific treatment, LPS induction, LPS induction+transfection of hsa-miR-375 inhibitor and LPS induction+transfection of hsa-miR-375 mimics.

pathophysiological processes by regulating gene expressions at post-transcriptional and translational levels. They also exert an important role in the pathogenesis of IBD[14-16]. Heinsbroek et al[13] have shown the crucial function of miRNAs in the TLR/NF-κB pathway. Expression changes of miRNAs may be closely related to the key genes in this pathway, thus leading to the development of various inflammatory diseases[13]. TLR4 is the most complex member of the TLRs family, and its ligand LPS is a component of the outer membrane of Gram-negative bacteria. TLR4 binds to LPS accompanied by the involvement of cofactors MD-2 and CD14 to activate NF-κB. Subsequently, effector cells are stimulated to secrete TNF-α and other cytokines, and thereafter induce the inflammatory response[17,18]. IBD is a systemic immune disease with a dysfunctional cytokine network, which may be related to the excessive activation of NF-κB. Abnormal increase in inflammatory cytokines and reduction of anti-inflammatory cytokines induce changes in intestinal mucosal inflammation[19,20]. IL-10 is a vital inhibitory cytokine, mainly secreted by Th cells. It is an anti-inflammatory cytokine with immunomodulatory and anti-inflammatory effects, participating in the intestinal immunity[20]. As a crucial pro-inflammatory cytokine, the major functions of IL-8 are to chemotaxis and activate neutrophils. The plasmin activity and phagocytosis of activated neutrophils exert a certain chemotactic effect on basophils and T cells[21]. It is currently believed that inflammation induced by TNF-α, IL-1β, and IL-6 is mainly mediated by chemokines, including IL-8. This study demonstrated that the lowly expressed hsa-miR-375 upregulated TLR4, which in turn induced the activation of NF-κB and promoted the expressions of pro-inflammatory factors TNF-α, IL-1β, IL-6 and IL-8, while inhibited IL-10 level.

The result revealed the protective effect of hsa-miR-375 on the intestinal epithelial barrier function, the most important immune defense barrier of the body to resist the invasion of foreign antigens and maintain the stability of the homeostasis[22]. Therefore, clarification of the structure and function of the intestinal mucosal barrier contribute to develop effective drugs to maintain and repair the intestinal mucosal barrier. The possible mechanism of hsa-miR-375 in the pathogenesis of IBD needs to be further validated in animal models to provide new ideas for the treatment of IBD.

Conclusions

Hsa-miR-375 is involved in the pathogenesis of IBD by upregulating TLR4 and inducing NF-κB activation.

Conflict of Interests

The authors declared no conflict of interest.

References

1) Sairenji T, Collins KL, Evans DV. An update on inflammatory bowel disease. Prim Care 2017; 44: 673-692.
2) Forbes JD, Bernstein CN, Tremlett H, Van Domselaar G, Knox NC. A fungal world: could the gut mycobiome be involved in neurological disease? Front Microbiol 2018; 9: 3249.
3) Ananthakrishnan AN, Bernstein CN, Iliopoulos D, Macpherson A, Neurath MF, Ali R, Vavricka SR, Fiocchi C. Environmental triggers in IBD: a review of progress and evidence. Nat Rev Gastroenterol Hepatol 2018; 15: 39-49.
4) Spagnuolo R, Cosco C, Mancina RM, Ruggiero G, Garieri P, Cosco V, Doldo P. Beta-glucan, inositol and digestive enzymes improve quality of life of patients with inflammatory bowel disease and irritable bowel syndrome. Eur Rev Med Pharmacol Sci 2017; 21: 102-107.
5) Graves P, Zeng Y. Biogenesis of mammalian microRNAs: a global view. Genomics Proteomics Bioinformatics 2012; 10: 239-245.
6) Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.
7) Liu Y, Xiong L, Zhou Y, Zheng B, Liu T, Xie W. Association of three polymorphisms rs11614913, rs2910146, and rs3746444 in miRNA-196a2, miRNA-146a, and miRNA-499 with inflammatory bowel disease: a systematic review and meta-analysis. Gastroenterol Res Pract 2018; 2018: 7295131.
8) Cheng X, Zhang X, Su J, Zhang Y, Zhou W, Zhou J, Wang C, Liang H, Chen X, Shi R, Zen K, Zhang CY, Zhang H. miR-19b downregulates intestinal SOCS3 to reduce intestinal inflammation in Crohn's disease. Sci Rep 2015; 5: 10397.
9) He C, Shi Y, Wu R, Sun M, Fang L, Wu W, Liu C, Tang M, Li Z, Wang P, Cong Y, Liu Z. miR-301a promotes intestinal mucosal inflammation through induction of IL-17A and TNF-alpha in IBD. Gut 2016; 65: 1938-1950.
10) Wang D, Wang X, Tong W, Cui Y, Li X, Sun H. Umbelliferone alleviates lipopolysaccharide-induced inflammatory responses in acute lung injury by down-regulating TLR4/MyD88/NF-kappaB signaling. Inflammation 2019 Jan 15. doi: 10.1007/s10753-018-00953-4. [Epub ahead of print]
11) Kamba A, Lee IA, Mizoguchi E. Potential association between TLR4 and chitinase 3-like 1 (CHI3L1/YKL-40) signaling on colonic epithelial cells in inflammatory bowel disease and colitis-associated cancer. Curr Mol Med 2013; 13: 1110-1121.
12) Wang H, Gu J, Hou X, Chen J, Yang N, Liu Y, Wang G, Du M, Qiu H, Luo Y, Jiang Z, Feng L. Anti-inflammatory effect of miltirone on inflammatory bowel disease via TLR4/NF-kappaB/IQGAP2 signaling pathway. Biomed Pharmacother 2017; 85: 531-540.
13) Heinsbroek SE, Squadrito ML, Schilderink R, Hilbers FW, Verseijden C, Hofmann M, Helmke A, Boon L, Wildenberg ME, Roelofs JJ, Ponsioen CY, Peters CP, Te VA, Gordon S, De Palma M, de Jonge WJ. miR-511-3p, embedded in the macrophage mannose receptor gene, contributes to intestinal inflammation. Mucosal Immunol 2016; 9: 960-973.
14) Wang S, Huang Y, Zhou C, Wu H, Zhao J, Wu L, Zhao M, Zhang F, Liu H. The role of autophagy and related microRNAs in inflammatory bowel disease. Gastroenterol Res Pract 2018; 2018: 7565076.
15) Lin XT, Zheng XB, Fan DJ, Yao QQ, Hu JC, Lian L, Wu XJ, Lan P, He XS. MicroRNA-143 targets ATG2B to inhibit autophagy and increase inflammatory responses in Crohn's disease. Inflamm Bowel Dis 2018; 24: 781-791.
16) Tili E, Michaille JJ, Piurowski V, Rigot B, Croce CM. MicroRNAs in intestinal barrier function, inflammatory bowel disease and related cancers-their effects and therapeutic potentials. Curr Opin Pharmacol 2017; 37: 142-150.
17) Shi H, Wang XL, Quan HF, Yan L, Pei XY, Wang R, Peng XD. Effects of betaine on LPS-stimulated activation of microglial M1/M2 phenotypes by suppressing TLR4/NF-kappaB pathways in N9 cells. Molecules 2019; 24: pii: E367.
18) Cochet F, Peri F. The role of carbohydrates in the lipopolysaccharide (LPS)/toll-like receptor 4 (TLR4) signalling. Int J Mol Sci 2017; 18: pii: E2318.
19) Hoesel B, Schmid JA. The complexity of NF-kappaB signaling in inflammation and cancer. Mol Cancer 2013; 12: 86.
20) DiDonato JA, Mercurio F, Karin M. NF-kappaB and the link between inflammation and cancer. Immunol Rev 2012; 246: 379-400.
21) Cabral-Santos C, de Lima JE, Fernandes I, Pinto RZ, Rosa-Neto JC, Bishop NC, Lira FS. Interleukin-10 responses from acute exercise in healthy subjects: a systematic review. J Cell Physiol 2019; 234: 9956-9965.
22) Shen Y, Zhou M, Yan J, Gong Z, Xiao Y, Zhang C, Du P, Chen Y. miR-200b inhibits TNF-alpha-induced IL-8 secretion and tight junction disruption of intestinal epithelial cells in vitro. Am J Physiol Gastrointest Liver Physiol 2017; 312: G123-G132.