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Original Research

Hsa-miRNA-31 regulates epithelial cell barrier function by inhibiting TNFSF15 expression

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Abstract: Ulcerative colitis (UC) is characterized by epithelial barrier disruption and alterations in immune regulation but with the etiology unknown. MicroRNA-31 is the most consistent differentially expressed miRNA in ulcerative colitis tissue. The aim of this project is to study the important roles of miRNA-31 in regulation of intestinal epithelial barrier function. We found that expression of miRNA-31 is proportional to the proliferation of Caco2-BBE cells and overexpression of miRNA-31 can increase its trans-epithelial resistance (TER) by decreasing the transepithelial permeability. miRNA-31 can directly bind to the 3-UTR of TNFSF15, thereafter negatively regulating its expression in Caco2-BBE cells. BrdU and TUNEL analysis demonstrated that transfection of miRNA-31 stimulates proliferation or apoptosis-resistance. Taken together, these results revealed a novel mechanism underlying the regulation of epithelial barrier function by miRNA-31 during its regulation on proliferation of epithelial cells.

Key words: TNFSF15, miRNA-31, epithelial proliferation, barrier function.

Introduction

Ulcerative colitis (UC), one of the two main forms of inflammatory bowel disease (IBD), is one relapsing, idiopathic intestinal inflammatory condition, characterized by intestinal barrier function loss and diarrhea. Intestinal epithelial cells undergo turnover every 3-5 days (1), during which cell proliferation, together with cell differentiation and migration, plays important roles in maintaining the integrity of intestinal epithelial monolayer and homeostasis of innate and adaptive immune system (2-4), evidenced by the fact that deregulated proliferation of epithelial cells is always accompanied with inflammation and tumorigenesis (5-7). Actually, over-proliferation of epithelial cells contributes to not only the pathogenesis of carcinomas but also the progression of most carcinomas toward malignancy characterized by increased cell motility and invasion, then resulting in tumor metastasis (8), and cancer-drug resistance (9). For example, kruppel-like factor 4 (Klf4) is critical for normal epithelial homeostasis and regulates goblet cell proliferation in the colon, loss of Klf4 in mice can lead to abnormal proliferation and differentiation and precancerous changes (10,11). In intestine, activation of transcription factor Oct-4 causes epithelial dysplasia by inhibiting cellular differentiation thus stimulating proliferation (12). In ulcerative colitis (UC), naïve T cells proliferate and predominately differentiate into Th2-like cells (13). Opposing effects of smoking in ulcerative colitis and Crohn's disease may attribute to its distinct differentiation or proliferation effect on dendritic cells (DC)(14). Even though these exciting advances, the underlying mechanisms involved in the deregulated proliferation of intestinal epithelium during inflammation and tumorigenesis are still largely unknown.

MicroRNAs (miRNAs), 20-23 nucleotides non-coding RNA, have reported to regulate up to the expression of 30% encoding genes by binding to specific mRNA targets and promoting their degradation and/or translational inhibition (15), thus plays important regulatory roles in various functions including proliferation in various cell types(16), such as embryonic stem cells (17), and intestinal epithelial cells (18). To explore the roles of miRNA in UC, many miRNA profiling studies were performed on different platforms using very different processing methods by different investigators. By systematic analysis (19), we found that only miRNA-31 is sorted out among all the miRNAs overexpressed in UC patients, none of others are highly consistently expressed (cut off>4), further, among the down-regulated miRNAs in UC, none of them are consistently expressed (cut off>4). But thus far, the underlying mechanism how miRNA-31 is involved in the pathogenesis is still illusive.

In this study, we employed intestinal epithelial cell line-the enterocyte-like Caco2-BBE cells to investigate how miRNA-31 is involved in pathogenesis of UC. We found that miRNA-31 can increase the overall transepithelial resistance (TER) by down-regulating the expression of TNFSF15, which further stimulate proliferation and apoptosis-resistance. Consequently, we established the concept that miRNA-31 regulates the proliferation and barrier function of Caco2-BBE cells by altering the expression of TNFSF15.

Materials and Methods

Cell culture

The human intestinal cancer cell line Caco2-BBE was grown in DMEM supplemented with 14 mM $NaHCO_3$, 10% fetal bovine serum, and 1.5 µg/ml plasmocin

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(Life technology, Carlsbad, CA, USA), at 37 °C in a 5% CO_2 atmosphere and 90% humidity.

miRNAs, plasmid construction, transfection,

Mature hsa-miRNA-31 is highly conserved in evolution with completely the RNA sequence (AGGCAAGAUGCUGGCAUAGCU). *mir*Vana® miRNA-31 mimic, Anti-miR™ miRNA-31 Inhibitor, pre-miR™ negative vector (scrambled, AM17110), and anti-miR™ negative control (scrambled, AM17010) were obtained from Thermo Fisher (Carlsbad, CA, USA). Caco2-BBE cells cultured on 6-well plastic plates, coverslips, or filter supports were transfected with 40 nM of different miRNA construct using Lipofectamine 2000 (Life technology, Carlsbad, CA, USA).

Measurement of transepithelial resistance (TER)

TER of Caco2-BBE cell, which can mirror cell proliferation and barrier function, was monitored by electric cell-substrate impedance sensing (ECIS) 1600theta device (Applied BioPhysics, Troy, NY) as described previously (20). Briefly, Cells were seeded in ECIS 8W1E electrodes (2×10^4 cells/400 µl/electrode), and resistance was measured in real-time at a frequency of 500 Hz and a voltage of 1 V.

Since TER is dominated by transepithelial permeability, we then investigated the contribution of permeability *in vitro* using a fluorescein isothiocyanate (FITC)-dextran method. Confluent and polarized Caco2-BBE cells grown on filters were treated with FITC-labeled dextrans (4-kDa, Sigma-Aldrich, St. Louis, MO) on the upper chamber for 2hrs at 37°C. The medium in the lower chamber was collected. Fluorescence intensity of each sample was measured (485Ex/520Em, Cytofluor 2300; Millipore, Waters Chromatography) and FITC-dextran concentrations were determined from standard curves generated by serial dilution of FITC-dextran.

RNA extraction and real time PCR

Total RNAs were extracted using TRIzol method (Invitrogen, Carlsbad, CA, USA) and reverse transcribed using the first-strand cDNA synthesis kit (Thermo Fisher Scientific, Waltham, MA). Real time RT-PCR was performed using an iCycler sequence detection system (Bio-Rad, Hercules, CA), with specific TNFSF15 primers (For 5' GAGGCCTGTGTGCAGTTCCA 3', Rev 5' CCTAGTTCATGTTCCCAGTGCAGA 3') and internal control GAPDH primers (For 5′ ACCACAGTCCATGCCATCAC 3′, Rev 5′ TCCACCACCCTGTTGCTGTA 3′). Real time PCR data were presented using the δ–δ cycle threshold ($\Delta\Delta C_t$) method (21).

Protein extraction and western blot analysis

Cells or tissue were re-suspended in radioimmune precipitation assay (RIPA) buffer (150 mM NaCl, 0.5% sodium deoxycholate, 50 mM Tris-HCl, pH 8, 0.1% SDS, 0.1% Nonidet P-40) supplemented with protease inhibitors (Roche Diagnostics, Indianapolis, IN), centrifuged, quantified, and subjected to western blot with relevant primary antibodies: anti-rabbit TNFSF15 (Cell Signaling Technology), anti-ALP (Santa Cruz Biotechnology), Lgr5 (Cell Signaling Technology), and monoclonal anti-GAPDH (Life technology, Carlsbad, CA, USA), followed by incubation appropriate horseradish peroxidase-conjugated secondary antibodies (Cell Signaling Technology), and blots were detected using the Enhanced Chemiluminescence Detectionkit (Amersham Biosciences).

Quantification of mature miRNA

Total RNAs were extracted with mirVana miRNA Isolation Kit (Life technology, Carlsbad, CA, USA), and polyadenylated and reverse transcribed using the NCode™ miRNA first-strand cDNA synthesis kit (Life technology, Carlsbad, CA, USA). Levels of mature miRNAs were quantified by real time PCR using specific primers, here, 18S is used as a housekeeping gene: miRNA-31: For 5'-GGAGAGGAGGCAAGATGCTGG 3'; Rev 5' GGAAAGATGGCAATATGTTGGC 3'. 18S: For 5′ CCCCTCGATGACTTTAGCTGAGT GT 3′, Rev 5′ CGCCGGTCCAAGAATTTCACCTCT 3′.

Luciferase assay

TNFSF15 mRNA 3'-UTR was cloned into the SpeI/HindIII sites of the pMIR-REPORT™ Luciferase vector (Life technology, Carlsbad, CA, USA). For luciferase assay, Caco2-BBE cells on 12-well plastic plates were transfected with 1 µg of the TNFSF15 3'-UTR-luciferase construct in the presence or absence of 20 nM the indicated miRNA constructs using Lipofectamine 2000 (Life technology, Carlsbad, CA, USA). Firefly luciferase activity was measured at 48-h post-transfection using the Dual-Luciferase Reporter Assay System (Promega, San Luis Obisop, CA, USA) and a Luminoskan Ascent luminometer (Thermo Electron Corp., Waltham, MA. USA). Values were normalized to lysate protein concentration.

Nuclear run-on assay

Nuclear run-on assay with nuclei isolated from Caco2-BBE cells was performed following the protocol described previously (22). cDNA was synthesized with the SuperScript III first-strand synthesis system (Invitrogen, Carlsbad, CA, USA) and amplified with Platinum TaqDNA polymerase (Invitrogen, Carlsbad, CA, USA), GAPDH acts as internal control, here, specific primers (TNFSF15 For, TNFSF15 Rev, GAPDH For and GAPDH Rev) are as same as the ones in real time PCR assay described previously in *Material & Methods*. The products were visualized with ethidium bromide agarose gel.

Detection of apoptosis

Apoptosis was determined using the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-FITC nick end-labeling (TUNEL) apoptosis assay kit (Roche Molecular Biochemicals, Basel, Switzerland) according to the manufacture's instructions. Briefly, confluent Caco2-BBE cells were fixed with 4% paraformaldehyde in PBS with calcium. The cells then were incubated with the TUNEL reaction mixture containing TdT and FITC-dUTP. Samples were mounted in p-phenylenediamine/glycerol (1:1) and analyzed by fluorescent microscopy (Zeiss dual-laser microscope). Hoechst 33258 (Invitrogen, Carlsbad, CA, USA) was performed on the same slides to visualize the nuclei.

Bromodeoxyuridine (BrdU) incorporation assay

Cell proliferation rate was measured by BrdU Cell Proliferation Assay Kit (Cell Signaling Technology) according to the manufacturer's instructions. The Caco2-BBE cells were fixed, washed and incubated with 10 μM BrdU for 1 h and stained with anti-BrdU antibody (Cell Signaling Technology). The cells were then incubated with anti-mouse Alexa Fluor 594-conjugated secondary antibody (Cell Signaling Technology).

Statistical analysis

Values in the current study were expressed as means±SE. Unpaired two-tailed Student's *t* test by InStat v3.06 (GraphPad) software was employed for the statistical analysis. $p < 0.05$ was considered statistically significant.

Results

Barrier function is increased during epithelial cell proliferation

The proliferation of Caco2-BBE cells was characterized by TER with ECIS technique at different time post-seeding as reported previously (20,23). Our data showed that Caco2-BBE monolayer underwent gradually and increasingly proliferation overtime, reaching a plateau after 5 days of culture as reflected by TER (Figure 1A).

The expression of miRNA-31 is positively associated with cell proliferation

After the synthesis of first-strand cDNA from tailed miRNA, the level of mature miRNA-31 in Caco2-BBE cells was quantified by real time PCR analysis. We found that the level of mature miRNA-31 was proportional to the proliferation of Caco2-BBE cell: miRNA-31 levels were higher in well-proliferated cells than in non-proliferated cells (Figure 1B). In contrast, expression of hsa-mir-626 (20), used as a control miRNA, was not significantly different between non-proliferated and well-proliferated Caco2-BBE cells (Figure 1B).

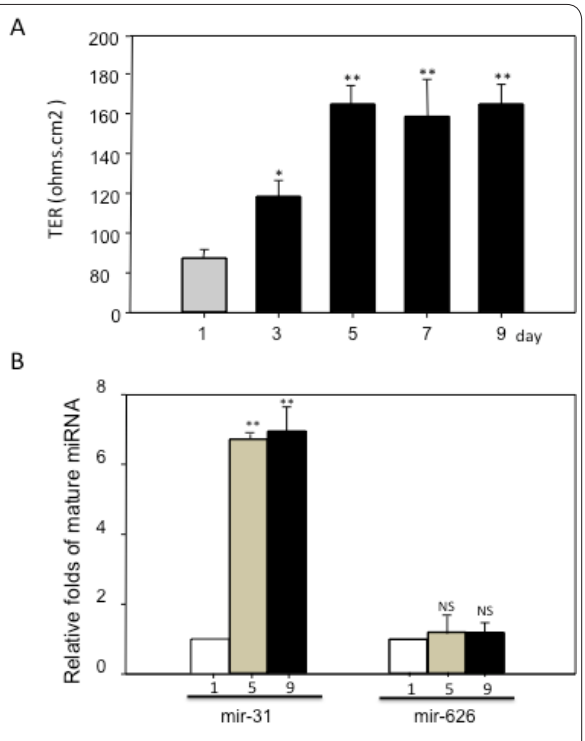

Figure 1. Expression of miRNA-31 is increased along with enhanced transepithelial resistance (TER) in intestinal epithelial cells. (A), TER of Caco2-BBE cells (2×10^4 cells/400 μl/electrode) was measured at 500 Hz, 1 V in real time using ECIS device. TER is increased post-seeding of Caco2-BBE cells, reaching to a plateau after the 5th day post-seeding. **(B)** levels of mature forms of miRNA-31 or hsa-miR-626 used as a internal control miRNA, in Caco2-BBE cells cultured on plastic plates for 1, 5, and 9 days were analyzed by real time PCR, Values represent means ± S.E. of three determinations. *, $p < 0.05$; **, $p < 0.01$.

miRNA-31 regulates barrier function

To test whether miRNA-31 is involved in epithelial barrier function, we first transfected Caco2-BBE cells with different miRNA-31 constructs (Figure 2A). By ECIS, we found that miRNA-31 mimics can increase significantly the TER in comparison with scramble RNA or negative control transfected counterparts, however, miRNA-31 specific inhibitor decreased the TER, implying that miRNA-31 can increase the epithelial TER.

To further investigate this hypothesis, we examined transepithelial permeability using the 4-kDa (Figure 2B) FITC- dextran method. As shown in Figure 2B, negative control treated cells showed a FITC-dextran flux (ng/ml/min) of 19.2±3.26. In comparison, a ~3-fold decrease in FITC-dextran flux was observed in miRNA-31-transfected cells (7.08±4.23), with no significant change in scramble RNA-transfected cells (17.19±3.2), but in miRNA-31 inhibitor transfected cells seen a significant increase (27.33±2.17).

miRNA-31 inhibits the expression of TNFSF15 at transcriptional level

By online program TargetScanHuman 7.0, it is indicated that TNFSF15 is one of the targets of miRNA-31, miRNA-31 potentially binds directly to 3UTR of TNFSF15 mRNA (Figure 3A). TNFSF15 is tumor necrosis factor ligand superfamily member 15, which is involved in apoptosis in variety of cells.

To directly examine the regulation of miRNA-31

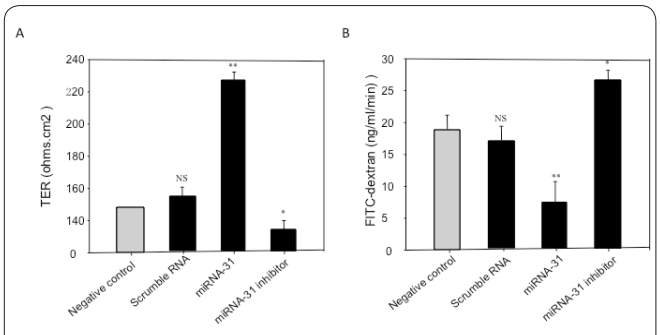

Figure 2. miRNA-31 increases the TER of Caco2-BBE cells by decreasing the permeability. (A) Transfection of miRNA-31 increased TER significantly in Caco2-BBE cells, however, overexpression of miRNA-31 inhibitor compromised TER; scramble RNA and negative control did not change the TER significantly. On the contrary, **(B)** Transfection of miRNA-31 decreased 4 kDa FITC-dextran concentration in the lower chamber significantly in Caco2-BBE cells, however, miRNA-31 inhibitor increased the concentration of FITC-dextran; scramble RNA and negative control did not change the FITC-dextran concentration significantly. Values represent means ± S.E. of three determinations. *, $p < 0.05$; **, $p < 0.01$; NS, not statistically significant.

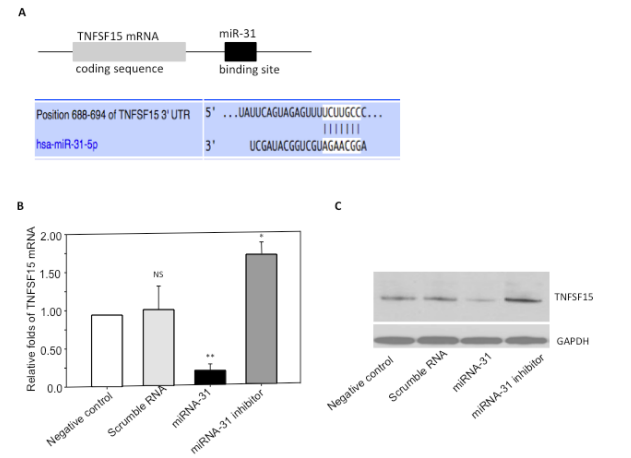

Figure 3. miRNA-31 inhibits TNFSF15 expression. (A) schematic representation of the human TNFSF15 mRNA with the 3′-UTR hsa-miRNA-31 binding site (upper panel) and sequence alignment of TNFSF15 3′-UTR miRNA-31 target site (bottom panel) predicted by miRBase. Caco2-BBE cells were transfected with 50 nM miRNA-31 mimic or the negative control scramble RNA, and miRNA-31 inhibitor. Expression of TNFSF15 was assessed by quantitative PCR **(B)** and Western blot **(C)**. Values represent means ± S.E. of three determinations. *, $p < 0.05$; **, $p < 0.01$; NS, not statistically significant.

in the expression of TNFSF15, Caco2-BBE cells were transiently transfected with different miRNA-31 constructs, and TNFSF15 expression levels were assessed by real time PCR and Western blot analyses. We found that miRNA-31 significantly decreased *TNFSF15* mRNA and protein levels in Caco2-BBE cells at 48 h post-transfection (Figure 3B and 3C). miRNA-31 inhibitor, as expected, increased the expression of TNFSF15 significantly in comparison with transfection with scramble RNA or negative control (Figure 3B and 3C).

miRNA-31 targets indirectly to the 3′-UTR of TNFSF15

To confirm the negative regulation of TNFSF15 expression by miRNA-31, TNFSF15 3′-UTR-luc, and miRNA-31 constructs were co-transfected into Caco2-BBE cells. Our data demonstrated that miRNA-31 mimic decreased significantly the luciferase activity, in the contrary, miRNA-31 inhibitor increased the luciferase value, further, miRNA-31 inhibitor restored the decreased luciferase activity from miRNA-31 mimic, compared to that no significant effect was noticed in scramble RNA and negative control treated cells (Figure 4A). This data suggested that miRNA-31 inhibitor competitively inhibits the interactions of miRNA-31 with TNFSF15 3′-UTR-luc, implying the functional specificity of miRNA and its inhibitor.

miRNA-31 inhibits the expression of TNFSF15 at transcriptional level

To determine whether the reduction of *TNFSF15* gene expression by miRNA-31 is mediated through transcriptional or posttranscriptional modification, nuclear run-on assays (Figure 4B) were performed on Caco2-BBE cells transfected with miRNA-31. Data demonstrated that overexpression of miRNA-31 significantly decreased the *TNFSF15* mRNA transcribed from nuclei *in vitro* in comparison with vector-transfect

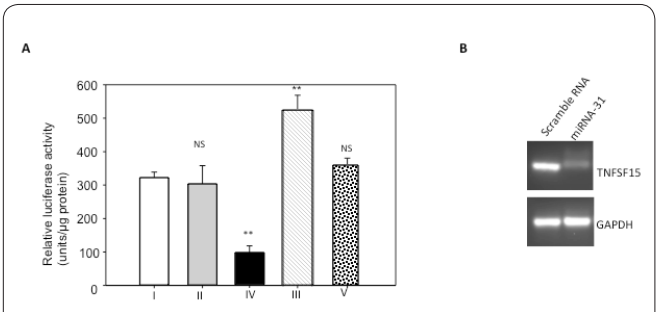

Figure 4. miRNA-31 inhibits the expression of TNFSF15 at transcriptional level. (A) Caco2-BBE cells were transfected with the 3′-UTR-luciferase reporter construct in the absence (control) or presence of 40 nM miRNA-31 constructs. Luciferase activity was measured at 2 days post-transfection and normalized to lysate protein concentration. Here, I, II, III, IV and V represents negative control; scramble RNA; miRNA-31 mimic; miRNA-31 inhibitor and miRNA-31 mimic/inhibitor antisense, respectively. **(B)** Nuclear run-on assay were performed in Caco2-BBE cells transfected with miRNA-31 mimic or scramble RNA and visualized by DNA electrophoresis. Values represent means ± S.E. of three determinations. **, $p < 0.01$; NS, not statistically significant versus control.

counterpart. This experiment was repeated three times with the similar result. Together, these findings indicate that the observed changes in TNFSF15 protein level are attributable to decreased transcriptional rate.

miRNA-31 regulates cell proliferation and apoptosis-resistance

Since TNFSF15 is involved in the cell proliferation and apoptosis, we then investigated the role of miRNA-31 in proliferation and apoptosis in Caco2-BBE cells. As showed in Figure 4A by immunostaining, overexpression of miRNA-31 significantly decreased the number of apoptotic cells in comparison with scramble RNA transfected counterparts. Further, miRNA-31 inhibitor dramatically increased the apoptosis rate. In parallel, we found that miRNA-31 mimics can significantly increase the proliferation of Caco2-BBE cells in comparison with cells transfected with negative control or scramble RNA, but the inhibitor can resume the proliferation rate to its normal level, which is consistent with previous study from another lab(24). In short, apoptosis-resistance and apoptosis may play more important roles in miRNA-31 mediated increase of barrier function.

Discussion

In the current study, we established for the first time the concept that miRNA-31 regulates the transepithelial barrier function by modulating its proliferation and apoptosis-resistance, which may shed some light on the mechanistic insight of the rapid turn over of intestinal epithelium.

It is known that proliferation is crucial for the integrity of intestinal epithelial cells, we found that miRNA-31 regulates epithelial barrier function and is predominantly expressed in proliferating cells, proportional to IEC proliferation, implying its distinct roles in differentiated and proliferating cells. Further studies showed that miRNA-31 could directly bind to 3'UTR of TNFSF15 mRNA, by which inhibits its transcription rate, which is consistent with the concept that transcriptional mecha-

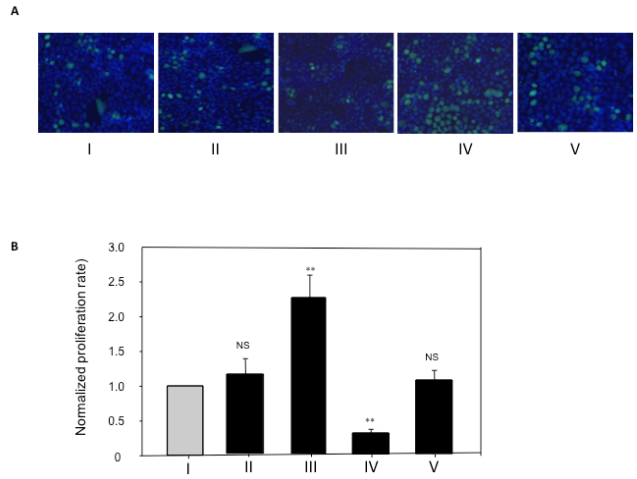

Figure 5. miRNA-31 regulates apoptosis-resistance and proliferation in Caco2-BBE cells. (A) Apoptosis was determined using TUNEL method. The cells were incubated with the TUNEL reaction mixture containing TdT and FITC-dUTP. Samples were mounted and visualized by fluorescent microscopy. Compared to negative control and scramble RNA transfected Caco2-BBE cells, miRNA-31 mimic decreases the apoptosis, but miRNA-31 inhibitor increase significantly the apoptosis rate, and co-transfection of miRNA-31 mimic and inhibitor can restore the apoptosis rate of Caco2-BBE cells. Here, I, II, III, IV and V represents negative control; scramble RNA; miRNA-31 mimic; miRNA-31 inhibitor and miRNA-31 mimic/inhibitor antisense, respectively. **(B)** Bromodeoxyuridine (BrdU) incorporation assay were performed to analyze cell proliferation state in Caco2-BBE cells transfected with miRNA-31 constructs. miRNA-31 mimic can increase dramatically the proliferation but the inhibitor decreased the proliferating cells significantly in comparison with negative control and scramble RNA transfected cells. The co-transfection of miRNA-31 mimic and inhibitor restored the proliferation rate to its normal level. Here, I, II, III, IV and V represents negative control; scramble RNA; miRNA-31 mimic; miRNA-31 inhibitor and miRNA-31 mimic/inhibitor antisense, respectively.

nisms play important roles in regulating gene expression (25) and confirmed our previous prediction that TNFSF15 is probably one target of miRNA-31 during proliferation. Further, consistent to our findings that miRNA-31 can regulate the proliferation and apoptosis, extensive evidence demonstrated that TNFSF15 is also involved in proliferation and apoptosis in different aspects, such as in HUVEC, adult bovine aortic endothelial (ABAE) cells and bovine pulmonary artery endothelial (BPAE) cells (26-29). Particularly, TNFSF15 was found to be involved in the pathogenesis of IBD and is expressed by lamina propia dendritic cells, in which it functions by increasing the proliferation of memory cells (29-31). To the best of our knowledge, it is the first time to show that miRNA-31 can regulate the intestinal barrier function by stimulating proliferation of intestinal epithelial cells mediated by TNFSF15.

miRNAs can regulate the expression of up to 30 percent of the protein-coding genes in human genome, highlighting their importance as regulators of gene expression, which further implies their intensive involvement in a variety of important biological processes like development, differentiation, auto-phagocytosis proliferation, and apoptosis (15,32,33). Besides what demonstrated in miRNA-31 in our study, many other miRNAs play important roles in proliferation of different cell types. For example, miR-362 induces proliferation and apoptosis resistance in gastric cancer cells (34); miRNA-301a promotes cell proliferation via PTEN targeting in Ewing's sarcoma cells (35). However, miR-141 inhibits cell proliferation and promotes apoptosis by targeting hepatocyte nuclear factor-3β in hepatocellular carcinoma cells (36); also, miRNA-302a suppresses tumor cell proliferation by inhibiting AKT in prostate cancer cells (37). Since many inflammations like inflammatory bowel diseases are T lymphocytes-mediated disorder, the role of miRNAs in proliferation of T cells has been recently intensively investigated; for example, miR-17~92 promotes CD4 T cell proliferation upon TCR stimulation and inhibits activation-induced cell death (AICD) (38). In human T cells miR-27b directly targets the Cyclin T1 3'UTR in resting CD4 T cells and is down-regulated with activation, thus promoting T cell proliferation (39). Interestingly, miRNA-31 has further been shown to negatively regulate FOXP3, the master regulator in regulatory T lymphocyte development and function(40), by binding directly to the 3'UTR of FOXP3 mRNA(41). These two studies indicate that miRNA-31 stimulates T lymphocytes proliferation but inhibits its differentiation into regulatory T cells, which is exactly the dominant mechanism underlying the pathogenesis of IBD, which is worth further investigating. Besides, recent studies have shown that miRNA-28 inhibits B lymphocyte proliferation (42). Thus, by the regulation of proliferation, miRNA is critical in the development of various diseases, such as miRNAs and viral diseases (43), Cardiovascular diseases (44), diabetes (45), liver diseases (46), kidney diseases (47), neurodegenerative disorders (48), generally human cancers and inflammation (49-51). Since UC is mainly lymphocyte-mediated autoimmune diseases, it would therefore be of interest to further study the mechanisms by which miRNA-31 regulates proliferation and apoptosis of lymphocytes.

Some other evidence showed that miRNA-31 is involved in intestinal disorders, for example, microarray analysis demonstrated that miRNA-31 is overexpressed in inflamed colon biopsies in UC patients (52-55), and its expression increases along with disease progression and can inhibit hypoxia inducible factor 1(HIP-1) (56), by which plays important roles in pathogenesis of IBD. One recent study found that overexpressed miRNA-31 could promote proliferation and tumor cell survival in colon cancer (24), as demonstrated in our current finding. Apart from UC, miRNA-31 is overexpressed in psoriasis and modulates inflammatory cytokine and chemokine production in keratinocytes via targeting serine/threonine kinase 40(57). In our previous work(19) by systematic analysis, miRNA-31 is overexpressed in ulcerative colitis which is supposed to lead the increase of permeability, but actually our data demonstrated that miRNA31 expression leads to decrease in permeability instead. It is known that the etiology of inflammatory bowel disease is unknown and the pathogenesis is complicated. miRNA-31, on the one hand, can decrease the permeability to increase the transepithelial barrier function, on the other hand, can haunt the foxp3+ T cell activity (40) to compromise the balance of host immunity. The onset and development of IBD is roughly the results of battle between pro-inflammatory factors and

anti-inflammatory factors.

In conclusion, miRNA-31 down-regulates TNFSF15 expression by directly targeting the 3′-UTR of TNFSF15 mRNA; miRNA-31 acts via regulating TNFSF15 expression to modulate intestinal epithelium barrier function by functioning in proliferation and/or apoptosis-resistance. Together, our study reveals a novel mechanism underlying the regulation of TNFSF15 during pathophysiological states, and raises miRNA-31 as a promising target for intestinal inflammatory disorders.

Acknowledgments

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