Role of miR-155 Inhibition in Reducing Inflammatory Responses in TNBS-Induced Colitis Models

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Abstract

Th17 cells play an important role in the immune imbalance and inflammatory state in colonic mucosa of Inflammatory Bowel Disease (IBD) and to clarify the mechanism that affect the differentiation of Th17 cells will help us find a new target for the treatment of IBD. MiR-155 which is reported to have an important role in regulating immune system function is also detected to be significantly up-regulated in colonic tissues of IBD patients. However, whether and how miR-155 affects the differentiation of Th17 cells in the colon of IBD patients is still worth studying. Here, we investigated the role of miR-155 in TNBS-induced rat colitis. Firstly, we found that the disease activity index (DAI) and Colon pathological changes were significantly reduced ($P < 0.05$) by using miR-155 inhibition sequences delivered by lentiviral vector, which revealed that miR-155 inhibition ameliorated TNBS-Induced experimental colitis. Then, we carried out flow cytometry, ELISA, qRT-PCR, and found that in TNBS+miR-155 inhibition group, the proportion of Th17 cells in spleens and mesenteric lymph nodes (MLNs) and the level of the Th17 cell-associated cytokines IL-6, IL-17A, IL-17F and IL-21 in colon tissues were significantly reduced ($P < 0.05$), which revealed that miR-155 inhibition regulated the differentiation and function of Th17 cells. Finally, we discovered that Jarid2 was significantly elevated ($P < 0.05$) by miR-155 inhibition and notch1 expression was inversely correlated with Jarid2 by using Immunohistochemistry and western blot. This study suggests that miR-155 inhibition ameliorates TNBS-induced colitis by regulating the Th17 cells differentiation and function and Jarid2/notch1 is closely related with the process.

1. Introduction

Inflammatory bowel disease (IBD), mainly comprising ulcerative colitis (UC) and Crohn's disease (CD), is a chronic non-specific inflammation of the gastrointestinal tract, which is characterized by diarrhea, rectal bleeding, abdominal pain and the passage of mucus [1]. Despite decades of research, the etiology of IBD is still not really clear. Most of the scholars think it is associated with genetics, immune responses, enteric flora, and environment factors [[1], [2], [3]]. These anomalies are closely related, and many researchers think IBD is caused by an abnormal immune response against the microbe of the intestinal flora in genetically susceptible individuals and both abnormal innate and adaptive immune pathways are associated with IBD [4]. As for the adaptive immune responses, CD has been thought to be a Th1-mediated disease, and UC has been thought to be a Th2-mediated disease for many years [5].

In recent years, a subpopulation of T lymphocytes, Th17 cells, characterized by producing abundant cytokines such as IL-17A, IL-17F, IL-21 and IL-22 and expressing the retinoic acid-related orphan receptor (ROR)-γt transcription factor [6], have got more and more attention in the pathogenesis of IBD. Numerous studies have found that Th17 cells play an important role in the development of intestinal inflammation in both IBD patients and animal models [[7], [8], [9], [10]]. Although many studies have found that the cytokines that affect the differentiation of human and mouse Th17 cells are different, but they are all regulated by a series of cytokines such as IL-6, TGF-β, IL-23, and IL-2 [11]. Furthermore, the

regulations are related with the higher order metabolic and epigenetic regulation [12]. Nevertheless, the differentiation mechanism of Th17 cells is still not yet fully clarified.

MicroRNAs (miRNAs), short non-coding RNA oligonucleotides, are closely related to the post-transcriptional regulation of target genes by pairing to the 3′ -untranslated region of their miRNAs [13]. Numerous miRNAs are associated with the causes and consequences of IBD pathogenesis [14]. MiR-155 is one of the highly expressed miRNAs in the inflamed colonic mucosa of both UC and CD patients [[14], [15], [16]]. Furthermore, miR-155 levels change dynamically during the course of the immune response and are implicated in a wide array of T cell subsets, involving Th17 cells [17], and regulation T cells [18], as well as Th1 cells [19], and Th2 cells [20]. However, the specific mechanisms by which miR-155 affects TH17 differentiation in IBD have not yet been fully understood. Interesting, notch1, one of the transmembrane receptors (Notch1–4) which coordinate a diverse set of cell fate events via interacting with ligands (Jagged 1, 2 and delta-like ligand 1, 3, 4), has been shown to affect Th17 differentiation by regulating both the IL-17 and ROR-γt promoters [21]. And Jumonji AT Rich Interactive Domain 2 (Jarid2), a DNA-binding protein, is found to regulate the expression of cytokines in Th17 cells [17,22].

Therefore, we hypothesize that miR-155 inhibition could ameliorate TNBS-Induced experimental acute colitis in rat via influencing the differentiation of Th17 cells, furthermore jarid2 and notch1 may participate in this process. In this study, we observed that the reduction expression of miR-155 could down-regulate Th17 cells differentiation and function in TNBS-induced acute colitis rat. Meanwhile, we found that miR-155 inhibition could inhibit main cytokines and a transcription factor in Th17 signaling pathway. In addition, we revealed that jarid2 and notch1 may be involved in the process of the regulation by finding that miR-155 inhibition could increase Jarid2 levels in colonic tissues but inhibit the notch1 levels on the contrary.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley (SD) rats (6–8 weeks old and specific-pathogen free (SPF)) were supplied by the Center for Disease Control of Hubei province and were housed under specific pathogen-free conditions in the experimental animal center of Huazhong University of Science and Technology (HUST, Wuhan, China).The rats were kept at room temperature (22–24 °C) and constant humidity, with 12/12 h darkness-light cycles and free access to regular laboratory chow and water. All experimental procedures in this study were conducted according to the Animal Research Institute Committee guidelines of HUST. And this study was approved by the Institutional Animal Care and Use Committee of HUST.

2.2. Lentivirus construction

The target sequence of miR-155 inhibition (Gene Bank accessionMIMAT0030409) is 5′ -ACCCCTATCACAATTAGCATTAA-3′ . Oligonucleotides encoding rno-miR-155-5p-inhibition sequences

were synthesized and annealed into double strands. The sequences of the oligonucleotides are 5′ -AATTCAAAAATTAATGCTAATTGTGATAGGGGT-3′ and 5′ -CcggACCCCTATCACAATTAGCATTAATTTTTg-3′ . Double-stranded DNAs were inserted intohU6-MCSU-biquitin-EGFP-IRES-puromycin (named GV280) which was supplied by Genechem Co. Shanghai, China. The lentivirus expression plasmid which contained positive recombinant clones were co-transfect into 293 T cells with packaging plasmids pHelper 1.0 and pHelper 2.0 (Genechem). Then the recombinant non-integrative lentiviral vectors (LV) of miR-155 inhibition (1 ∗ 10^9TU/ml) were gained from the supernatants. Before intracolonic administration, miR-155 inhibition (50 µl) and LV negative control were resuspended in polybrene and enhanced infection solution supplied by Genechem.

2.3. Model creation and sample collection

As shown in Fig. 1, after acclimating to the conditions of the center for 7 days, male SD rats were randomly divided into four Groups: normal control group (normal group, n = 8), TNBS+ polybrene and enhanced infection solution group (TNBS group, n = 8), TNBS+ miR-155 inhibition group (n = 8) and TNBS+ Lentivirus negative control group (TNBS + LV negative control group, n = 8). On day 1, each rat was lightly anesthetized by intraperitoneal injection of sodium pentobarbital (3%, 30 mg/kg body weight) after a 24 h fast. A polyethylene catheter of 2 mm external diameter was inserted 8 cm proximal to the anus of rats. In all the groups except normal group, TNBS (Sigma-Aldrich, St. Louis, MO, USA, 150 mg/kg body weight) dissolved in 0.25 ml 50% solution of ethanol, was administered into the colon through the catheter. And equal volume of PBS was administered into the colon of normal group. Then the rats were kept in the head-down position for 60s, and were placed in the Trendelenburg position to ensure distribution of TNBS throughout the whole colon after instillation. On day 3, 100 µL lentiviral vector solution of miR-155 inhibition was intracolonicaly administered into the colon of TNBS+ miR-155 inhibition group. 100 µL lentiviral vector solution of negative control was instilled intracolonicaly into TNBS+ LV negative control group. And 100 µL polybrene and enhanced infection solution was instilled intracolonicaly into TNBS group. On day 10, all the rats were sacrificed. Spleens and mesenteric lymph nodes (MLNs) were obtained for flow cytometry. The entire colon and ileum of each rat was removed, measured and then opened longitudinally. After rinsing in physiological saline to remove faecal residues, tissue samples were obtained. And then most of the samples were frozen in liquid nitrogen for the subsequent experiments, the rest were fixed in 4% paraformaldehyde for Histopathological examination.

Fig. 1

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Fig. 1. The process of animal experiment. In Day 3, TNBS+miR-155 inhibition group and TNBS+LV negative control group were intracolonicaly administered lentiviral vector solution of miR-155 inhibition and lentiviral vector solution of negative control correspondingly.

2.4. Assessment of colitis

During the last 7 days, body weights, stool occult blood and stool formation were monitored every day. The disease activity index (DAI) and the Colon Macroscopic Damage Index (CMDI) were scored in accordance with the method described previously [23]. The tissue sections were stained with hematoxylin and eosin (H&E) for histological analysis by microscopy (Nikon TE200-U, Tokyo, Japan).

2.5. In situ hybridization

In situ hybridization was performed according to the previous description [24]. Sections were hybridized with probes (rno-miR-155 and scrambled negative control probe).

2.6. Real-time polymerase chain reaction (RT-PCR) for miRNA and mRNA

The expression of miR-155 and the mRNA of Jarid2, notch1, IL-6,TGF-β1,retinoid-related orphan nuclear receptor (ROR)-γt,IL-17A, IL-17F, andIL-21 were quantified through quantitative RT-PCR according to the previous description [25]. The primers of all miRNAs and mRNAs were listed in Table 1.

Table 1. Primer sequences used for polymerase chain reaction.

Gene	Primer sequences (5′ to 3′)
U6	Reverse transcription AAAATATGGAACGCTTCACGA
	Forward CGCTTCGGCAGCACATATAC
	ReverseAAAATATGGAACGCTTCACGA
miR-155-5p	Reverse transcription GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACacccct
	Forward GGCAGGTTAATGCTAATTGTGATAG
	Reverse(RP2) CAGTGCAGGGTCCGAGGTAT
β-actin	Forward CGTTGACATCCGTAAAGACCTC
	ReverseTAGGAGCCAGGGCAGTAATCT
IL-6	Forward CTTCCAGCCAGTTGCCTTCT
	ReverseGTTTTCTGACAGTGCATCATCG
TGF-β	Forward TGGTGGACCGCAACAACG
	ReverseGGCACTGCTTCCCGAATG
Jarid2	Forward GTCGTGTTCTGCCTGGAGTG

Reverse	CTCTTGCGGGGACCTCTTT	
IL-17A	Forward	GCCCTCAGACTACCTCAACCG
Reverse	GGCTCCCTCTTCAGGACCAG	
IL-17F	Forward	CATTGGTTGGATTCTATTGCG
Reverse	GCCCTACTTTGGGGTTCCT	
IL-21	Forward	TCATCTTCTTGGGGACCGT
Reverse	ATGGCTTGAGTTTGGCTTTCT	
Notch1	Forward	ACTGCCCTCTGCCCTATACA
Reverse	GACACGGGCTTTTCACACAC	
ROR-γt	Forward	CTTCCCAATGGACGCAAAC
Reverse	TGATGGAGGGGTAGAGGTGAC	

2.7. Western blot and immunohistochemistry

Western blot and Immunohistochemistry were performed to quantify Jarid2 and notch1 protein expression levels in the colonic tissues according to the previous description [26]. Anti-activated Notch1 antibody (ab52301) was used to Western blot and Immunohistochemistry as primary antibodies to detect activated Notch1. To detect Jarid2, anti-Jarid2 antibody (ab213679) and anti-Jarid2 polyclonal antibody (BS71337) were used to Western blot and Immunohistochemistry as primary antibodies, respectively.

2.8. Enzyme-linked immunosorbent assay

The levels of IL-6, TGF-β, IL-17A, IL-17F and IL-23 in colon homogenate supernatants were measured using ELISA kits (NeoBioscience, Shenzhen, China) according to the manufacturer's instructions. The assays of these cytokines employ the quantitative sandwich enzyme immunoassay technique and the absorbance was measured at 450 nm using microplate reader.

2.9. Flow cytometry

Monocyte isolated from spleen and MLNs were first treated with ionomycin, phorbol myristate acetate (PMA) (Abcam, Cambridge, UK), and GolgiPlug protein transport inhibition (BD Biosciences, San Diego, USA) for 6 h in 5% CO2 at 37 °C. Anti-rat CD4 antibody (OX35) and anti-IL-17A antibody (eBio17B7) were used to stain cells. Stained cells were analyzed by flow cytometry.

2.10. Statistical analysis

Measurement data were presented as mean ± standard deviation (SD). Significant differences between two groups were analyzed by using unpaired two tailed Student t-test; more than two groups were analyzed by using One-way ANOVA with Bonferroni post hoc test. $P < 0.05$ was regarded as statistically significant. SPSS 20.0 was used for data analysis.

3. Result

3.1. miR-155 inhibition attenuates TNBS-induced colonic damage

The distribution and expression of miR-155 in colonic tissues of rat were examined by in suit hybridization. As showed in Fig. 2, miR-155 was characteristically distributed in colonic epithelial cells and miR-155 levels in active TNBS-induced rat were significantly higher than that in normal rat. The miR-155 inhibition was effectively taken up by epithelial and submucosal cells after intracolonic administration of the lentiviral vector (Fig. 3A). We further confirmed the relative miR-155 expression by qRT-PCR. The levels of miR-155 in TNBS-induced group were increased 2.2-fold as compared with that in normal group. However, miR-155 inhibition significantly decreased miR-155 levels ($P < 0.01$) (Fig. 3B).

Fig. 2

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Fig. 2. In situ hybridization analysis of miR-155 in rat colon. Blue violet was positive signal for miR-155 (magnification ×400, labeled by red arrow). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 3

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Fig. 3. (A) Location of miR-155 inhibition in rat colon (magnification ×100). (B) qRT-PCR analysis of miR-155. Each bar represents mean ± SD, n = 8 from each group. #$P > 0.05$, ∗$P < 0.05$, ∗∗$P < 0.01$ vs. TNBS group.

Many symptoms of all rats, such as weight and appetite loss, activity, diarrhea and bloody stools, were observed and recorded every day during the experiment. The TNBS-induced rats displayed significant weight loss, severe diarrhea and even bloody stool softer TNBS enema. The DAI score (Fig. 4A) in TNBS-induced rats increased signally as compared with that in normal rats ($P < 0.01$). However, the DAI score was gradually decreased from day 4 after intracolonic administration of the miR-155 inhibition. The shortening of colon length is a reliable marker of the damage of colon. The colon length in TNBS group reduced observably (Fig. 4C and D) as compared with that in normal group ($P < 0.01$). However, the intracolonic administration of miR-155 inhibition significantly alleviated this change (1.35-fold that of TNBS group) ($P < 0.01$). Ulceration and inflammation of colonic mucosa were observed in TNBS group. In TNBS+ miR-155 inhibition group, the damage of colonic mucosa was reduced and the macroscopic

damage score was 76% that of TNBS group (Fig. 4B).The pathophysiologic structure of rat colon was showed by hematoxylin and eosin (HE) staining. Massive mononuclear cell infiltration, mucosal erosion, goblet cell depletion, and submucosa edema were observed throughout the colons in the TNBS group. There are colonic mucosal ulcers that can be observed, and the colonic submucosa is incomplete. While, mucosal lesions in the colon of TNBS+ miR-155 inhibition group were alleviated (Fig. 5). In conclusion, these results show that miR-155 is closely linked with the colon inflammation and miR-155 inhibition has ability to alleviate colonic mucosal inflammation induced by TNBS in rat.

Fig. 4

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Fig. 4. miR-155 inhibition attenuates TNBS-induced colonic damage.(A) DAI of rat was presented. (B) CMDI was showed. (C) Colons gross appearances were showed. (D) colonic length was measured. Each bar represents mean ± SD, n = 8 from each group. #$P > 0.05$, $*P < 0.05$, $**P < 0.01$ vs. TNBS group.

Fig. 5

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Fig. 5. Hematoxylin and eosin (HE) analysis of colon specimens (magnification ×100).Massive mononuclear cell infiltration, mucosal erosion, goblet cell depletion, and submucosa edema were observed throughout the colons in the TNBS group. There are colonic mucosal ulcers, and colonic submucosa is incomplete (labeled by black arrow).

3.2. miR-155 inhibition affects Th17 cells differentiation and function

To gain an insight into the influence of miR-155 inhibition on Th17 cells differentiation, we examined the frequency of CD4+ IL17A+ cell subsets isolated from fresh spleen and MLNs. Compared with normal group, the frequency of CD4+ IL17A+ cell subsets increased 1.83-fold in spleen and 1.86-fold in MLNs in TNBS group (Fig. 2B). While, the frequency of CD4+ IL17A+ cell subsets in spleen and MLNs obtained from TNBS+ miR-155 inhibition group was lower than that from TNBS group (Fig. 6A and B). Furthermore, the differentiation of Th17 cells is affected by IL-6 and TGF-β which are associated with the activation of the transcription factor ROR-γt (Rorc). To investigate the influence of miR-155 inhibition on these cytokines and transcription factor, we examined both protein and mRNA levels of IL-6, TGF-β, and Rorc. IL-6 mRNA and protein in TNBS+ miR-155 inhibition group were lower than that in TNBS group ($P < 0.01$). However, miR-155 inhibition increased the mRNA and protein levels of TGF-β. Moreover, ROR-γt mRNA was significantly decreased due to silencing of miR-155(Fig. 6C and D).

Fig. 6

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Fig. 6. miR-155 inhibition affects Th17 cells differentiation. (A) The frequency of Th17 cells in spleen and MLNs were showed by flow cytometry. (B) Averages of frequency of Th17 cells in spleen and MLNs. (C) ELISA analysis of IL-6 and TGF-β protein expression in colon. (D) qRT-PCR analysis of IL-6, TGF-β, and ROR-γt mRNA in colon. #P > 0.05, ∗P < 0.05, ∗∗P < 0.01 vs. TNBS group.

In addition, we also assessed the level of Th17 cell-related cytokines including IL-17A, IL-17F and IL-21 in colonic tissues. Compared with normal group, both the protein and mRNA level of these cytokines were significantly increased in TNBS group, but the levels of these cytokines in TNBS+ miR-155 inhibition group were lower than that in TNBS group(Fig. 7A and B). In summary, our results indicate that miR-155 inhibition can influence the differentiation and function of Th17 cells in TNBS-induced rat colitis.

Fig. 7

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Fig. 7. miR-155 inhibition affects Th17 cells function. (A) ELISA analysis of IL-17A, IL-17F, and IL-21 protein expression in colon. (B) qRT-PCR analysis of IL-17A and TGF-17F mRNA in colon. #P > 0.05, ∗P < 0.05, ∗∗P < 0.01 vs. TNBS group.

3.3. MiR-155 and Jarid2 expression is inversely correlated in TNBS-induced colonic tissues

To assess the relationship between Jarid2 and miR-155 in TNBS-induced colonic tissues, we detected the relative level of Jarid2 protein by Immunohistochemistry and western blotting analysis. As showed in Fig. 8B, the level of Jarid2 protein in TNBS group was lower than that in normal group. However, the expression of Jarid2 increased in TNBS+ miR-155 inhibition group as compared with that in TNBS group. Furthermore, the expression of Jarid2 mRNA in TNBS-induced colonic tissues was decreased2.15-fold as compared with that in normal control tissues (Fig. 8A).However, compared with the TNBS group, the expression of the Jarid2 mRNA significantly elevated in TNBS+ miR-155 inhibition group (P < 0.01). These results show miR-155 and Jarid2 expression is inversely correlated in TNBS-induced rat.

Fig. 8

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Fig. 8. Jarid2 and notch1 participate in the process miR-155 inhibition affects Th17 cells. (A) qRT-PCR analysis of Jarid2 and notch1 mRNA in colon. (B) Western blotting analysis of Jarid2 and notch1 protein in colon and representative protein levels of Jarid2/β-actin and activated notch1/β-actin. #P > 0.05, ∗P < 0.05, ∗∗P < 0.01 vs. TNBS group.

3.4. The target of miR-155/Jarid2 axis in TNBS-induced colonic tissues may be notch1

Notch1 which is associated with the differentiation of Th17 cells [21] is reported to be regulated by Jarid2 in cardiac tissues [27]. So we also explored the relationship between Jarid2 and notch1 in TNBS-induced colonic tissues. Immunohistochemistry and western blotting analysis showed notch1 protein was increased in rat treated with TNBS compared to normal rat (Fig. 8B and Fig. 9). And the levels of notch1 mRNA in TNBS-induced group were increased1.84-fold as compared with that in normal group (Fig. 8A).However, both the protein and mRNA level of notch1 were decreased obviously in TNBS+ miR-155 inhibition group as compared with that in TNBS group($P < 0.01$). As described previously, Jarid2's expression is just the opposite of notch1. These results show the target of miR-155/Jarid2 axis in TNBS-induced colonic tissues may be notch1.

Fig. 9

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Fig. 9. Immunohistochemistry analysis of Jarid2 and activated notch1 in colon. Yellowish-brown was positive signal (magnification ×200, labeled by black arrow). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

Revealing the underlying mechanisms that regulate the homeostasis of immune system is essential to develop novel therapeutics in autoimmune diseases, such as IBD. Thus far, the ability of microRNAs to target functional genes and influence immune balance has drawn great attention from bench to bedside [28]. Though the role of miRNAs in the pathogenesis of IBD has not been broadly revealed, the role of miR-155 in regulation of immune system homeostasis is gaining increasing attention [29]. The levels of miR-155 are significantly increased in both circulating system and inflamed colonic mucosa of IBD patients [15,16,30]. Our current study showed that miR-155 significantly increased in TNBS-induced inflammatory colonic mucosa. Moreover, intracolonic administration miR-155 inhibition significantly alleviated the disease activity, the colonic damage and degree of intestinal inflammation. These data provide a delightful proof-of-concept for miR-155-based therapeutic approaches that could regulate inflammation in IBD.

There are several animal models of experimental colitis thought to resemble IBD that are used to investigate the immunopathogenesis of IBD [31]. Our understanding of mucosal immunology and the mechanisms of chronic inflammation and mucosal inflammation has significantly enhanced with the development of these animal models [32]. Intrarectal application of TNBS and oral administration of dextran sulfate sodium (DSS) are two common way to modeling the experimental colitis induced by exogenous agents. However, TNBS colitis which develops as a delayed-type hypersensitivity reaction to haptenized proteins shares pathogenic features with CD, whereas DSS colitis that is the result of a change in epithelial barrier function is more like UC [32].Just as our research team's previous study [25] in DSS-induced colitis mouse model, in TNBS-induced rat model that our current study used, miR-155 inhibition significantly relieved the colitis. And, we showed that the frequency of CD4+IL17+ cell subsets

isolated from spleen and MLNs of TNBS-induced rats was more than that of normal rats, while miR-155 inhibition had significantly reduced Th17 cells levels of spleen and MLNs. Besides, IL-6 along with combination of low concentration of TGF-β, which associated with activation of the transcription factor ROR-γt, can induce naïve CD4+T cells towards Th17 cells. Our current study revealed that miR-155 inhibition effected Th17 cells differentiation by regulating the levels of IL-6, TGF-β, and ROR-γt in colitis tissues. Furthermore, the expression of Th17 cells associated cytokines such as IL-17A, IL-17F, and IL-21 were also regulated by silencing of miR-155.These results reinforce the concept that miR-155 is closely linked with the pathogenesis of IBD by contributing to the differentiation and function of Th17 cells. And they also point out some promising miR-155-based therapeutic approaches for IBD.

Subsequently, we investigated the possible mechanisms with which miR-155 affected the differentiation of Th17 cells. By targeting many mRNAs, miRNAs can regulate various biological processes. Several studies have found that Jarid2 may be an important target of miR-155 [33,34]. Ruggiero et al. [35] had found that overexpression of miR-155-5p and the resulting downregulation of JARID2 may contribute to megakaryocyte hyperplasia in Primary myelofibrosis. Furthermore, our previous study [25] had demonstrated that miR-155 can bind to Jarid2 3′ -UTR and inhibit its expression by luciferase reporter assays. Our present study showed that the mRNA and protein levels of Jarid2 are inversely correlated with the levels of miR-155 in TNBS induced colonic tissues. All of these studies suggest that Jarid2 seems to be a novel downstream target of miR-155 in IBD.

In addition, Escobar et al. [17] link miR-155-mediated posttranscriptional regulation of Jarid2 to the transcriptional regulation of cytokine genes in Th17 cells.Jarid2 recruits PRC2 which inhibit RNA polymerase II and compact chromatin by catalyzing the repressive histone modifications H3K27me3 and H2AK119ub1 to repress transcription [22]. Escobar et al. [17] reveal that loss of miR-155 results in added Jarid2 expression, augmented PRC2 recruitment, and reduced IL22 transcription; while, in Jarid2-deficient Th17 cells, PRC2 recruitment is reduced, and the expression of a subset Jarid2 target genes is increased. This study elaborates on the mechanism that Jarid2 directly bindIl22loci. However, the effect of Jarid2 on the expression IL-17A and IL-17F is still worthy of further study. Matthew et al. [27] report that Jarid2 occupies a regulatory region at the endogenous Notch1 loci, suggesting that Jarid2 directly controls Notch1 expression in mouse embryonic heart tissue. And notch1 activated by ligands release an intracellular fragment (N1ICD) which directly binds to both ROR-γt and IL-17 promoters and regulates Th17 differentiation. Notch1, one of the four mammalian Notch receptors (Notch1, 2, 3 or 4), plays a crucial role in cell fate determination in series immune cell lineages, such as αβ versus γδ T cell differentiation [36] and CD4+ versus CD8+ T lineage decision [37]. Furthermore, the expression of notch1 and its ligand was increased in colonic tissues during the acute phase in the TNBS and DSS induced models of colitis, and transient blocking of Notch signaling ameliorates inflammation during the onset of acute colitis [38]. Our current study showed that both the mRNA and protein levels of notch1 were increased in TNBS induced acute colitis, which was inversely correlated with the levels of Jarid2. Collectively, we suppose that notch1 may be involved in the process that miR-155/Jarid2 axis affect Th17 cells differentiation in IBD.

5. Conclusion

Overall, our study revealed that miR-155 inhibition significantly ameliorated TNBS-Induced experimental colitis in rat. This result was associated with the differentiation of Th17 cells. We also showed that Jarid2 and notch1 may be participated in the process that miR-155 inhibition impact Th17 cells differentiation and function. Thus, our study pointed out some promising miR-155 inhibition-based therapeutic approaches for human IBD.

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