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MiR-155 inhibition ameliorates 2, 4, 6-Trinitrobenzenesulfonic acid (TNBS)-induced experimental colitis in rat via influencing the differentiation of Th17 cells by Jarid2

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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 [1–3]. 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 [7–10]. 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

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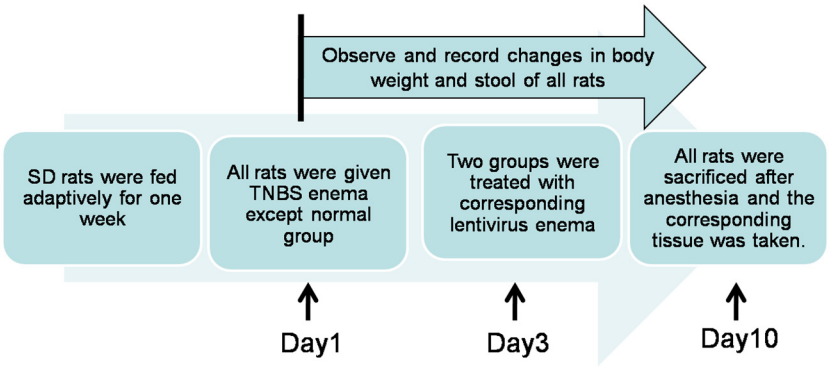

Fig. 1. The process of animal experiment. In Day 3, TNBS + miR-155 inhibition group and TNBS + LV negative control group were intracolonicaly administered lentiviral vector solution of miR-155 inhibition and lentiviral vector solution of negative control correspondingly.

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 [14–16]. 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′-AATTCAAAAATTAATGCTAATTGTGATAGG GGT-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 ($1 * 10\hat{}9$TU/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,