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Role of miR-22 in intestinal mucosa tissues and peripheral blood CD4+ T cells of inflammatory bowel disease

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ABSTRACT

Objective: miR-22 is known to be involved in the pathogenesis of several autoimmune diseases, but it remains unclear whether miR-22 is associated with inflammatory intestinal disease (IBD).
Methods: The patients with ulcerative colitis (UC) and Crohn's disease (CD) were enrolled in this study. After the CD4+ T cells from healthy controls and active IBD patients were isolated and then transfected with miR-22 mimics/inhibitors, Quantitative real-time polymerase chain reaction (qRT-PCR) was conducted to measure expressions of miR-22, HDAC4, specific transcription factors in intestinal mucosa tissue and CD4+ T cells, while enzyme-linked immuno sorbent assay (ELISA) to detect expressions of inflammatory cytokines in PB. Antisense miR-22 was administered into mice during trinitrobenzene sulphoni cacid (TNBS)-induced colitis to determine its role in IBD.
Results: A significant elevation of miR-22 but an evident decrease of HDAC4 was found in CD4+ T cells in PB and intestinal mucosa tissues from IBD patients. In addition, there was a great reduction in HDAC4 and a dramatic enhancement in Th17 cell specific transcription factor (RORC) and inflammatory cytokines (IL-17A, IL-6 and TNF-α) after overexpression miR-22, which was opposite to the effect of inhibition of miR-22. Furthermore, administration of antisense miR-22 in TNBS-induced mouse colitis model significantly decreased numbers of interleukin (IL)-17A+ CD4+ T cells and the expressions of IL-17A, RORC, IL-6 and TNF-α.
Conclusion: MiR-22 was up-regulated in CD4+ T cells in PB and intestinal mucosa tissues of IBD patients, which could promote Th17 cell differentiation via targeting HDAC4 to be involved in IBD progression.

1. Introduction

Inflammatory bowel disease (IBD) is a typical chronic relapsing inflammatory disorder occurring in the gastrointestinal tract, which was mainly including Crohn's disease (CD) and ulcerative colitis (UC) [25]. According to epidemiological survey in recent years, there has been presented a global rising trend in the incidence of IBD [21], with the characteristics of long duration, easy recurrence and treatment difficulty, seriously affecting patients' life and resulting in a certain burden to society [6,27]. Thus, it is extremely urgent to study and develop effective IBD therapies. Although the etiology and pathogenesis of IBD is still unknown, it is generally believed to be related to various factors, such as environment, genetics, microbial infection and immunity, among which the immune factors may be the most direct and important factors of IBD [9,11].

CD4+ T cells, a cell type abundant in the intestine, is the main participant in the adaptive immune response to play an immune-regulatory role in the occurrence and development of IBD [39]. On the other hand, miRNA, as a class of endogenous and non-coding single chain small molecule RNA, has been reported to be not only involved in the regulation of CD4+ T cell proliferation, differentiation and apoptosis, but also acted as a negative modulator of mRNA to be differentially expressed in autoimmune diseases, including IBD [24,30]. For example, miR-21 in the study by Wu et al. was up-regulated in IBD, and

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Table 1
Clinical characteristics of Healthy control and IBD patients.

–	Healthy control	CD(A/R)	UC(A/R)	*P*
Number of patients	15	45(25/20)	54(30/24)	–
Age(years)	35.14 ± 6.45	33.58 ± 7.36	36.51 ± 8.14	0.169
Gender				
Male	8	24(13/11)	25(14/11)	0.563
Female	7	21(12/9)	29(16/13)	
Disease duration (months)		40.25 ± 8.54	43.25 ± 9.45	0.104
Mayo scores(UC)			7.48 ± 1.31/1.26 ± 0.78	
CDAI(CD)		168.63 ± 26.25/65.53 ± 19.23		
Disease extent (UC)				
E1			10(7/3)	
E2			21(12/9)	
E3			23(11/12)	
Disease location (CD)				
L1		8(3/5)		
L2		12(7/5)		
L3		25(15/10)		
L4		0		
CRP (mg/L)	–	34.25 ± 7.58	35.69 ± 8.47	0.379

A/R: Active/Remission; CD, Crohn's disease; UC, ulcerative colitis.

the deletion of miR-21 exerted a significant function in CD4+ T-cell-mediated colitis [35]. Besides, He et al. identified miR-301a to be elevated in IBD CD4+ T cells and inflammatory mucosal tissues, which could down-regulate SNIP1 to enhance Th17 cell differentiation [14]. In terms of miR-22, a novel miRNA with an identical seed sequence from fruit fly to human [17], was observed in the study by Feng Wu and his group to be significantly up-regulated in IBD patients [36]. Moreover, miR-22, associated with immune-regulatory activity, could facilitate the differentiation of CD4+ T cells into Th17 cells in lung myeloid dendritic cells (mDCs) of smokers via inhibiting HDAC4, as indicate by Lu et al. [20], which was rare investigated in IBD patients.

Therefore, this study aims to explore miR-22 in inflammatory intestinal mucosa tissues and the peripheral blood (PB) CD4+ T cells from IBD patients, and to further investigate the regulatory role of miR-22 in the differentiation of CD4+ T cells, thereby providing a new theoretical basis for elucidating the pathogenesis of IBD.

2. Materials and methods

2.1. Ethics statement

Before experiments, all the subjects and relatives of the study were informed and singed the informed consents. This experiment got the permission from the Ethics Committee of Jingzhou Central Hospital, the Second Clinical Medical College, Yangtze University, and followed the Declaration of Helsinki [23]. And all the animals were maintained and handled following the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (PMID: 8854724).

2.2. Study subjects

This study enrolled patients with IBD who admitted at the gastroenterology department of our hospital from December 2014 to December 2016. According to diagnostic criteria issued by American College of Gastroenterology (ACG) in 2010 [10], the study included 54 UC patients (30 cases in active period and 24 cases in remission period) and 45 CD patients (25 cases in active period and 20 cases in remission period). Before enrollment, all patients did not receive following treatments like corticosteroids, immunosuppressive agents or biological agents, and were without other autoimmune diseases, infectious diseases and neoplastic diseases. The normal intestinal mucosa tissues were collected in the same period respectively from 15 patients with colonic polyps and peripheral venous blood was taken from 20 healthy examinees as Healthy Control group. The severity of diseases was assessed according to international standard criteria such as Crohn's disease activity index (CDAI) for the diagnosis of patients with CD and Mayo scores for patients with UC. All the subjects presented no significant differences in gender and age with comparability (all $P > 0.05$). The baseline characteristics of patients have been described in Table 1. During colonoscopy, 8–10 biopsies were taken to place in 10 ml phosphate buffer solution (PBS) containing 1% streptomycin and penicillin and persevered on the ice. Intestinal epithelial cells (IECs), lamina propria CD4+ T cells, B cells and dendritic cells (DCs) were isolated from normal intestinal mucosa using immune-magnetic beads obtained from Miltenyi Biotec, Bergisch Gladbach, Germany. Then miR-22 and HDAC4 expressions were analyzed by qRT-PCR.

2.3. Isolation and transfection of CD4+ T cells

The blood collection tube containing heparin anticoagulant was used to take a total of 10 ml peripheral venous blood from IBD patients and healthy controls to isolate peripheral blood mononuclear cells (PBMCs) using Ficoll-Hypaque density-gradient centrifugation (Organon Teknika, Durham, NC, USA). After isolation by magnetic beads (BD Biosciences,; the purity was generally > 95%), CD4+ T cells were incubated in human T cell culture medium (Amaxa) supplemented with 15% fetal bovine serum (FBS) and 100 g/ml penicillin G and streptomycin. CD4+ T cells from active IBD patients and Control group were classified into Blank group, negative control (NC) group, miR-22 mimics group and miR-22 inhibitors group. In order to get high efficiency, Amaxa nucleofection technology (Amaxa, Koeln, Germany) with the V-24 program was used to transfect CD4+ T cells with 3 mg miR-22 mimics/inhibitors/NC (miR-22 negative control sequence), following the instructions of manufacturer's (Human T cell Nucleofector Kit for unstimulated human T cells, Lonza).

Table 2
The primer sequences used in the detection of qRT-PCR.

	Forward (5′–3′)	Reverse (5′–3′)
miR-22	GCCTGAAGCTGCCAGTTGA	GTGCAGGGTCGAGGT
HDAC4	AGGAGAAGGGCAAAGAGAGT	GAGGGTCGCTGGAAATGC
RORC	CAATGGAAGTGGTGCTGGTTAG	GGGAGTGGGACAAGTCAAAGAT
GATA-3	CGGCAGGACGAGAAAGAGTG	TAGGGCGGGTAGGTGGTGAT
T-bet	TGTGACCCAGATGATTGTCT	TGCGTGTTGGAAGCGTTG
GAPDH	GGTGAAGGTCGGTGTGAACGGA	TGTTAGTGGGGTCTCGCTCCTG
U6	TGGGGTTATACATTGTGAGAGGA	GTGTGCTACGGAGTTCAGAGGTT