THE CORRELATION BETWEEN MIRNA-10A EXPRESSION AND SERUM TNF- A AND IL-6 LEVELS IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE AND ITS DIAGNOSTIC VALUE

YINGQI SHI*, JIANWEI QIU, JUNBO QIAN, YAMIN WANG, ZHENMING GE
Department of Gastroenterology, Nantong First People's Hospital, Nantong, PR China

ABSTRACT

Objective: *To investigate the correlation between miRNA-10a expression and serum TNF-α and IL-6 levels in patients with inflammatory bowel disease and its diagnostic value.*

Methods: *65 patients with inflammatory bowel disease treated in our hospital from May 2017 to September 2018 were selected as observation group, and other 65 patients without digestive system diseases were selected as control group. The expression of miRNA-10a in intestinal mucosa, serum and peripheral blood mononuclear cells (PBMC), serum tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6) levels were detected by fluorescence quantitative PCR (FQ-PCR). Pearson correlation test was used to analyze the correlation between miRNA-10a expression and serum TNF-α and IL-6 levels. ROC curve was used to evaluate the value of miRNA-10a in the diagnosis of inflammatory bowel disease.*

Results: *Compared with the control group, the relative expression of miRNA-10a in serum and PBMC in UC patients and CD patients in the observation group was significantly lower than that in the control group, and the levels of TNF-α and IL-6 in the serum of UC patients and CD patients in the observation group were significantly higher than those in the control group (P<0.05), and the expression of miRNA-10a was positively correlated with the levels of serum TNF-α and IL-6 (r = 0.538, 0.613, P<0.01). The results of ROC curve showed that the area under the curve of miRNA-10a was 0.891 (95%CI: 0.810~0.971), the sensitivity was 90.8%, and the specificity was 93.3%.*

Conclusion: *The expression of miRNA-10a in patients with inflammatory bowel disease is significantly higher than that in healthy patients, and is closely related to the levels of TNF-α and IL-6 in serum of inflammatory factors. The sensitivity and specificity of miRNA-10a expression in the diagnosis of IBD are higher. It may be a new target for IBD therapy.*

Keywords: *Inflammatory bowel disease, miRNA-10a, serum TNF- α, IL-6, correlation.*

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Introduction

Inflammatory bowel disease (IBD) is a common digestive system disease, and the pathogenesis is not clear, mainly including ulcerative colitis (Ulcerative, UC) and Ceohn disease (CD)[1]. Immune abnormality and genetic factors in the intestinal mucosa are the main factors of IBD. The relationship between the changes of the intestinal mucosa and the development of the continuous infection of the susceptible population is closely related to the occurrence and development of the disease[2]. The microRNA (miRNA) is a class of small-molecule non-coding single-chain RNA (miRNA) which is found in eukaryotes and has a length of about 20-25 nucleotides. MiRNA can further regulate the differentiation and function of the immune cells and improve the intestinal mucosa barrier function[3]. The expression of miRNA-10a was found in the intestinal epithelial cells of the mice with enteritis and the expression of IL-12/ IL-23 p40 in the intestinal epithelial cells of the mice[4]. In addition, the expression of the intestinal miRNA can also promote the high expression of the miR-146b and the miR-155 by affecting the IL-23 and the receptor thereof, release a plurality of inflammatory cytokines such as IL-6, TNF-α and the like, and attack the intestinal tract; and meanwhile, the inflammatory cytokine can lead to the activity of the nitric oxide synthase to be enhanced, and the intestinal inflammation reaction is aggravated[5].

The relationship between the expression of miRNA-10a and the level of TNF-α and IL-6 in the patients with inflammatory bowel disease was studied, and the application value of miRNA-10a in the diagnosis of IBD was analyzed.

Materials and methods

General Information

65 patients with inflammatory bowel disease treated in our hospital from May 2017 to September 2018 were selected as the observation group, and all the patients were in accordance with the relevant diagnostic criteria of the inflammatory bowel disease[6] developed by the Society of Digestive Diseases of the Chinese Medical Association. The study has been approved by the Ethics Committee of the Hospital, and all patients and control subjects have signed the informed consent form prior to the withdrawal. Among them,40 were UC, 24 in male, 16 in female, age (38.77±9.21) years, 25 in CD, 13 in male, 12 in female, age (34.15±7.68) years, and in the same period,65 of the patients with medical history and physical examination without digestive system disease were control group, of which 34 were male. 31 cases were female, age (41.62±9.45) years.

Exclusion criteria:

• The combination of severe liver and kidney function is not complete;

• Other autoimmune diseases and infectious diseases are combined;

• Gastrointestinal motility is used within 1 month prior to the prenatal examination, and other factors which can affect the intestinal flora and the like;

• The withdrawal of the clinical data in the middle of the study, leading to the missing of the clinical data. There was no significant difference in the average age and gender distribution of the two groups ($P > 0.05$).

Method

Specimen Collection

The samples of the peripheral blood mononuclear cells of all patients and all the patients with colonoscopy and the colon mucosa of the healthy physical examination were collected, and the fasting venous blood in the morning of the patient was extracted for 5 ml, and the supernatant was taken to be examined after centrifugation.

Main Reagents

Tissue cell culture solution, double antibody, fetal bovine serum (Beijing Life Technologies Corporation); miRcute miRNA extraction and separation kit, fluorescence detection kit, miRNA-10a and internal control primer (Beijing Tigen Biochemical Technology); reverse transcription kit (Invitrogen, USA); Human lymphocyte separator (GE Healthcare).

Determination of miRNA-10a content

The samples of all the intestinal mucosa endoscope are taken out from a -80°C refrigerator to be detected in a vacuum cup filled with liquid nitrogen, and the sample is added into the liquid nitrogen holding tissue to be hard and crisp to be sufficiently ground to the powder when the RNA is extracted. The extraction procedures of intestinal mucosa, serum and PBMC RNA are described with reference to the instructions of the kit. The RNA extracted from the intestinal mucosa specimen, the serum and the PBMC is put into a PCR instrument, and is carried out at 37°C for 60 minutes, and the miRNA reverse transcription process follows the operation of the first strand synthesis kit of the cDNA.

Determination of Inflammatory Factors

The serum TNF-α and IL-6 levels in the two groups were completed by the automatic biochemical analyzer of our hospital. The collected blood of the patients was centrifuged at 3000 rpm for 15 min, and the supernatant was taken, and the levels of TNF-α and IL-6 were detected by enzyme-linked immunosorbent assay (ELISA).

Statistical Methods

The whole study data were analyzed for statistical analysis using the SPSS20.0 software package. The standard deviation ($\bar{x} \pm s$) was used for the measurement data, and t-test was adopted between the groups; [n (%)] was used for counting the data, and the comparison between the groups was carried out by the standard deviation test. Pearson correlation analysis was used to assess the value of miRNA-10a in the diagnosis of inflammatory bowel disease by ROC, and $P<0.05$ was considered to be of statistical significance.

Results

Expression of miRNA-10a in the Tissues of Two Groups of Patients

Compared with the control group, the relative expression of miRNA-10a in the intestinal muco-

sa, serum and PBMC of patients with UC and CD decreased significantly (P<0.05), and there was no significant difference in the expression of miRNA-10a between patients with UC and CD (P> 0.05). See Table 1.

Group	n	Intestinal mucosa	Serum	PBMC
Observation group				
UC group	40	0.35±0.04*	7.02±0.52*	5.52±0.44*
CD group	25	0.41±0.04*	8.48±2.05*	4.84±0.52*
Control group	65	2.08±0.27	62.67±11.04	22.13±4.57
F		778.020	322.319	184.406
P		<0.001	<0.001	<0.001

Table. 1: Expression of miRNA-10a in the tissues of two groups of patients ($\bar{x}\pm s$).
*Note: *means P<0.05 compared with the control group.*

Comparison of Serum TNF- A and IL-6 Levels between the Two Groups

Compared with the control group, serum TNF-α and IL-6 levels in UC patients and CD patients in the observation group were significantly increased (P<0.05), while there was no statistically significant difference in serum TNF-α and IL-6 levels between UC patients and CD patients in the observation group (P>0.05). See table 2.

Group	n	TNF-α (ng/L)	IL-6 (ng/L)
Observation group			
UC group	40	61.53±4.27*	72.34±6.87*
CD group	25	60.87±4.16*	71.17±6.71*
Control group	65	22.26±2.45	29.41±2.28
F		17.886	63.931
P		<0.001	<0.001

Table. 2: Comparison of serum TNF-α and IL-6 levels between the two groups ($\bar{x}\pm s$).

Correlation Analysis between miRNA-10a Expression and Serum TNF-α and IL-6 Levels

Correlation analysis results showed that the expression of miRNA-10a was negatively correlated with serum TNF-α and IL-6 levels (r=-0.538, -0.613, P<0.01), as shown in figure 1 and 2.

Application Value of miRNA-10a Expression in Diagnosis of Inflammatory Bowel Disease

ROC curve results showed that the area under the curve for the diagnosis of IBD by miRNA-10a was 0.891 (95%CI: 0.810-0.971), the sensitivity was 90.8%, and the specificity was 93.3%, which could be used as biomarkers for the diagnosis of IBD.

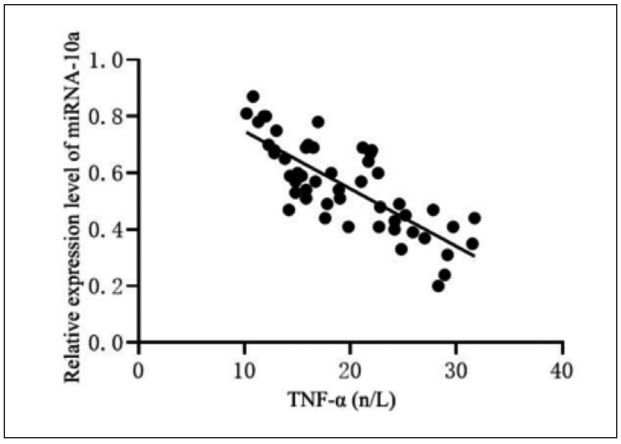

Figure 1: Trend of correlation between miRNA-10a expression and serum TNF-α.

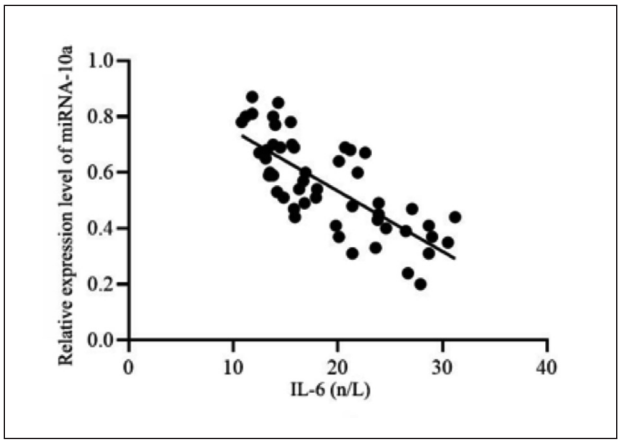

Figure 2: Trend of correlation between miRNA-10a expression and serum IL-6.

Discussion

Inflammatory bowel disease (IBD) is the common diseases of the digestive system in our country, usually involving the ileum and rectum and colon, and clinical often characterized by diarrhea, abdominal pain, some patients blood. With the further research of its pathogenesis, many scholars believe that is the susceptible factors and the combination of immune dysfunction in susceptible people. Recent research has also attached great importance to the role of intestinal epithelial cells in immune inflammation, and this effect can be reflected in the abnormal gene expression changes, and with IBD, with a better understanding of the cellular and molecular pathogenesis can through gene therapy to cure the disease completely[7-8]. MinRNA expression of certain genes have certain regulation, it is more and more used to study the pathogenesis of many diseases, including IBD, minRNA involved in immune regulation, and can further adjust the intestinal immune system and intestinal mucosal barrier func-

tion. Its role in innate and adaptive immunity has become the focus of the clinical study. MinRNA as an activity indicator established in the treatment of patients with IBD has been direction in some clinical study[9]. In this study, the expression of miRNA-10a and serum TNF-α and IL-6 levels were analyzed to explore their clinical significance in the pathogenesis of IBD.

Small RNA (minRNA) as a new kind of endogenous non-coding RNA, is the key to the gene expression regulation factor, and signal transduction and cell proliferation, differentiation and apoptosis is closely related to the process. Small RNA could have been important Bridges between the body and intestinal flora. Gut microbiota can be combined with toll-like receptor (TLR), and regulate minRNA generation[10-11]. Data shows minRNA in IBD patients exist in the peripheral blood and target organ specificity expression, its rich content in the intestinal mucosa, is the key factor of the intestinal barrier and immune regulating[12]. Since the pathogenesis of IBD is not clear, it has been shown that under the continuous action of antigen stimulation and immune regulation, the expression of mRNA is targeted to regulate and participate in the process of intestinal chronic inflammatory response[13].

Inflammatory factors play a role in regulating cell function and maintaining physiological balance of the body. Pro-inflammatory factor IL-6 is a multi-directional cytokine produced by endothelial cells and T cells. Its overexpression can affect electrolyte secretion of intestinal epithelial cells, increase cell permeability, and release a large number of neutrophils to infiltrate into inflammatory sites[14]. TNF-α is a pro-inflammatory factor secreted by activated mononuclear macrophages, which can lead to mucosal microcirculation disorder and reduce the barrier function of gastrointestinal mucosa by inducing the formation of thrombin. It has been shown that TNF-α is significantly highly expressed in the damaged intestinal mucosa, and its content gradually increases with the increase of the damaged area[15]. Some scholars have found that reducing TNF-α and IL-6 levels in mice can significantly improve the progress of ulcerative colitis in mice in animal experiments[16].

The results of this study showed that compared with the control group, the relative expression of miRNA-10a in intestinal mucosa, serum and PBMC of UC patients and CD patients in the observation group was significantly reduced ($P<0.05$). This indicates that the expression of miRNA-10a in different tissues is significantly different, and the expression of miRNA-10a in peripheral blood monocytes is significantly reduced, which indicates that the lack of expression of miRNA-10a in the immune system of the whole body further aggravates the disease progression of patients. Serum TNF-α and IL-6 levels in patients with UC and CD in the observation group were significantly higher than those in the control group ($P<0.05$). The overexpression of TNF-α and IL-6 enhances the cellular immune response and worsens the intestinal mucosal injury in INB patients. Correlation analysis results showed that miRNA-10a expression was negatively correlated with serum TNF-α and IL-6 levels ($r=-0.538$, -0.613, $P<0.01$), suggesting that miRNA-10a expression and inflammatory factors play a systematic role in mediating IBD.ROC curve results showed that the area under the curve for the diagnosis of IBD by miRNA-10a was 0.891 (95%CI: 0.810-0.971), the sensitivity was 90.8%, and the specificity was 93.3%, which could be used as the sensitivity index for clinical detection of IBD.

In summary, miRNA-10a expression in inflammatory bowel disease patients was significantly increased compared with healthy patients, and was closely correlated with serum TNF-α and IL-6 levels of inflammatory factors. The sensitivity and specificity of miRNA-10a expression in the diagnosis of IBD were both high. It can provide new ideas for the diagnosis and treatment of IBD.

References

1) Shang AJ, Yang CH, Cheng C, Tao BZ, Zhang YZ, et al. Microsurgical efficacy in 326 children with tethered cord syndrome: a retrospective analysis. Neural Regen Res 2019; 14: 72.
2) Altmann C, Schmidt MHH. The Role of Microglia in Diabetic Retinopathy: Inflammation, Microvasculature Defects and Neurodegeneration. Int J Mol Sci 2018; 19: 110.
3) Wei JC, An J. Role of micrornas in virus-host interactions. Microorganisms infect 2017; 12: 57-64.
4) Paydas S, Acikalin A, Ergin M, Celik H, Yavuz B, et al. Micro-RNA (miRNA) profile in Hodgkin lymphoma: association between clinical and pathological variables. Med Oncol 2016; 33: 34.
5) Shen F, Wang BW. Anti-inflammatory effect and mechanism of hydrolyzed whey protein on rats with inflammatory bowel disease. Guangzhou Med 2015; 46: 18-20.
6) Wu XP. 2012 consensus interpretation of diagnosis and treatment of inflammatory bowel disease in China (ulcerative colitis part). J Clin Med 2015; 32: 70-71.

7) Benchimol EI, Bernstein CN, Bitton A, Carroll MW, Singh H, et al. Trends in Epidemiology of Pediatric Inflammatory Bowel Disease in Canada: Distributed Network Analysis of Multiple Population-Based Provincial Health Administrative Databases. Am J Gastroenterol 2017; 112: 1120-1134.
8) Kanegane H. Inflammatory bowel diseases and primary immunodeficiency diseases. Gastroenterology Research and Practice 2019; 2019: 1-25.
9) Chapman CG, Pekow J. The emerging role of miRNAs in inflammatory bowel disease: a review. Therap Adv Gastroenterol 2015; 8: 4-22.
10) Cao J, Liu WQ, Yang XH, Peng YQ. Application of micrornas (mirnas) in the treatment of ulcerative colitis. Shandong J Tradit Chin Med 2017; 10: 902-905.
11) Wang S, Yan H, Zhou CL, Wu HG, Zhao JM, et al. The Role of Autophagy and Related MicroRNAs in Inflammatory Bowel Disease. Gastroenterol Res Pract 2018; 2018: 7565076.
12) Zhang J, Wang C, Wang J, Zhang F. Relationship between intestinal flora and inflammatory factors in patients with nonalcoholic steatohepatitis. Exp Ther Med 2018; 15: 723-726.
13) Wei XX, Liu ZJ, Hu Y, Du DM, Wang ZW. Correlation of the Intestinal Flora Distribution with the Serum TNF-α and IL-6 Levels in the Inflammatory Bowel Disease. Progress Mod Biomed 2017; 17: 3076-3078.
14) Wang T, Wang X, Fan HF, Guo N, Wu JF, et al. Evaluation on the Effects of Baitouweng Decoction on the Colonic Mucosa, Serum TNF-α and IL-6 in Ulcerative Colitis Mouse Model. J Liaoning Univ Tradit Chin Med 2017; 19: 34-37.
15) Li Z, Sheng L. Significance of dynamic evolution of TNF-α, IL-6 and intestinal fatty acid-binding protein levels in neonatal necrotizing enterocolitis. Exp Ther Med 2018; 15: 1289-1292.
16) Bhatti FUR, Hasty KA, Cho H. Anti-inflammatory role of TPCA-1 encapsulated nanosomes in porcine chondrocytes against TNF-α stimulation. Inflammopharmacology 2019; 19: 1-9.

Corresponding Author:
Yingqi Shi
Email: px4bkq@163.com
(China)