European Review for Medical and Pharmacological Sciences 2018; 22: 4941-4948

KFL2 participates in the development of ulcerative colitis through inhibiting inflammation via regulating cytokines

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Abstract. – OBJECTIVE: Ulcerative colitis (UC) is a kind of chronic inflammatory bowel diseases that seriously endangers human health. The pathogenesis of UC is closely related to the intestinal immune response. Cytokines exert a key role in the regulation of intestinal inflammatory and immune responses. Abnormalities in the function and quantity of various cytokines or imbalance of inflammatory factors and immune factors would lead to UC development. We aimed to investigate whether Kruppel-like transcription factor 2 (KFL2) participates in the development of ulcerative colitis by regulating inflammation, so as to provide a new direction for the clinical treatment.

PATIENTS AND METHODS: 40 UC patients were enrolled in this study, including 20 patients with mild ulcerative colitis (MUC) and 20 with severe ulcerative colitis (SUC). 20 normal end of intestinal tissues surgically resected from patients with colorectal cancer in the same period were selected as the control group. Hematoxylin-eosin (HE) staining was used to detect the inflammatory infiltration of intestinal mucosa tissues. Expressions of interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10) and tumor necrosis factor-alpha (TNF-α) in peripheral blood mononuclear cells (PBMCs) of each group were detected by qRT-PCR (quantitative Real-Time Polymerase Chain Reaction). Immunohistochemistry was performed to observe the infiltration of IL-6 and TNF-α in intestinal mucosal tissues. Protein and mRNA levels of KLF2 in PBMCs of each group were detected by Western blot and qRT-PCR, respectively. The relationship between the mRNA level of KLF2 in PBMCs and expressions of IL-6, IL-8, IL-10, TNF-α were analyzed using qRT-PCR.

RESULTS: Inflammatory cells and cytokines were infiltrated in the intestinal mucosa of UC patients. IL-6, IL-8, IL-10, and TNF-α were overexpressed in PBMCs of UC patients than those of controls. Protein and mRNA levels of KLF2 in PBMCs of UC patients were remarkably lower than those of controls, which were more significant in SUC patients. Meanwhile, KLF2 was closely related to expressions of IL-6, IL-8, IL-10, and TNF-α in PBMCs of UC patients.

CONCLUSIONS: KLF2 was downregulated in PBMCs of UC patients than that of normal controls, which participated in the inflammatory response of UC by regulating expressions of IL-6, IL-8, IL-10, and TNF-α. KLF2 may suggest new treatments for ulcerative colitis.

Key Words
KLF2, Ulcerative colitis, Inflammation.

Introduction

Inflammatory bowel disease (IBD) is a group of chronic intestinal inflammatory diseases, including chronic nonspecific ulcerative colitis (UC) and Crohn's disease (CD). The pathogenesis of UC, however, has not been fully elucidated[1]. Currently, it is believed that UC is caused by multiple factors, such as environmental, immune, and genetic factors[2-4]. Infection, diet and other environmental factors together affect genetically susceptible populations, leading to hyperactive immune response in intestine and intestinal mucosal injury[5,6]. Clinical and pathological manifestations of UC may be explained by abnormalities of intestinal mucosal immune function[7,8]. At present, autoantibodies, lymphocytes, granulocytes, macrophages, adhesion molecules and cytokines have been well studied. In particular, the imbalance between pre-inflammatory cytokines and anti-inflammatory cytokines is related to the pathogenesis of UC[9,10]. Certain cytokines play a key role in mucosal inflammation and the balance among various cytokines helps to control the inflammatory development. So far, anti-TNF monoclonal antibodies have been applied in the treatment of refractory CD and achieved certain efficacy[11]. Our study detected expressions of tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), soluble interleukin-2 receptor (sIL-2R) and other factors in peripheral blood mononuclear cells (PBMCs)

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of UC patients to further reflect the disease condition of UC, so as to provide new directions in better treatment. KLF2 is a member of the Kruppel-like transcription factor family (KLFs). KLFs are widely expressed in various tissues, which can encode transcription activators and repressors. KLFs family members possess three tandem zinc finger structures in the protein C-terminus F/Y XC-X2-4-C-X3-F-X5-L-X2-HXR/KXH, where a specific DNA sequence enriched in GC (such as the CACCC box) is bound[12]. N-terminus of KIF protein is highly variable with some contain special regions of transcriptional activators and/or repressors. In addition, N-terminus of some KIF proteins contains phosphorylation and acetylation sites that may contribute to the regulation of KLF activity[13]. KIF2 is also known as LKIF, since it is widely expressed in lung tissues. Functionally, KIF2 is involved in the quiescence, activation and migration of T cells. It was initially found that KIF2 functions on T cells to maintain $CD4^+$ or $CD8^+$ T cells in quiescence[14]. Further investigations[15] have found that overexpressed KLF2 in acute T-cell leukemia cells (Jurkat T cells) can reduce protein synthesis and expressions of surface activation markers, thus arresting cell cycle.

In addition, KIF2 is a novel transcriptional regulator that stimulates inflammatory activation of endothelial cells[16]. KIF2 increases expressions of endothelial nitric oxide synthase and nitric oxide activity in endothelial cells[17]. Under certain circumstances, KIF2 inhibits the activation of endothelial cells induced by many pro-inflammatory cytokines, including IL-1β, TNF-α, lipopolysaccharide, thrombin, pro-inflammatory molecules, vascular cell adhesion molecule 1 and E-selectin secretion[18, 19], so as to keep endothelial cells in a stable state.

Accumulating studies have shown that KLF2 is closely related to the occurrence and development of inflammation. The specific role of KLF2 in UC, however, is rarely studied. This study aimed to investigate the role of KLF2 in UC and its underlying mechanism.

Patients and Methods

Patients

A total of 40 UC patients diagnosed at Department of Anorectal, Chongqing Three Gorges Central Hospital from September 2016 to December 2017 were enrolled, including 20 patients with mild ulcerative colitis (MUC) and 20 with severe ulcerative colitis (SUC). There were 12 male and 8 female MUC patients, with 24-66 (37.2±12.0) years. There were 9 male and 11 female SUC patients, with 18-63 (37.8±10.7) years. All UC patients underwent pathological, physical, laboratory and image examinations. The disease stage was evaluated based on Witts and Truelove Standard[20]. Normal end of intestinal tissues surgically resected from 20 patients with colorectal cancer in the same period were selected as the control group, including 11 males and 9 females with 28-74 (56.8±11.7) years. All enrolled subjects did not have any viral hepatitis and other autoimmune diseases. Baseline characteristics of subjects were listed in Table I. This study was approved by the Ethics Committee of Chongqing Three Gorges Central Hospital. Signed written informed consents were obtained from the patients.

HE Staining and Immunohistochemical Staining

Five 5-μm slices were cut from each paraffin block for negative control, hematoxylin-eosin (HE) staining and immunohistochemical staining, respectively. Scores of histopathological injuries were listed in Table II. For HE staining, two pathologists examined the slices independently, and the average inflammation score of each field was calculated and recorded. For immunohistochemical staining, positive cells were expressed as brown granules in the cytoplasm or nuclei. Immunohistochemical grade was evaluated based on the proportion and intensity of staining under an optical microscope. Five typical fields without edge effect or unstructured folds were selected for calculating the amount of positive cells by two independent pathologists. Proportion score was calculated by counting 100 cells and ranged between 0-4 (0 = 0-5%, 1 = 6-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%). Intensity score was ranged between 0 and 3 (0 = no staining, 1 = light yellow, 2 = brown or tan, 3 = sepia). Positive cells were those higher than 3 scores of proportion plus intensity score.

Table I. Characteristics of the patients.

	Control group	MUC group	SUC group
N	20	20	20
Age (year)*	56.8±11.7	37.2±12.0	37.8±10.7
Male number	11(55%)	12(60%)	9(45%)

Note: *mean±SD

Table II. Scores of histopathological damage.

	Item	Score
Depth of lesion	None	0
	Submucosa	1
	Muscular layer	2
	Slurry layer	3
Range of lesion	≤25%	1
	26-50%	2
	51-75%	3
	≥76%	4
Crypt destruction	None	0
	1/3 submucosa destroyed	1
	2/3 submucosa destroyed	2
	Only epithelium was complete	3
	Crypts and epithelium were all destroyed	4
Inflammation	None	0
	Mild	1
	Severe	2

Western Blot

The total protein was extracted by the RIPA (radioimmunoprecipitation assay) lysate (Yeasen, Shanghai, China). The concentration of each protein sample was determined by a BCA (bicinchoninic acid) kit (Abcam, Cambridge, MA, USA). Briefly, total protein was separated by SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) under denaturing conditions and then transferred to PVDF (polyvinylidene difluoride) membranes (Merck Millipore, Billerica, MA, USA). Membranes were blocked with 5% skimmed milk for 1 h, followed by the incubation of specific primary antibodies (Cell Signaling Technology, Danvers, MA, USA) overnight. After washing with TBST-20 (Tris-buffered saline and Tween-20) (Yeasen, Shanghai, China) for 3 times, membranes were then incubated with the secondary antibody (Cell Signaling Technology, Danvers, MA, USA) at room temperature for 1 h. Immunoreactive bands were exposed by enhanced chemiluminescence method.

RNA Extraction and qRT-PCR (Quantitative Real-Time Polymerase Chain Reaction)

The TRIzol kit (Invitrogen, Carlsbad, CA, USA) was used to extract the total RNA, which was then reversely transcribed into complementary Deoxyribose Nucleic Acid (cDNA). After the cDNA was amplified, qRT-PCR was performed to detect the expressions of related genes. Primers used in this study were listed below. KLF2 Forward: ACAGACTGCTATTTATTGGACCTTAG, Reverse: CAGAACTGGTGGCAGAGTCATTT; glyceraldheyde 3-phosphate dehydrogenase (GADPH) Forward: TGAAGGTCGGAGTCAACGGATT, Reverse: CCTGGAAGATGGTGATGGGATT

PBMCs Extraction

2 mL of peripheral blood of UC patients and negative controls were collected and preserved in an Ethylene Diamine Tetraacetic Acid (EDTA)-Na tube. After centrifugation, PBS (phosphate-buffered saline) was mixed with plasma, followed by lysate precipitation. Cell suspension was re-suspended into 100 μL of PBS, which were PBMCs suspension.

Statistical Analysis

We used Graphpad Prism (v6.0) for statistical analysis (La Jolla, CA, USA). The quantitative data were represented as mean ± standard deviation ($\bar{x}\pm s$). The *t*-test was used for comparing differences between the two groups. One-way ANOVA was utilized to compare among different groups, followed by significance analysis through Bonferroni test. $p<0.05$ was considered statistically significant.

Results

Inflammatory Infiltration in UC

HE staining showed that the intestinal mucosa was intact and epithelial cells were continuous in paracancerous tissues. Besides, the glands were arranged regularly in a clear structure. We did not observe inflammatory infiltration of lymphocytes and phlogocytes in paracancerous tissues. On the contrary, damaged epithelium and glands, inflammatory infiltration in lamina propria, and vasodilatation of mucous membrane and submucosa were seen in UC tissues. More seriously, recess abscess and destructed recess were observed. The inflammatory contents in the crypt were released into the surrounding lamina propria (Figures 1 and 2, Table III). The results showed that UC development is closely related to inflammatory infiltration.

Effect of Cytokines on UC Development

To further explore the effect of cytokines on UC development, we detected mRNA levels of cytokines in PBMCs of UC patients by qRT-PCR.

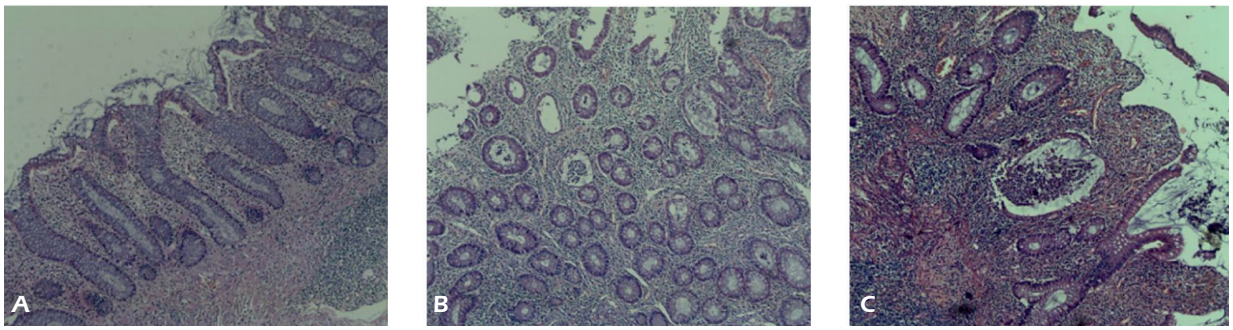

Figure 1. HE staining of inflammatory infiltration in UC (100×). Lesion depth, range, inflammatory cell number, fiber cells, crypt destruction and goblet cell death of intestinal mucosa in controls (**A**), MUC patients (**B**) and SUC patients (**C**).

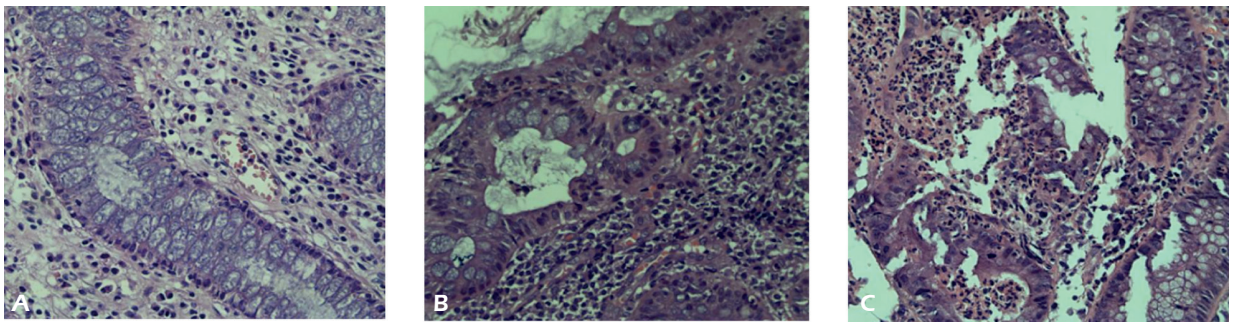

Figure 2. HE staining of inflammatory infiltration in UC (400×).

IL-6, IL-8, IL-10 and TNF-α were overexpressed in PBMCs of UC patients than those of controls, which were more significant in SUC patients (Figure 3). Besides, immunohistochemical-staining results demonstrated that the proportion and intensity of TNF-α (Figure 4) and IL-6 (Figure 5) in UC tissues were higher than those of controls. The positive rate of TNF-α in controls, MUC and SUC was 10.0% (2/20), 50.0% (10/20) and 85.0% (17/20), respectively (Table IV). The positive rate of IL-6 in controls, MUC and SUC was 5.0% (1/20), 45.0% (9/20) and 75.0% (15/20), respectively (Table V). The data further indicated that cytokines are closely related the occurrence and development of UC.

Table III. HI scores of intestinal mucosa in each group.

Group	HI	F	*p*
Control group	1.43±0.64	154.496	0.001
MUC group	5.68±0.52*		
SUC group	8.27±0.79*		

Note: *$p<0.05$ compared with control group; $^{\Delta}p<0.05$ compared with mild UC group.

KLF2 Participated in UC Development

Previous studies have elucidated that KLF2 is involved in inflammatory activation of endothelial cells. In the present study, we found that both mRNA (Figure 6A) and protein (Figure 6B) levels of KLF2 were decreased in PBMCs of UC patients than those of controls. Moreover, SUC patients expressed the lowest expression of KLF2, suggesting that KLF2 exerted a crucial role in UC development, which was negatively correlated with the inflammatory level.

KLF2 Regulated Inflammatory Response in UC

We next explored whether KLF2 participated in UC development *via* regulating expression levels of cytokines. The relationship between KLF2 and inflammatory factors (IL-6, IL-8, IL-10, TNF-α) in PBMCs were analyzed by qRT-PCR. The data showed that KLF2 negatively regulated expressions of IL-6, IL-8, IL-10 and TNF-α in PBMCs of UC patients, which were more significant in SUC patients (Table VI). The above results confirmed that KLF2 participated in UC development *via* regulating cytokines, providing new targets for better prediction and treatment of UC.

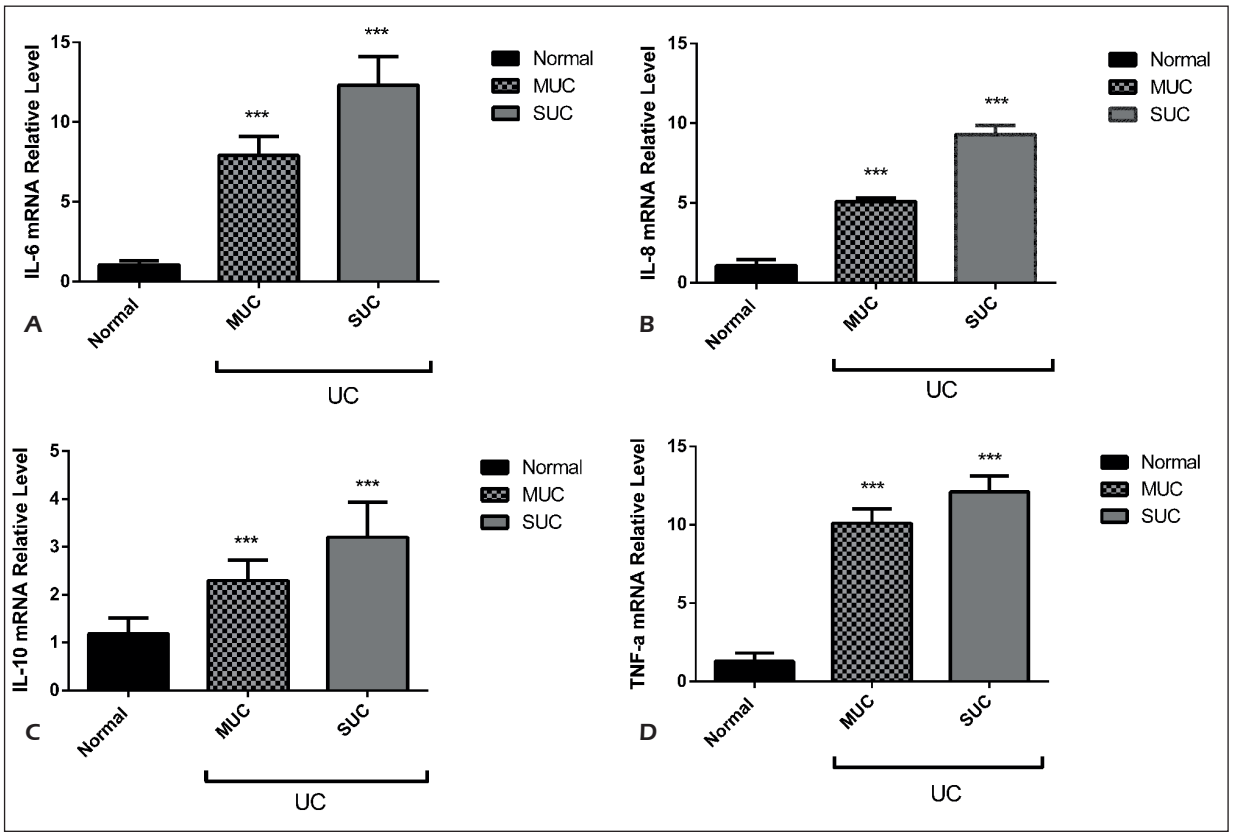

Figure 3. The mRNA levels of IL-6, IL-8, IL-10 and TNF-α in PBMCs of controls, MUC patients and SUC patients (***p<0.001).

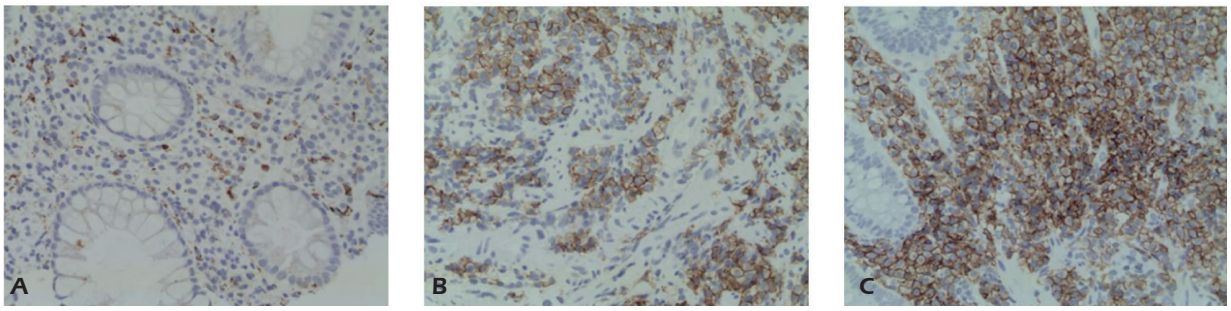

Figure 4. Immunohistochemical staining of TNF-α in intestinal mucosa tissues of controls, MUC patients and SUC patients (400×).

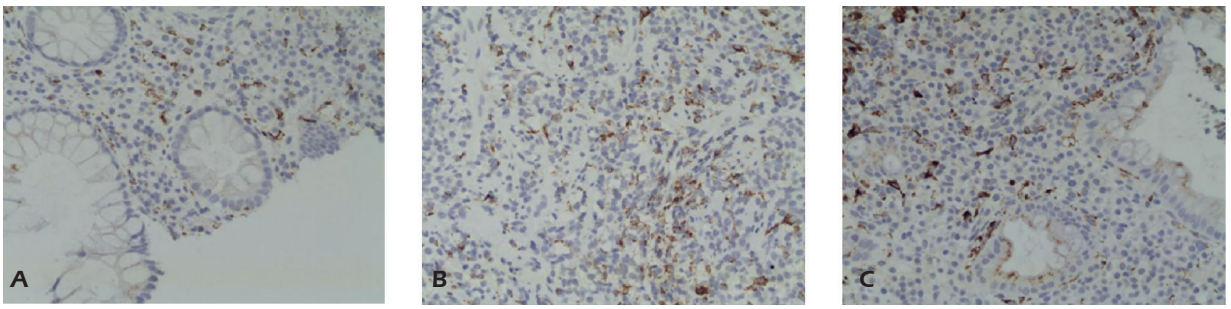

Figure 5. Immunohistochemical staining of IL-6 in intestinal mucosa tissues of controls, MUC patients and SUC patients (400×).

Table IV. Expression of TNF-α in intestinal epithelium.

Group	n	Positive number	χ^2	*p*
Control group	20	2		
MUC group	20	10*	7.619	<0.01
SUC group	20	17*$^{\Delta}$	22.556	<0.01

Note: *p<0.05 compared with control group; $^{\Delta}$ p<0.05 compared with mild UC group.

Table V. Expression of IL-6 in intestinal epithelium.

Group	n	Positive number	χ^2	*p*
Control group	20	1		
MUC group	20	9*	8.533	<0.01
SUC group	20	15*$^{\Delta}$	20.417	<0.01

Note: *p<0.05 compared with control group; $^{\Delta}$ p<0.05 compared with mild UC group.

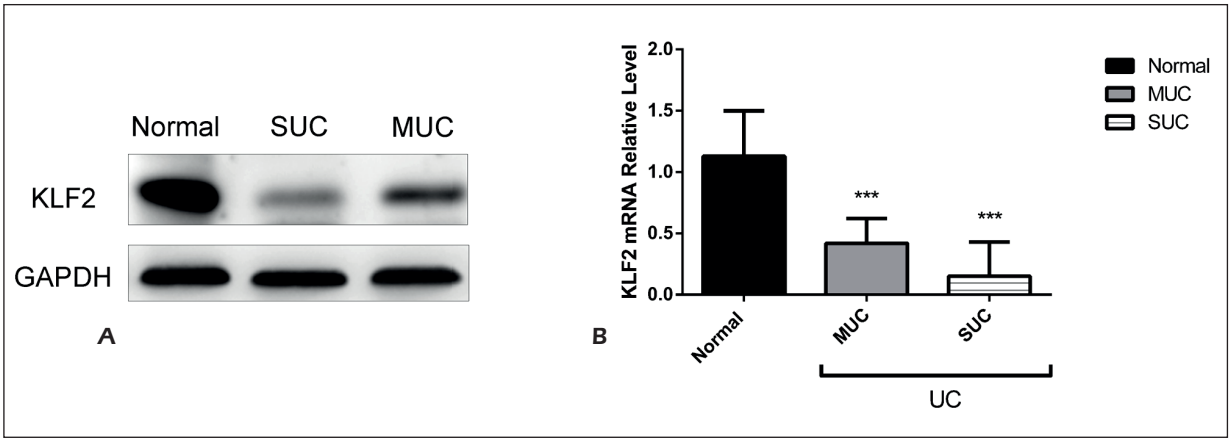

Figure 6. Effect of KLF2 on UC development. Protein (**A**) and mRNA (**B**) expressions of KLF2 in PBMCs of controls, MUC patients and SUC patients (***p<0.001).

Discussion

Cytokines have been well applied in treating UC, including administration of pro-inflammatory cytokine antagonist, anti-inflammatory cytokine antibody, and recombinant anti-inflammatory cytokine. Animal experiments have showed that anti-IL-8 antibodies are effective in preventing immune complex-induced colitis[21]. Nowadays, anti-TNF-α monoclonal antibodies have been used in the treatment of Crohn's disease. Experimental studies of IL-lra and IL-12 antagonists determined promising results in clinical application. Other studies have pointed out that IL-10 can reduce acute colitis caused by immune complexes, which can be used in improving intestinal inflammation of DSS-induced (dextran sulfate sodium) colitis.

TNF-α is mainly produced by lipopolysaccharide-activated mononuclear macrophages. It has a wide range of biological activities that participates in immune responses, body metabolism and inflammation. TNF-α is generally recognized as a cytokine that mediates UC. Relative researches have shown that TNF-α expression in UC is higher than that of the colon mucosa without involvement[22].

IL-6 is a widely expressed pro-inflammatory cytokine. A series of studies have found that serum IL-6 levels in UC patients are remarkably elevated and positively correlated with the extent and severity of UC[23]. Grottup et al[24] reported that mRNA and protein expressions of IL-6 in mucous membranes of patients with active UC were higher than those of healthy mucous tissues. IL-6 expression was positively correlated with the inflammation grade. These studies all demonstrated that IL-6 exerts an essential role in UC pathogenesis and can be used to monitor disease activity and therapeutic effects.

Table VI. Relationship between mRNA level of KLF2 and cytokines in UC patients.

	Cardiac marker	r	p
SUC group	IL-6	-0.711	0.000
	IL-8	-0.681	0.017
	IL-10	-0.692	0.021
	TNF-a	-0.726	0.005
MUC group	IL-6	-0.805	0.000
	IL-8	-0.713	0.012
	IL-10	-0.722	0.031
	TNF-a	-0.821	0.000

In our work, inflammatory infiltration was significant in UC tissues. Meanwhile, cytokines expressions in PBMCs of UC patients were also remarkably higher than those of controls, which were more remarkable in SUC patients, all indicating that inflammation and cytokines are closely related to UC development.

KLF2 is a member of the zinc finger Kruppel-like transcription factor family (KLFs) and is widely expressed in lung, spleen, heart, skeletal muscle, pancreas and placenta. KLF2 encodes transcriptional activators and repressor proteins[25]. Since it was originally found to be mainly expressed in the lung, it is also called lung Kruppel-like transcription factor (LKLF). KLF2 is involved in the development of lungs and blood vessels, as well as survival, quiescence and migration of mature T-cells[26]. Downregulated KIF2 leads to rapid secretion of cytokines such as IL-1β, IL-8, TNF-α and monocyte chemotactic protein-1 (MCP-1)[27]. KLF2 exerts a significant anti-inflammatory effect on lung inflammatory diseases[28]. Inflammation can upregulate KLF2, and KLF2 in turn inhibits inflammatory progress. However, KLF2 expression is reduced in chronic lung diseases.

Therefore, we detected KLF2 expressions in PBMCs of UC patients and normal controls. The results indicated that UC patients expressed lower KLF2 expression than that of controls, which was more significant in SUC patients. It is concluded that KLF2 exerts a crucial in the occurrence and development of UC.

Previous studies have already demonstrated the important role of cytokines in UC. We therefore speculated whether KLF2 deficiency would lead to abnormal expressions of cytokines, so as to stimulate inflammation. After detecting mRNA levels of KLF2 and relative cytokines, it was found that KLF2 negatively regulates expression levels of IL-6, IL-8, IL-10 and TNF-α, further suggesting the importance of KLF2 in UC development.

To sum up, KLF2 was downregulated in UC patients, which could be served as a monitor in evaluating disease condition. KLF2 was expected to be a new hallmark of preventing inflammation in UC.

Conclusions

We observed that KLF2 was downregulated in PBMCs of UC patients and participated in the inflammatory response of UC via regulating expressions of IL-6, IL-8, IL-10 and TNF-ich could be served as ainsights in the treatment of ulcerative colitis.

Conflict of Interests

The authors declared no conflict of interest.

References

1) Price AB. Overlap in the spectrum of non-specific inflammatory bowel disease--'colitis indeterminate'. J Clin Pathol 1978; 31: 567-577.
2) van Heel DA, Satsangi J, Carey AH, Jewell DP. Inflammatory bowel disease: progress toward a gene. Can J Gastroenterol 2000; 14: 207-218.
3) Laterza L, Piscaglia AC, Lecce S, Gasbarrini A, Stefanelli ML. Onset of ulcerative colitis after thyrotoxicosis: a case report and review of the literature. Eur Rev Med Pharmacol Sci 2016; 20: 685-688.
4) Moore K, Walters MT, Jones DB, Garvey E, Harvey J, Cawley MI, Smith JL. An immunohistological study of CD4+ lymphocyte subsets within inflammatory lesions with special reference to rheumatoid arthritis and inflammatory bowel disease. Immunology 1988; 65: 457-463.
5) Mueller C, Knoflach P, Zielinski CC. T-cell activation in Crohn's disease. Increased levels of soluble interleukin-2 receptor in serum and in supernatants of stimulated peripheral blood mononuclear cells. Gastroenterology 1990; 98: 639-646.
6) Mullin GE, Maycon ZR, Braun-Elwert L, Cerchia R, James SP, Katz S, Weissman GS, McKinley MJ, Fisher SE. Inflammatory bowel disease mucosal biopsies have specialized lymphokine mRNA profiles. Inflamm Bowel Dis 1996; 2: 16-26.
7) Strober W, Kelsall B, Fuss I, Marth T, Ludviksson B, Ehrhardt R, Neurath M. Reciprocal IFN-gamma and TGF-beta responses regulate the occurrence of mucosal inflammation. Immunol Today 1997; 18: 61-64.
8) Schmitz H, Barmeyer C, Fromm M, Runkel N, Foss HD, Bentzel CJ, Riecken EO, Schulzke JD. Altered tight junction structure contributes to the impaired epithelial barrier function in ulcerative colitis. Gastroenterology 1999; 116: 301-309.
9) Xia B, Crusius J, Meuwissen S, Pe AA. Inflammatory bowel disease: definition, epidemiology, etiologic aspects, and immunogenetic studies. World J Gastroenterol 1998; 4: 446-458.
10) Anand AC, Adya CM. Cytokines and inflammatory bowel disease. Trop Gastroenterol 1999; 20: 97-106.
11) Baert FJ, Rutgeerts PR. Anti-TNF strategies in Crohn's disease: mechanisms, clinical effects, indications. Int J Colorectal Dis 1999; 14: 47-51.
12) Ruppert JM, Kinzler KW, Wong AJ, Bigner SH, Kao FT, Law ML, Seuanez HN, O'Brien SJ, Vogelstein B. The GLI-Kruppel family of human genes. Mol Cell Biol 1988; 8: 3104-3113.
13) Anderson KP, Kern CB, Crable SC, Lingrel JB. Isolation of a gene encoding a functional zinc finger protein homologous to erythroid Kruppel-like factor: Identification of a new multigene family. Mol Cell Biol 1995; 15: 5957-5965.

14) Kuo CT, Veselits ML, Leiden JM. LKLF: a transcriptional regulator of single-positive T cell quiescence and survival. Science 1997; 277: 1986-1990.

15) Buckley AF, Kuo CT, Leiden JM. Transcription factor LKLF is sufficient to program T cell quiescence via a c-Myc-dependent pathway. Nat Immunol 2001; 2: 698-704.

16) Lin Z, Kumar A, SenBanerjee S, Staniszewski K, Parmar K, Vaughan DE, Gimbrone MJ, Balasubramanian V, Garcia-Cardena G, Jain MK. Kruppel-like factor 2 (KLF2) regulates endothelial thrombotic function. Circ Res 2005; 96: e48-e57.

17) Dekker RJ, Boon RA, Rondaij MG, Kragt A, Volger OL, Elderkamp YW, Meijers JC, Voorberg J, Pannekoek H, Horrevoets AJ. KLF2 provokes a gene expression pattern that establishes functional quiescent differentiation of the endothelium. Blood 2006; 107: 4354-4363.

18) Parmar KM, Larman HB, Dai G, Zhang Y, Wang ET, Moorthy SN, Kratz JR, Lin Z, Jain MK, Gimbrone MJ, Garcia-Cardena G. Integration of flow-dependent endothelial phenotypes by Kruppel-like factor 2. J Clin Invest 2006; 116: 49-58.

19) SenBanerjee S, Lin Z, Atkins GB, Greif DM, Rao RM, Kumar A, Feinberg MW, Chen Z, Simon DI, Luscinskas FW, Michel TM, Gimbrone MJ, Garcia-Cardena G, Jain MK. KLF2 is a novel transcriptional regulator of endothelial proinflammatory activation. J Exp Med 2004; 199: 1305-1315.

20) Baumgart DC. The diagnosis and treatment of Crohn's disease and ulcerative colitis. Dtsch Arztebl Int 2009; 106: 123-133.

21) Braunstein J, Qiao L, Autschbach F, Schurmann G, Meuer S. T cells of the human intestinal lamina propria are high producers of interleukin-10. Gut 1997; 41: 215-220.

22) Ishiguro Y. Mucosal proinflammatory cytokine production correlates with endoscopic activity of ulcerative colitis. J Gastroenterol 1999; 34: 66-74.

23) Holtkamp W, Stollberg T, Reis HE. Serum interleukin-6 is related to disease activity but not disease specificity in inflammatory bowel disease. J Clin Gastroenterol 1995; 20: 123-126.

24) Grottrup-Wolfers E, Moeller J, Karbach U, Muller-Lissner S, Endres S. Elevated cell-associated levels of interleukin 1beta and interleukin 6 in inflamed mucosa of inflammatory bowel disease. Eur J Clin Invest 1996; 26: 115-122.

25) Huddleson JP, Ahmad N, Srinivasan S, Lingrel JB. Induction of KLF2 by fluid shear stress requires a novel promoter element activated by a phosphatidylinositol 3-kinase-dependent chromatin-remodeling pathway. J Biol Chem 2005; 280: 23371-23379.

26) Bu DX, Tarrio M, Grabie N, Zhang Y, Yamazaki H, Stavrakis G, Maganto-Garcia E, Pepper-Cunningham Z, Jarolim P, Aikawa M, Garcia-Cardena G, Lichtman AH. Statin-induced Kruppel-like factor 2 expression in human and mouse T cells reduces inflammatory and pathogenic responses. J Clin Invest 2010; 120: 1961-1970.

27) Cullingford TE, Butler MJ, Marshall AK, Tham EL, Sugden PH, Clerk A. Differential regulation of Kruppel-like factor family transcription factor expression in neonatal rat cardiac myocytes: effects of endothelin-1, oxidative stress and cytokines. Biochim Biophys Acta 2008; 1783: 1229-1236.

28) Panigrahi S, Freeman ML, Funderburg NT, Mudd JC, Younes SA, Sieg SF, Zidar DA, Paiardini M, Villinger F, Calabrese LH, Ransohoff RM, Jain MK, Lederman MM. SIV/SHIV infection triggers vascular inflammation, diminished expression of kruppel-like factor 2 and endothelial dysfunction. J Infect Dis 2016; 213: 1419-1427.