

Critical roles of RGS16 in the mucosal inflammation of ulcerative colitis

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Background Ulcerative colitis is a chronic and progressive inflammatory disorder. The regulator of the G-protein signaling (RGS) is involved in the pathogenesis of several immune system disorders. RGS16, a member of the RGS protein superfamily, has been shown to play critical roles in several immune system-related diseases. However, the roles of RGS16 in ulcerative colitis remain to be elucidated.

Methods We analyzed the expression of RGS16 in peripheral blood mononuclear cells (PBMCs) and inflamed mucosa of ulcerative colitis patients using quantitative reverse transcription-PCR, western blotting and immunohistochemistry. We performed Spearman's correlation to analyze the correlation between RGS16 expression and the ulcerative colitis endoscopic index of severity (UCEIS), Mayo index, erythrocyte sedimentation rate (ESR) and serum tumor necrosis factor alpha (TNF- α) and IL-17A levels. Further, PBMCs were stimulated with inflammatory cytokines *in vitro*.

Results RGS16 expression significantly increased in the colonic mucosa and PBMCs from patients with ulcerative colitis and significantly correlated with the Mayo index, UCEIS, ESR and serum TNF- α and IL-17A levels. TNF- α upregulated RGS16 expression in PBMCs in a dose- and time-dependent manner via the nuclear factor kappa beta (NF- κ B) signaling pathway. Moreover, anti-TNF treatment with infliximab significantly decreased RGS16 expression in PBMCs and intestinal mucosa of patients with ulcerative colitis.

Conclusion Our study revealed a novel mechanism by which RGS16 expression in ulcerative colitis is positively correlated with disease activity. Thus, RGS16 might serve as a potential therapeutic marker for the treatment of ulcerative colitis. *Eur J Gastroenterol Hepatol* 34: 993–999

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Introduction

Ulcerative colitis, a subtype of inflammatory bowel disease (IBD), is a chronic, relapsing intestinal inflammatory disease of the colorectum, with a high prevalence among the American, European and Asian populations [1,2]. Although the pathogenesis of ulcerative colitis is a result of abnormal activation of the intestinal mucosal immune system in response to commensal bacteria in a genetically susceptible host [3–5], the comprehensive etiology of ulcerative colitis is still unclear. At present, therapeutic strategies to treat ulcerative colitis involve suppressing inflammation and repressing the abnormal activation of the immune response and include 5-aminosalicylic acid (5-ASA), immunosuppressants and biologics [6,7]. However, some patients with ulcerative colitis still do not

achieve clinical remission after treatment. Hence, there is an urgent need for more research on the pathogenesis and effective treatment of ulcerative colitis.

To date, genome-wide association studies (GWAS) have identified several genetic risk loci in patients with ulcerative colitis, including genes associated with epithelial barrier function, innate and adaptive immune responses, autophagy and microbial defense pathways [8–11]. The G-protein coupled receptor (GPCR) signaling modulates B and T lymphocyte chemotaxis and entry and exit from the lymph nodes and thymus [12]. The regulator of the G-protein signaling (RGS) family is an important and ubiquitous effector that mediates the strength and duration of GPCR signaling. RGS16, also known as A28-RGS14 or RGS-r, belongs to the B/R4 subfamily of the RGS proteins. Although initially cloned from the retina, RGS16 is widely expressed in several tissues, including the pituitary gland, gastrointestinal tract, bone marrow and liver [13–15]. RGS16 is expressed in most immune cell subsets, such as natural killer cells, dendritic cells, and T lymphocytes [12]. Further, RGS16 acts as an oncogene that promotes the progression of numerous cancers [16–19]. In addition, RGS16 is critical for immune and inflammatory responses.

In one study, RGS16 was shown to be upregulated in inflammatory dendritic cells stimulated with lipopolysaccharide [20]. Related studies have demonstrated that RGS16 can regulate T cell-mediated inflammatory responses by affecting T cell activation and promoting T cell migration via chemokine receptors, such as CXCR4, CCR3 and CCR5 [21,22]. Further, RGS16 negatively

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regulates monocyte-mediated immune response by inhibiting monocytes-related inflammatory cytokines [23]. Recent discoveries have also suggested a role of RGS16 in the regulation of circadian rhythms [24]. However, the role of RGS16 in ulcerative colitis remains unclear.

Hence, in this study, we aimed to elucidate the role of RGS16 in ulcerative colitis using patient samples. We detected RGS16 expression in inflamed colonic mucosa and peripheral blood mononuclear cells (PBMCs) and observed that RGS16 expression significantly increased in inflamed mucosa and PBMCs from patients with ulcerative colitis and positively correlated with disease activity. Moreover, we found that TNF- α upregulated RGS16 expression in PBMCs from ulcerative colitis patients via the nuclear factor kappa beta (NF- κ B) signaling pathway, and anti-TNF treatment downregulated RGS16 expression.

Materials and methods

Patients and samples

Patients with ulcerative colitis were recruited from the Department of Gastroenterology of the Affiliated Hospital of Jining Medical University (Jining, Shandong, China) between 2018 and 2020. Colonic biopsy samples were obtained from patients with ulcerative colitis ($n=28$) and healthy controls ($n=22$) who underwent endoscopy. EDTA anticoagulated peripheral blood samples were obtained from patients with ulcerative colitis ($n=22$) and healthy controls ($n=15$) after overnight fasting. The diagnosis of ulcerative colitis was based on clinical characteristics, endoscopic examination and histological findings [25,26]. The clinical characteristics of the patients with ulcerative colitis are shown in Table 1. This study was approved by the Institutional Review Board for Clinical Research of the Affiliated Hospital of Jining Medical University. Written informed consent was obtained from all participants before the study.

Table 1. Clinical characteristics of patients with ulcerative colitis

	Blood samples		Biopsy samples	
	Healthy controls	Ulcerative colitis	Healthy controls	Ulcerative colitis
Number of patients	15	22	22	28
Age (years)	27.59 \pm 4.63	41.26 \pm 15.16	29.16 \pm 4.16	39.46 \pm 9.12
Gender				
Male	8	9	8	15
Female	7	12	14	13
Disease duration (month)		16.46 \pm 17.45		20.46 \pm 10.4
Disease extent (ulcerative colitis)				
E1		7		9
E2		10		13
E3		5		6
Mayo index				
Slight				15
Mild				8
severe				5
CRP		32.15 \pm 8.16		31.16 \pm 17.46
ESR		27.63 \pm 8.16		35.16 \pm 8.13

According to the Montreal classification system. (CRP, mg/L) (ESR, mm/h). ESR, erythrocyte sedimentation rate.

Isolation and stimulation of peripheral blood mononuclear cells

EDTA anticoagulated peripheral blood samples (10 mL) were collected from ulcerative colitis and healthy controls, and PBMCs were isolated by density gradient centrifugation using Ficoll-Paque (2000 rpm, 20 °C, 20 min). PBMCs were collected from the interface and washed thrice with PBS. PBMCs were isolated from the peripheral blood of healthy donors and stimulated with tumor necrosis factor alpha (TNF- α , 20 ng/mL) IL-4 (20 ng/mL), IL-6 (20 ng/mL), IL-12 (20 ng/mL), IL-23 (20 ng/mL) and lipopolysaccharide (200 ng/mL) in the presence of immobilized anti-CD3 and anti-CD28 antibodies *in vitro* for 8 h. RGS16 mRNA levels were measured using real-time reverse transcription-PCR (qRT-PCR).

Anti-TNF mAb treatment *in vivo* and *ex vivo*

Treatment with anti-TNF mAb [i.e., infliximab (IFX)] is therapeutically effective in patients with ulcerative colitis. Endoscopic colonic biopsies were obtained from active ulcerative colitis patients and subsequently incubated with IFX (50 μ g/mL) or control at 37 °C in 24-well plates. After 24 h, the biopsies were harvested for analysis of RGS16 expression. PBMCs isolated from patients with active ulcerative colitis were incubated with IFX (50 ng/mL) and anti-CD3/anti-CD28 for 24 h, and RGS16 mRNA levels were measured using qRT-PCR.

Quantitative real-time PCR

Total RNA was extracted according to the manufacturer's instructions and reverse-transcribed with the All-in-one 5 \times RT MasterMix (abm, Vancouver, Canada). qRT-PCR assays were performed using SYBR green. The relative levels of target gene expression were calculated using the $2^{-\Delta\Delta C_t}$ method [27]. The primers used are listed in Table 2.

Enzyme-linked immunosorbent assay

Blood samples were obtained from ulcerative colitis patients and healthy controls after overnight fasting. Serum was collected via centrifugation and stored at -80 °C until use. Each serum sample was tested for TNF- α and IL-17A using ELISA according to the manufacturer's instructions (BioLegend, San Diego, California, USA).

Western blot

Cell lysates were quantified by the bicinchoninic acid assay method and the proteins were resolved by 15% SDS-PAGE followed by transfer onto polyvinylidene fluoride membranes for 2 h at 100 V with a standard

Table 2. The primers using in real-time reverse transcription-PCR analysis

Gene	Species	DNA sequence (sense 5'-3')	DNA sequence (anti-sense 5'-3')
RGS16	Human	ATCAGAGCTGGGCTG-CGATA	CAGGTGCAACGACTCTCTCC
GAPDH	Human	CTGGGCTACACTGAG-CACC	AAGTGGTCGTTGAGGG-CAATG

transfer solution. After blocking with 3% BSA, membranes were incubated with primary antibodies against RGS16 (1:1000, ab119424, Abcam, Cambridge, UK) at 4 °C overnight, followed by incubation with horseradish peroxidase-labeled goat secondary antibody. An ECL kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA) was used to detect the protein bands using chemiluminescence. Glyceraldehyde-3-phosphate dehydrogenase was used as an internal control.

Statistical analysis

Data were analyzed with GraphPad Prism 8.0 (San Diego, California, USA), and all values in the graphs are given as means \pm standard error of the mean (SEM). All experiments were performed at least in triplicates. All data were normally distributed. Data were analyzed using the non-parametric Mann–Whitney and Wilcoxon tests with false discovery rate (FDR) correction. Spearman correlation was performed to analyze the correlation between RGS16 expression and the ulcerative colitis endoscopic index of severity (UCEIS), Mayo index, erythrocyte sedimentation rate (ESR) and serum TNF- α and IL-17A levels. The statistical significance was set at $P < 0.05$.

Results

RGS16 expression increases in colonic mucosa from patients with ulcerative colitis

Colonic mucosa was collected from patients with ulcerative colitis and RGS16 expression was determined by qRT-PCR, western blot and immunohistochemistry. Immunohistochemistry analysis exhibited that the percentage of RGS16 positive cells in inflamed colon mucosa from ulcerative colitis was markedly increased compared with that from healthy controls (Fig. 1a and b). The RGS16 expression was markedly higher in the colonic mucosa of patients with ulcerative colitis than that of healthy controls (Fig. 1c). Further, the expression of RGS16 protein in the inflamed mucosa from patients with active ulcerative colitis was significantly higher than that in healthy controls (Fig. 1d and e). We also observed a higher RGS16 mRNA (Fig. 1f) and protein (Fig. 1g and h) expression in the inflamed mucosa than in the unaffected intestinal mucosa from the same patients with ulcerative colitis. Therefore, our findings indicate that RGS16 may play a role in the pathogenesis of ulcerative colitis.

RGS16 expression positively correlates with disease activity in colonic mucosa from patients with ulcerative colitis

To test our hypothesis of whether RGS16 expression is associated with the disease activity in patients with ulcerative colitis, the disease severity of the patients was evaluated according to international standard criteria, such as the Mayo index. Slight ulcerative colitis was defined by a Mayo index of 3–5, mild ulcerative colitis by a Mayo index of 6–10 and severe ulcerative colitis by a Mayo index of 11–12 [28]. We performed Spearman's correlation analysis and found a significant correlation between RGS16 expression in inflamed mucosa with the

Mayo index and UCEIS in patients with ulcerative colitis (Fig. 2a). Interestingly, with respect to the Mayo index, RGS16 expression was highest in the severe group, followed by the middle and slight groups (Fig. 2b), which further indicated that RGS16 expression positively correlated with the disease activity of ulcerative colitis.

Intestinal mucosal lesions in patients with ulcerative colitis were graded using the UCEIS, and Spearman's correlation analysis was performed between RGS16 expression and UCEIS. RGS16 expression significantly correlated with UCEIS (Fig. 2c). Because 5-ASA and prednisone have been used to treat mild or moderate-to-severe ulcerative colitis, we examined RGS16 expression in the colonic mucosa of patients who received 5-ASA or prednisone and achieved clinical remission. We observed that RGS16 expression was significantly downregulated in the colonic mucosa of patients after 5-ASA (Fig. 2d) or prednisone treatment (Fig. 2e) than that before treatment. Collectively, these data indicate that RGS16 expression positively correlates with disease activity in the colonic mucosa of ulcerative colitis patients.

RGS16 expression positively correlates with serum TNF- α and IL-17A levels

Next, we examined the RGS16 expression in PBMCs of patients with ulcerative colitis. RGS16 expression was markedly higher in PBMCs from patients with ulcerative colitis than that in healthy controls (Fig. 3a). The ESR, a peripheral laboratory measure of inflammation, is frequently used to evaluate the clinical disease activity in ulcerative colitis. In this study, ESR significantly correlated with RGS16 expression in PBMCs of patients with ulcerative colitis (Fig. 3b). Further, because TNF- α and IL-17A are markedly increased in patients with ulcerative colitis [29], we hypothesized that RGS16 expression positively correlates with the levels of TNF- α and IL-17A. As shown in Fig. 3c,d, RGS16 expression was highly correlated with serum levels of TNF- α and IL-17A, suggesting a positive correlation between RGS16 levels in PBMCs with the disease activity in ulcerative colitis patients.

TNF- α upregulates RGS16 expression in peripheral blood mononuclear cells

Cytokines (e.g. IL-4, IL-6, IL-10 and TNF- α) are notably increased in the colonic mucosa or serum of patients with ulcerative colitis. Hence, we investigated whether these cytokines could regulate RGS16 expression in ulcerative colitis. We isolated PBMCs from the peripheral blood of healthy donors and stimulated them with TNF- α , IL-4, IL-6, IL-10, IL-12, IL-23 and lipopolysaccharide in the presence of immobilized anti-CD3 and anti-CD28 *in vitro*. Intriguingly, we found that TNF- α greatly, whereas lipopolysaccharide and IL-10 modestly, enhanced RGS16 mRNA expression in PBMCs (Fig. 4a). However, IL-4, IL-6, IL-12 and IL-23 had no significant effect on RGS16 expression. Furthermore, we found that TNF- α upregulated RGS16 expression in PBMCs in a dose- and time-dependent manner (Fig. 4b,c). Because the NF- κ B signaling pathway is involved in the pathogenesis of ulcerative colitis, we hypothesized that TNF- α participates in the modulation of RGS16 expression through the NF- κ B signaling pathway.

To this end, PBMCs were pretreated with a caspase-3/8 inhibitor (Z-DEVD-FMK), JNK inhibitor (JNK-IN-7) or NF- κ B inhibitor (BAY 11-7082), followed by stimulation with TNF- α in the presence of anti-CD3/28 for 48 h. BAY 11-7082 blocked the TNF- α -induced RGS16 expression, whereas Z-DEVD-FMK and JNK-IN-7 had no effect. These data indicate that TNF- α upregulates RGS16 expression in PBMCs from patients with ulcerative colitis via the NF- κ B signaling pathway.

Anti-TNF treatment downregulates RGS16 expression in peripheral blood mononuclear cells

TNF- α is crucial for the pathogenesis of ulcerative colitis, and anti-TNF treatment can ameliorate intestinal mucosal inflammation in patients with ulcerative colitis. Hence, we hypothesized that anti-TNF participates in the TNF- α -induced increase in RGS16 expression in ulcerative colitis patients. IFX, an anti-TNF agent, is often used to treat ulcerative colitis [30]. Freshly inflamed colonic tissue from ulcerative colitis patients was obtained and treated with IFX for 24h. The levels of RGS16 significantly reduced after IFX treatment than after the control treatment (Fig. 5a). The same result was also observed in ulcerative colitis patient-isolated PBMCs treated with IFX for 24h

(Fig. 5b). Collectively, anti-TNF treatment downregulated RGS16 expression in inflamed intestinal tissues and PBMCs from patients with ulcerative colitis.

Discussion

Ulcerative colitis, characterized by a chronic relapsing-remitting gastrointestinal tract, is caused by a dysregulated immune response against the intestinal microbiota with an imbalance in the pro- or anti-inflammatory pathway [31]. In this study, we observed that RGS16 expression was significantly increased in inflamed mucosa and PBMCs from patients with ulcerative colitis and positively correlated with disease activity. Moreover, TNF- α upregulated RGS16 expression in PBMCs from ulcerative colitis patients via the NF- κ B signaling pathway, whereas anti-TNF treatment downregulated RGS16 expression.

GWAS have demonstrated several susceptible genes involved in abnormal immune responses in the pathogenesis of IBD, including genes involved in circulating T-cells (ADA, CD40, TAP1/2, NBS1, BLM and DNMT3B) or specific subsets such as Th17 (STAT3), memory (SP110) or regulatory T-cells (STAT5) [10,32]. Genetic defects in some IBD loci, such as STAT3 and CARD9, lead to

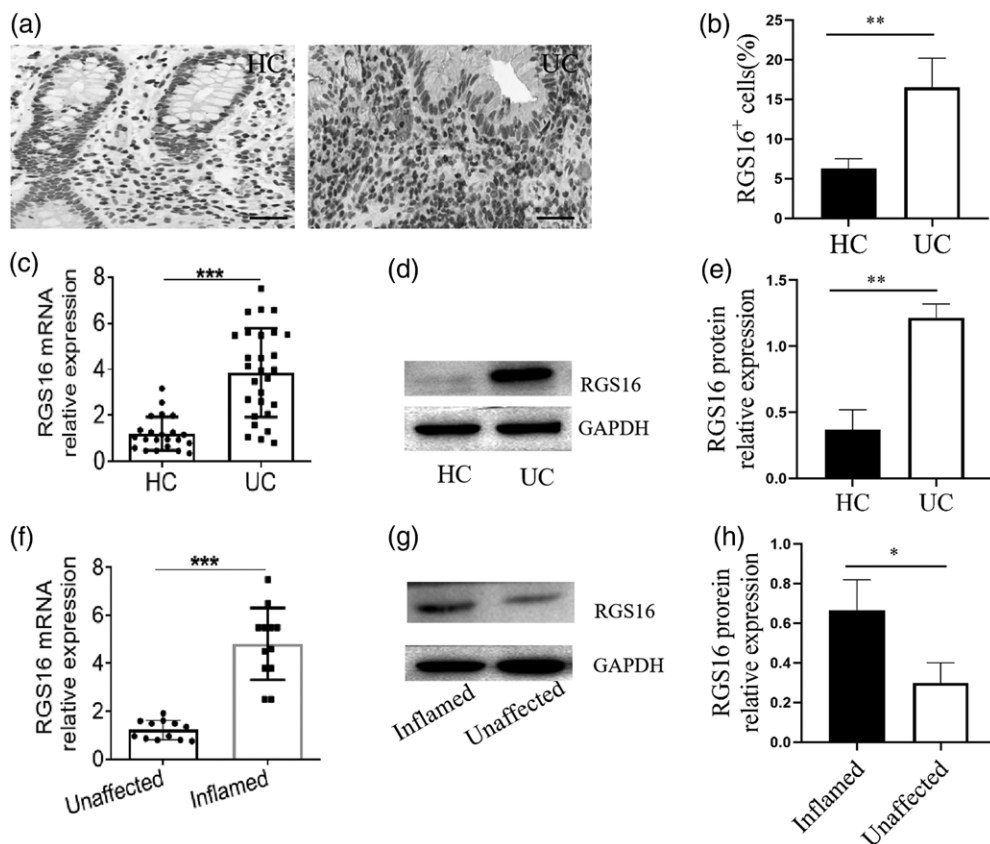


Fig. 1. RGS16 expression increased significantly in inflamed mucosa from patients with ulcerative colitis (UC). (a) Representative images of immune-histochemical staining of RGS16 expression in inflamed colon from patients with UC and normal colonic mucosa from healthy controls (HC). Scar bar represents 50 μ m. (b) Percentages of RGS16+ cells in colonic mucosa in a were shown in the bar. ** $P < 0.01$. (c) Colon biopsy specimens were collected from patients with UC ($n = 28$), and HC ($n = 22$). RGS16 mRNA expression was detected by using real-time reverse transcription-PCR (qRT-PCR). Gene expression was normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in each group. *** $P < 0.001$. (d) RGS16 protein expression in colonic mucosa from patients with UC ($n = 11$), and HC ($n = 9$) was examined by western blot, with GAPDH as reference. (e) Relative expression of RGS16 protein expression in D. ** $P < 0.01$. (f) RGS16 mRNA expression in inflamed and unaffected intestinal mucosa from the same patients with UC ($n = 12$) was examined by qRT-PCR. *** $P < 0.001$. (g). RGS16 protein expression in inflamed and unaffected intestinal mucosa from the same patients with UC ($n = 8$) was examined by western blot. (h) Relative expression of RGS16 protein expression in g. ** $P < 0.01$. RGS, regulator of the G-protein signaling.

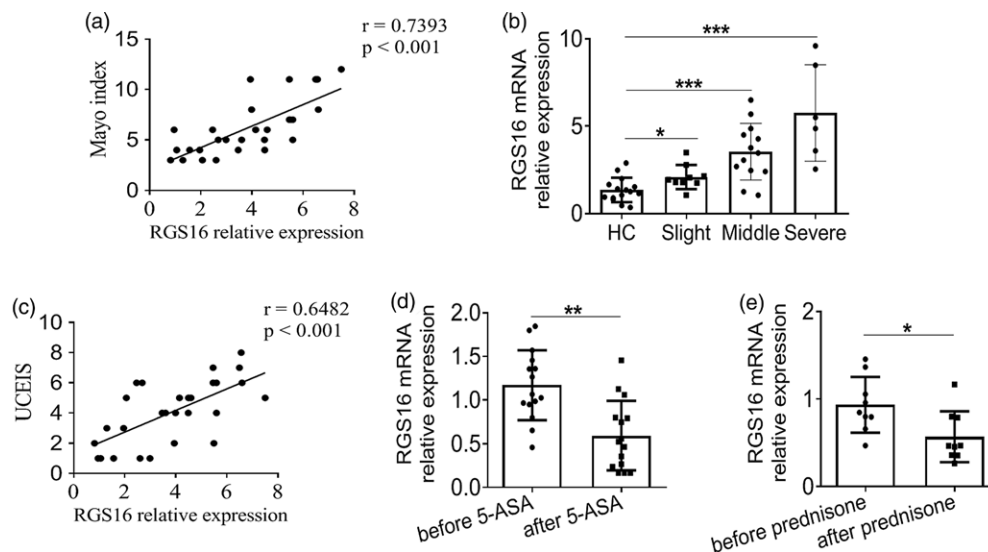


Fig. 2. Increased RGS16 expression in colonic mucosa was correlated with disease activity. (a) Correlation analysis was performed between Mayo index and RGS16 mRNA expression in inflamed mucosa of patients with ulcerative colitis (UC) ($***P < 0.001$). (b) RGS16 mRNA expression in colonic mucosa from patients with slight UC ($n = 9$), mild UC ($n = 13$), severe UC ($n = 6$) and healthy controls (HC) ($n = 15$) was analyzed. Gene expression was normalized to GAPDH in each group. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. (c) Correlation analysis was performed between UCEIS and RGS16 mRNA expression in inflamed mucosa of patients with UC. $***P < 0.001$. (d,e) RGS16 expression was examined in colonic mucosa from patients after or before the treatment of 5-ASA (d, $n = 15$) or prednisone (e, $n = 9$). 5-ASA, 5-aminosalicylic acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RGS, regulator of the G-protein signaling; UCEIS, ulcerative colitis endoscopic index of severity.

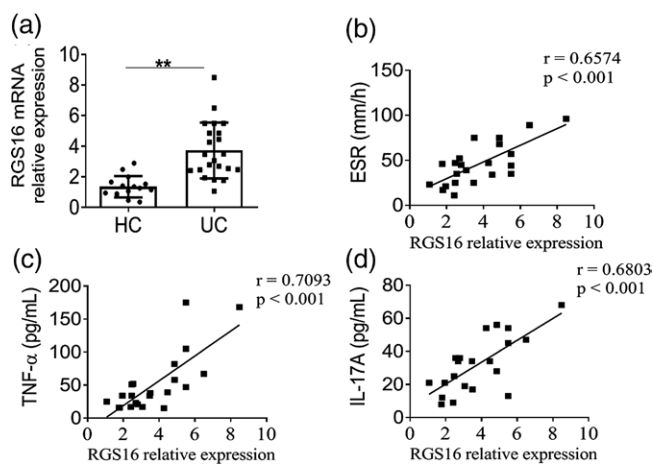


Fig. 3. RGS16 expression significantly increased in PBMCs from patients with ulcerative colitis (UC). (a) RGS16 mRNA expression in PBMCs from patients with UC ($n = 22$), and healthy controls (HC) ($n = 15$) was examined by real-time reverse transcription-PCR (qRT-PCR). Gene expression was normalized to GAPDH. $**P < 0.01$. (b–d) Correlation analysis was performed between ESR (b), serum TNF- α (c) or IL-17A levels (d) and RGS16 mRNA expression in PBMCs from patients with UC. ESR, erythrocyte sedimentation rate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PBMCs, peripheral blood mononuclear cells; RGS, regulator of the G-protein signaling.

primary immunodeficiencies involving skin infections caused by *Staphylococcus* and *Candida*, respectively [10,33–35]. The RGS protein superfamily negatively controls the GPCR signal transduction pathways. RGS16, an important member of the RGS protein superfamily, has been reported to have several roles. It is enriched in activated T lymphocytes and inhibits IL-8 and CCR5 mediated signals in lymphocytes [31]. The overexpression of RGS16 in the megakaryocytic MO7e cell line inhibits SDF-1-induced migration, mitogen-activated protein kinase and protein kinase B activation and negatively regulates

CXCR4 signaling in megakaryocytes [36]. Besides, RGS16 inhibits breast cancer cell growth by mitigating the phosphatidylinositol 3-kinase signaling [37]. Hepatic RGS16 suppresses hepatic fat oxidation by upregulating the carbohydrate response element-binding protein, and it is subsequently identified to reverse the protective effects of Arg2 overexpression during fasting [38,39]. Further, RGS16 expression is related to tumor growth in several gastrointestinal cancer cell lines and is a novel predictive marker for the prognosis of colorectal cancer [15]. Fructooligosaccharides and wheat bran maintain colon health by regulating RGS16 expression and G-protein signaling pathways in the colon epithelia of rats [40]. In this study, we found that the protein and mRNA expression of RGS16 significantly increased in the inflamed colonic mucosa and PBMCs from patients with ulcerative colitis, which indicated that RGS16 might be involved in the immune response of ulcerative colitis.

Patients with crohn's disease and ulcerative colitis often suffer from alternating relapse and remission periods, which affect them physically and psychologically. The diagnostic approaches for ulcerative colitis are based on clinical, endoscopic, histological, radiological and biochemical criteria. However, patients often refuse to undergo these diagnostic tests due to their invasive nature [41]. Therefore, novel biomarkers are needed to easily and noninvasively diagnose and predict the disease course, monitor therapeutic efficacy and predict the relapse of the disease. In this study, we hypothesized that increased RGS16 expression can be used as a marker for ulcerative colitis. The Mayo index and UCEIS are often used to evaluate clinical and endoscopic activities in patients with ulcerative colitis [42]. We observed that RGS16 expression positively correlated with the Mayo index and UCEIS. 5-ASA and prednisone are common therapeutic agents for the treatment of ulcerative colitis. Further, the RGS16 expression in the colonic mucosa of

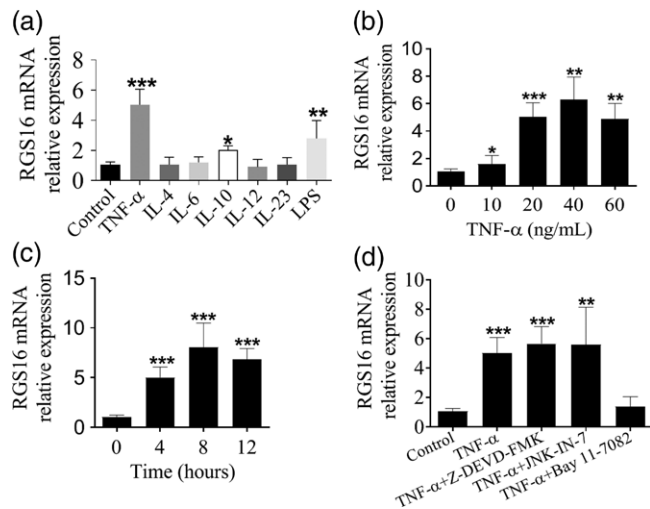


Fig. 4 TNF- α upregulated RGS16 expression in PBMCs. PBMCs were stimulated with TNF- α (20 ng/mL), IL-4 (20 ng/mL), IL-6 (20 ng/mL), IL-12 (20 ng/mL), IL-23 (20 ng/mL) and lipopolysaccharide (LPS) (200 ng/mL) for 8 h, respectively. Expression of RGS16 mRNA was detected by real-time reverse transcription-PCR (qRT-PCR). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus control. (b) PBMCs were stimulated with different concentrations of TNF- α for 8 h, and expression of RGS16 mRNA was detected by qRT-PCR. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus control. (c) PBMCs were stimulated with TNF- α (20 ng/mL) for 4, 8 and 12 h, and expression of RGS16 mRNA was detected by qRT-PCR. *** $P < 0.001$ versus control. (d) PBMCs were pretreated with caspase-3/8 inhibitor (Z-DEVD-FMK, 50 μ M), JNK inhibitor (JNK-IN-7, 1 μ M), or NF- κ B inhibitor (BAY 11-7082, 1 μ M) followed by TNF- α (20 ng/mL), respectively. Expression of RGS16 mRNA was detected by qRT-PCR. ** $P < 0.01$, *** $P < 0.001$ versus control. PBMCs, peripheral blood mononuclear cells; RGS, regulator of the G-protein signaling.

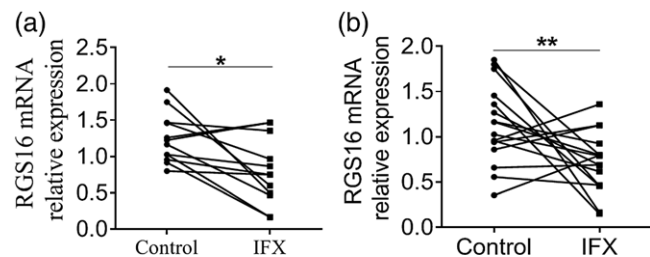


Fig. 5 Anti-TNF treatment downregulated RGS16 expression. (a) Freshly obtained inflamed colonic tissue from patients with active ulcerative colitis (UC) ($n = 12$) were treated with IFX (50 μ g/mL) or control for 24 h, and RGS16 mRNA expression was measured by qRT-PCR. (b) PBMCs isolated from UC patients ($n = 16$) were stimulated with IFX (50 ng/mL) for 24 h, and RGS16 mRNA expression was measured by qRT-PCR. * $P < 0.05$, ** $P < 0.01$. IFX, infliximab; PBMCs, peripheral blood mononuclear cells; RGS, regulator of the G-protein signaling.

patients who received 5-ASA or prednisone treatment and achieved clinical remission was significantly downregulated. Moreover, the RGS16 expression in PBMCs also positively correlated with ESR and serum TNF- α and IL-17A levels, which are critical markers of disease activity. Hence, RGS16 expression significantly correlated with disease activity and could, to some extent, predict therapeutic efficacy.

The typical characteristics of ulcerative colitis are hyperactivation of pro-inflammatory pathways, defective anti-inflammatory signaling pathways and the production of large amounts of pro-inflammatory cytokines in the inflamed intestinal mucosa and peripheral blood of ulcerative colitis patients [26]. We hypothesized that these

inflammatory cytokines modulate RGS16 expression. We observed that TNF- α , IL-10 and lipopolysaccharide significantly induced the expression of RGS16. Further, TNF- α exerts its effect on RGS16 expression via the NF- κ B pathway. These data indicate that increased RGS16 expression in patients with ulcerative colitis might partly result from the excessive production of TNF- α , thus contributing to disease progression. TNF- α is a major pathological cytokine in the pathogenesis of ulcerative colitis, and monoclonal antibody against TNF- α is a critical therapeutic strategy in ulcerative colitis treatment [43]. We found that anti-TNF treatment markedly downregulated RGS16 expression in inflamed intestinal tissues and PBMCs from patients with ulcerative colitis *ex vivo*, which, to some extent, indicates that RGS16 might predict the therapeutic efficacy of anti-TNF treatment.

In summary, our data demonstrate that RGS16 expression is significantly increased in inflamed intestinal mucosa and PBMCs from patients with ulcerative colitis and is positively correlated with disease activity. Moreover, TNF- α could upregulate RGS16 expression in PBMCs from ulcerative colitis patients via the NF- κ B signaling pathway, and anti-TNF treatment could downregulate RGS16 expression. Therefore, our study provides novel insights into the roles of RGS16 in ulcerative colitis, and RGS16 may serve as a novel diagnostic and therapeutic target for ulcerative colitis.

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G.Z. and Y.W. conceived and designed the experiments. F.Z. performed the experiments. Y.Q. and Y.W. analyzed the data. F.Z., Z.X., F.D. and W.C. contributed to the clinical data and specimens. F.Z., Y.W. and G.Z. wrote the manuscript. All authors discussed and revised the manuscript.

Conflicts of interests

There are no conflicts of interest.

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