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ORIGINAL ARTICLE

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Knockdown of TRIM8 alleviates dextran sulfate sodium-induced colitis in mice by inhibiting the NF-κB signaling pathway

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Abstract
Background: Although *TRIpartite Motif containing 8* (*TRIM8*) gene plays an important role in a number of biological processes, such as inflammation, its function and mechanism in ulcerative colitis (UC) remain unknown.
Methods: The UC model was established by feeding mice with 3.5% dextran sulfate sodium (DSS). The animals were divided into the following four groups: control group, DSS group, DSS+short hairpin (sh)-NC group, and DSS+sh-TRIM8 group. Changes in body weight and disease activity index (DAI) score of mice in all the groups were recorded for 7 days. The animals were executed at the end of the experiment, and the expression of TRIM8 in colon tissue was detected by polymerase chain reaction and Western blot assays. The length of colon was measured, and the histopathological changes in mice colon were examined by hematoxylin and eosin staining. The expression of pro-inflammatory factors in mice serum and colonic tissue homogenate was detected by enzyme-linked-immunosorbent serologic assay. The expression of nuclear factor kappa B (NF-κB) pathway-related proteins in colonic tissues was detected by Western-blot analysis.
Results: TRIM8 was highly expressed in the colonic tissues of UC mice. Knockdown of TRIM8 improved DSS-induced weight loss, increased DAI score, shortened colon length, and alleviated colonic injury and inflammation in mice. Western-blot experiments showed that knockdown of TRIM8 inhibited DSS-induced phosphorylation of p65 and nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (IκBα) protein but increased IκBα expression.
Conclusion: Knockdown of TRIM8 inhibits UC injury and inflammatory response caused by DSS. This could be related to the regulation of NF-κB signaling pathway by TRIM8 protein.

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Introduction

Ulcerative colitis (UC) is one of the most common inflammatory bowel diseases caused by a combination of environmental factors, host genetics, immune disorders, intestinal microbiota, and intestinal barrier dysfunction. It is characterized by chronic and recurrent inflammation of colonic and rectal mucosa, resulting in abdominal pain, diarrhea, intestinal blood loss, and weight loss. The incidence of UC has been increasing over the past decades and has become a serious public health problem. However, the exact pathophysiological mechanisms of UC remains unknown.[1-3] Therefore, investigation of molecular targets of UC is crucial for treatment of UC.

The TRIM (TRIpartite Motif)/N terminal RING domain, one or two B-Box motifs, and Coiled-Coil (RBCC) protein family is a subfamily of the RING (Really Interesting New Gene) finger E3 ubiquitin ligases family. The human TRIM family contains more than 70 proteins, making it one of the largest subfamilies of RING finger E(3) ubiquitin ligases family.[4,5] TRIM8 is a member of TRIM family, with a molecular weight of 61.5 kDa and a gene located on chromosome 10q24.3. TRIM8 participates in a number of crucial biological processes, including cell survival, innate immunological response, oncogenesis, autophagy, apoptosis, differentiation, and inflammation.[6] Previous research has demonstrated that TRIM8 plays a role in both tumor development and inflammatory disorders. As an anti-oncogene,[7] TRIM8 is a potential target for treating colorectal cancer.[8] Besides, TRIM8 acts as a pro-inflammatory factor in disorders with an inflammatory phenotype. For example, TRIM8 positively regulates brain ischemia-reperfusion injury by boosting inflammation and apoptosis through activation of nuclear factor kappa B (NF-κB) pathway.[9]

NF-κB is a key regulator of inflammation, innate immune response, and tissue integrity. In 3.5% dextran sulfate sodium (DSS)-induced colitis mice model, the NF-κB signaling pathway is strongly activated, resulting in increased levels of inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin(IL)-1β, and IL-6. These cytokines then trigger an inflammatory cascade response, in which aggregated neutrophils cause a number of pathological changes, such as intestinal epithelial cell damage, crypt abscess, and small vessel inflammation.[10-13]

Although it has been noted that TRIM8 has pro-inflammatory properties, its function in UC and the underlying mechanism is unclear. In the present work, we investigated the role of TRIM8 in UC-induced mice model and discovered that knockdown of TRIM8 reduced the severity of UC and prevented the production of pro-inflammatory factors by inhibiting the NF-κB pathway.

Materials and Methods

Animal experimental design

Male C57BL/6 J mice (6–8 weeks old, 20–24 g) were purchased from Shanghai Jihui Experimental Animal Breeding Co. All experiments were approved by the Biomedical Ethics Committee of the Department of Medicine, Xi 'an Jiaotong University, for the use of animals and conducted in accordance with the National Institutes of Health Laboratory Animal Care and Use Guidelines. The animals were kept in a stable environment of 12-h light/dark cycle, temperature of 25 ± 2°C, and 55% humidity. The animals were housed for 7 days to acclimate with the environment and were given access to water without restriction. Six mice were randomly assigned to the following four groups: control (healthy control), DSS (ulcerative colitis), DSS+short hairpin (sh)-NC, and DSS+sh-TRIM8. For 7 days, 3.5% DSS was added in the drinking water of the mice to establish the model. Mice in the DSS+sh-NC and DSS+sh-TRIM8 groups were transfected with adeno associated virus (AAV)-sh-NC and AAV-sh-TRIM8, respectively, 1 week prior to establishment of the model. Mice were fasted overnight as described previously,[14,15] and the intestines were cleansed with N-acetyl-1-cysteine. The mice were anesthetized with isoflurane, and phosphate-buffered saline (PBS) solution containing AAV-sh-NC and AAV-sh-TRIM8 was instilled in the intestines and kept in an inverted position for 1 min to distribute it throughout the colon. Body weight was observed and recorded during animal experiments. Disease activity index (DAI) score[16] was determined as the sum of weight loss, stool consistency, and total bleeding as follows: (a) weight loss: 0 = none; 1 = 1–5%; 2 = 5–10%; 3 = 10–15%; 4 = more than 15%; (b) stool consistency: 0 = normal; 2 = loose stool; 4 = diarrhea; and (c) bleeding in the stool: 0 = negative; 2 = positive; 4 = rectal hemorrhage. Mice were sacrificed by decortication at the end of the experiment.

Histopathology analysis

Hematoxylin-eosin (H&E) staining was used to analyze histopathology of the colon. First colon tissues were fixed in 4% paraformaldehyde, followed by gradient ethanol solution dehydration, xylene transparency, and paraffin embedding. Colon tissues were sliced into 4-μm pieces, stained with H&E, and examined under a microscope for histopathological alterations in each group of animals.

Enzyme-linked-immunosorbent serologic assay (ELISA) analysis

The serum and intestinal tissue homogenates were collected from mice of each group. The contents of IL-1β, IL-6, and TNF-α (Neobioscience, China) in serum and colon tissue homogenates were measured according to the manufacturer's instructions.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis

The colon tissue was subjected to total RNA extraction using TRIzol (Thermo Fisher, MA, USA) reagent, reversely transcribed into complementary DNA (cDNA) using a reverse transcription kit (Thermo Fisher), quantitative polymerase chain reaction using SYBR Premix ExTaq Mix (TAKARA, Japan), and quantitative measurements using the ABI Prism 7900HT Sequence Detection System (Life

Technologies, CA, USA). The following thermocycler settings were used: one cycle at 95°C lasting for 30 s, 40 denaturation cycles lasting for 5 s at 95°C, and combined annealing and extension cycles lasting for 30 s at 60°C. Fold change in mRNA expression of gene was normalized to β-actin using the $2^{-\Delta\Delta CT}$ method. The TRIM8 primer sequence is as follows: 5'-TCTCTCCCTCCCCCTTCCGTTTCTA-3' (forward); 5'-ATGAAATTCCAGTCCCCGGCCA-3'(reverse).

Western-blot analysis

The colonic tissue was frozen in liquid nitrogen and crushed into powder. Radioimmunoprecipitation (RIPA) buffer lysate was added and centrifuged to obtain total protein samples. The protein concentration of samples was measured by bicinchoninic acid assay (BCA) kit and the loading volume was calculated. The total protein was separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride (PVDF) membrane, which was placed in 5% skimmed milk powder at room temperature for 1 h.[17] The membranes were washed with tris buffered saline with tween (TBST) and incubated overnight at 4°C with TRIM8 (ab155674, 1:1000; Abcam, Cambridge, UK), phosphorylated (p)-p65 (ab76302, 1:1000; Abcam), p65 (ab16502, 1:1000; Abcam), phosphorylated nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (p-IκBα) (ab133462, 1:10,000; Abcam), and IκBα (ab32518, 1:10,000; Abcam) antibodies. Next, they were washed with TBST and incubated with horseradish peroxidase (HRP)-coupled secondary antibody (ab6721, 1: 10,000; Abcam) for 1 h. After washing, the strips were treated with enhanced chemiluminescence (ECL) kit (Beyotime, China). β-actin (ab8227, 1:1000; Abcam) was used as an internal control to normalize protein expression.

Statistical analysis

All data were expressed as the mean ± standard error of mean (SEM). The statistical product and service solutions (SPSS) software was used to analyze the data conformed to a normal distribution. One-way ANOVA was used for statistical analysis, followed by Fisher's Least Significant Difference (LSD) test and Dunnett's test. Statistical significance was defined as $P < 0.05$.

Results

TRIM8 expression was up-regulated in DSS-induced mouse colonic tissue

We examined the expression of TRIM8 in the colonic tissues of mice in the control, DSS, DSS+sh-NC, and DSS+sh-TRIM8 groups. The results revealed that the expression levels of TRIM8 mRNA and protein were increased in the colonic tissues of mice in DSS-induced UC group compared with that in the control group (Figures 1A and B), and decreased in the colonic tissues of mice in the DSS+sh-TRIM8 group compared with the DSS+sh-NC group, indicating successful transfection.

Knockdown of TRIM8 ameliorated DSS-induced colonic injury in mice

In order to establish a model of UC, we fed mice with 3.5% DDS instead of water for 7 days and found that mice in the DSS group showed an overall decreasing trend in body weight, an increasing trend in DAI score, and a shorter colon length, compared to DSS and DSS+sh-TRIM8 groups. Animals in the DSS group demonstrated an increasing body weight curve (Figure 2A), a decreasing DAI score (Figure 2B), and a longer colon length (Figure 2C). H&E staining indicated that the colonic tissue of mice in the DSS group had infiltration of inflammatory cells, mucosal erosion, loss of epithelial cells and cup cells, shortening and collapse of crypt, and submucosal edema, which were improved after transfection with sh-TRIM8 (Figure 2D). It indicated that knockdown of TRIM8 alleviated DSS-induced UC in mice.

Knockdown of TRIM8 attenuated colonic inflammation in DSS-induced ulcerative colitis mice model

We measured the levels of IL-1β, IL-6, and TNF-α in mice serum and colonic tissues to investigate the impact of TRIM8 on inflammatory factors. We discovered that these levels in mouse colonic tissue homogenates (Figure 3A) and serum (Figure 3B) in the DSS group were significantly higher than that in the control group whereas the levels of these inflammatory cytokines were significantly lowered after TRIM8 was knocked down. This suggested that TRIM8 knockdown would lessen colonic inflammation in DSS-induced UC animal model.

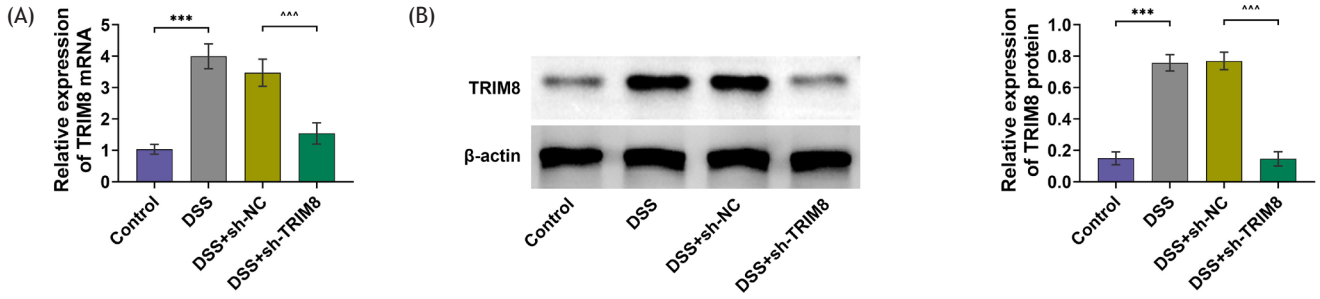

Figure 1 TRIM8 expression was up-regulated in DSS-induced mouse colonic tissue. (A) The mRNA expression of TRIM8 in each group. (B) The protein expression of TRIM8 in each group (vs. control group, ***$P < 0.001$; vs. DSS+sh-NC, ^^^$P < 0.001$).

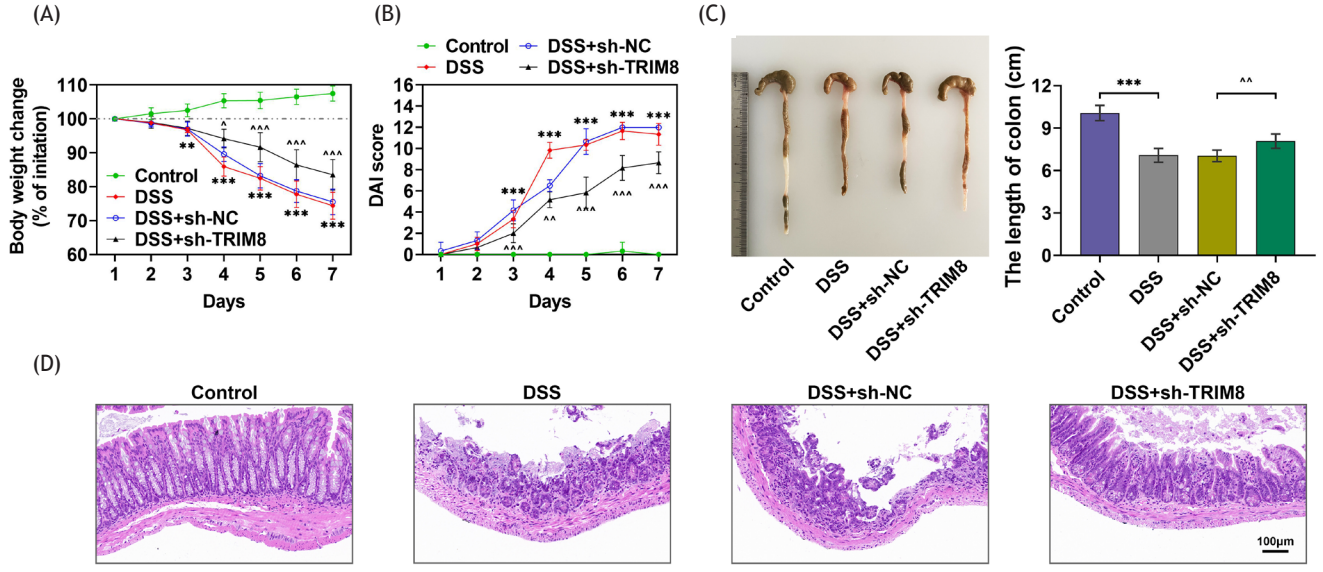

Figure 2 Knockdown of TRIM8 ameliorated DSS-induced colonic injury in mice. (A) Change in body weight of mice in each group during 7 days. (B) Change in DAI score of mice in each group during 7 days. (C) H&E staining of colonic tissues of various groups of mice (vs. control group, *P < 0.05, **P < 0.01, ***P < 0.001; vs. DSS+sh-NC, ^P < 0.05, ^^P < 0.01, ^^^P < 0.001).

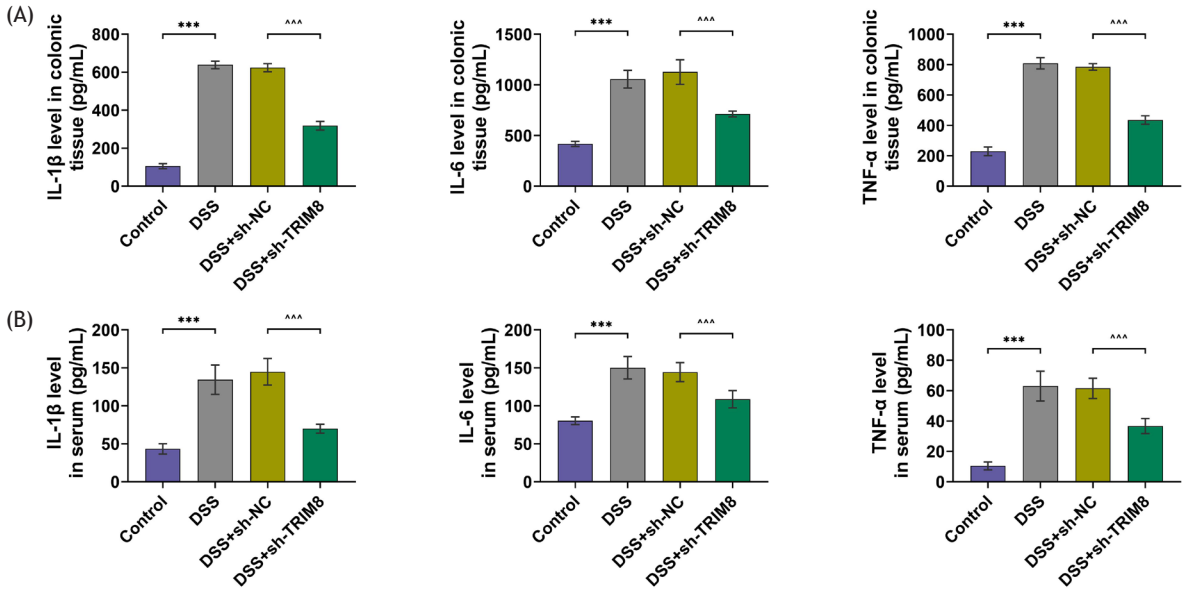

Figure 3 Knockdown of TRIM8 attenuated colonic inflammation in DSS-induced ulcerative colitis mouse model. (A) Levels of IL-1β, IL-6, and TNF-α in colon tissue homogenates. (B) Serum levels of IL-1β, IL-6, and TNF-α in mice (vs. control group, ***P < 0.001; vs. DSS+sh-NC, ^^^P < 0.001).

Knockdown of TRIM8 inhibited activation of NF-κB signaling pathway

NF-κB, being a major regulator of innate immunity and inflammatory response, regulated the production of pro-inflammatory cytokines. Therefore, we further investigated the regulatory effect of TRIM8 on NF-κB signaling. The detection of NF-κB-related proteins revealed that the expression levels of p-p65 and p-IκBα were increased and the expression level of IκBα was decreased in the colonic tissues of mice in the DSS group, while these were reversed by transfection with sh-TRIM8. This indicated that knockdown of TRIM8 would inhibit activation of NF-κB signaling pathway.

Discussion

Ulcerative colitis has evolved into a difficult disease globally due to its age-neutral onset and simple recurrence.[18] DSS-induced UC model is a common method that consistently induces UC-related characteristics at a high rate.[19]

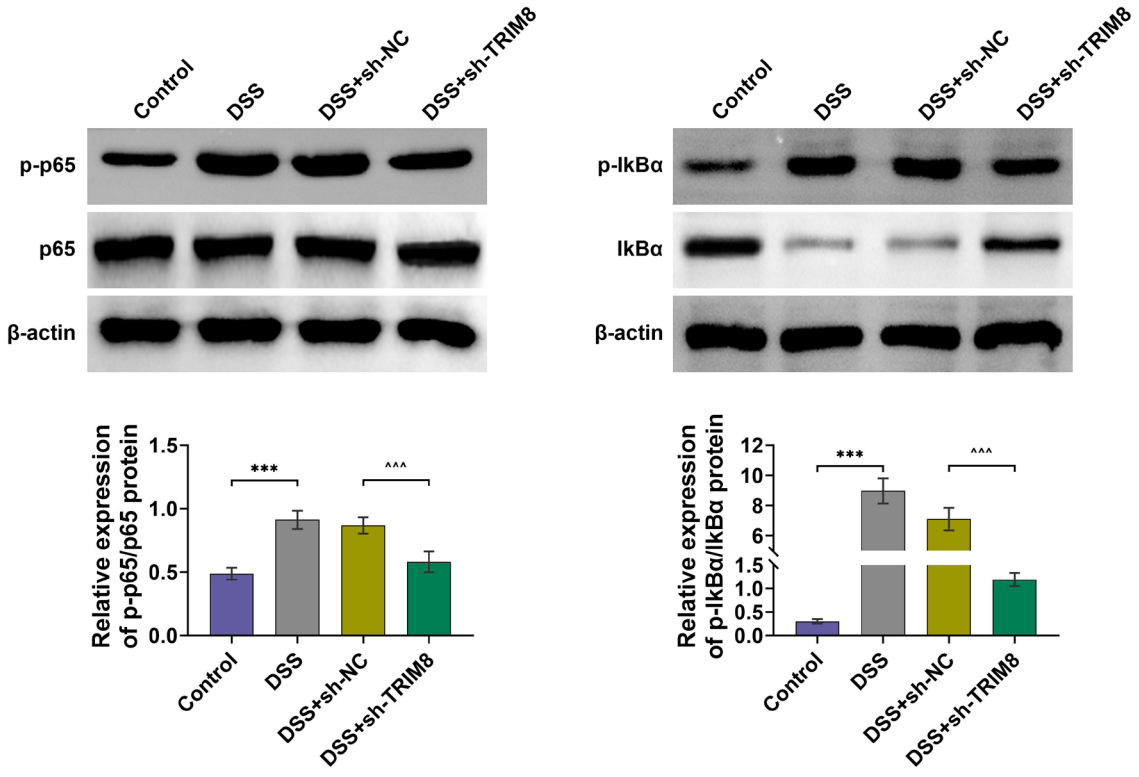

Figure 4 Knockdown of TRIM8 inhibited activation of NF-κB pathway. Expression levels of p65, p-p65, IκBα, and p-IκBα proteins in colon tissue homogenates.

This approach was used to mimic UC in this research, and as predicted, the DSS-induced model demonstrated clinical traits resembling those of UC patients, such as elevated DAI scores.

A considerable level of emphasis has been placed on anomalies in inflammation and immunological responses despite the fact that the pathophysiology of UC is not entirely understood. Evidence from earlier studies indicates that intestinal homeostasis is impacted and tight junction proteins are disrupted as a result of interactions between the intestinal mucosal immune system and pro-inflammatory cytokines.[20] An unbalanced production of pro- and anti-inflammatory cytokines has been linked to immune system disturbances, and increased expression of pro-inflammatory cytokines is a key component of UC.[21] As a result, determining pertinent targets is essential for treating UC. It has been established that TRIM8, a pro-inflammatory factor, was highly expressed in hepatic ischemia-reperfusion, and knockdown of TRIM8 was able to reduce inflammatory response during liver injury. In our study, TRIM8 expression was increased in colonic tissues of DSS mice, and knockdown of TRIM8 reduced DAI scores, increased body weight and colonic length, repaired DSS-induced colonic tissue injury, and reduced TNF-α, IL-6, and IL-1β levels in serum and colonic tissue homogenates in DSS-induced UC animal model. This indicated that knockdown of TRIM8 could alleviate UC injury and suppress inflammatory response during UC.

There is accumulating evidence that the NF-κB signaling pathway is crucial for controlling the expression and release of inflammatory cytokines. When sedated, NF-KB is found in an inactive state in the cytoplasm, and its activation is strictly regulated by nuclear factor of kappa light polypeptide gene enhancer in B-cells (IκB), which is phosphorylated and degraded upon external stimulation and NF-κB nuclear translocation. The nuclear translocation of NF-κB activates its target genes to increase the expression of a variety of pro-inflammatory mediators and cytokines.[22] Previous research has demonstrated that inhibiting NF-κB improves experimental colitis by lowering the production of pro-inflammatory cytokines.[23,24] The NF-κB pathway can be inhibited by TRIM8 knockdown.[25] In our research, we discovered that TRIM8 knockdown decreased p-p65/p-65 and p-IκB expression while increasing IκB expression, indicating that TRIM8 was involved in NF-κB regulation in UC.

Conclusion

We discovered that TRIM8 knockdown could prevent DSS-induced UC, which can be attributed to the inhibition of NF-κB pathway. Our study provides a potential therapeutic targets for treating UC, although some limitations were observed during the research. We will continue with in-depth studies in the future, such as determining the effects of TRIM8 overexpression on UC progression, apoptosis, oxidative stress, and other phenotypes in the intestinal tissues of UC mice, and incorporating NF-κB inhibitors for rescue experiments.

Competing Interests

The authors stated that there were no conflicts of interest to disclose.

Data Availability

All data supporting the findings of this study are available within the paper and any raw data can be obtained from the corresponding author upon request.

Author Contributions

Ting Qiu and Yifei Lv designed and carried out the study. Ting Qiu, Yifei Lv, Lu Niu, and Yu Zhang supervised collection, analysis, and interpretation of data; they prepared and reviewed draft of the manuscript for publication. All authors read and approved the final manuscript.

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