FOSL1 knockdown ameliorates DSS-induced inflammation and barrier damage in ulcerative colitis via MMP13 downregulation

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Abstract. Ulcerative colitis (UC) is a chronic inflammatory disease of the colon. Fos-related antigen 1 is highly expressed in mild UC and affects the maintenance and delayed recurrence of inflammatory bowel disease. Therefore the present study aimed to investigate the role of Fos-like antigen-1 (FOSL1) in UC. Use of the JASPAR database predicted the possible interaction of FOSL1 and the MMP13 promoter. FOSL1 and MMP13 mRNA and protein expression levels in dextran sodium sulfate (DSS)-induced HT29 cells and colon tissues of a UC mice model were determined using reverse transcription-quantitative PCR and western blotting, respectively. MMP13 promoter activity was determined using the dual-luciferase reporter assay, the relationship between FOSL1 and MMP13 promoter was determined using chromatin immunoprecipitation, inflammatory factor levels were quantified using ELISA, cell monolayer permeability analysis was performed using transepithelial electrical resistance measurements and tight junction protein expression levels were determined by western blotting. After constructing a UC mice model, mice colonic injury was determined with the quantification of colon length, H&E staining and disease activity index. The results demonstrated that FOSL1 and MMP13 were upregulated in DSS-induced HT29 cells and tissues. FOSL1 was also determined to be bound to the MMP13 promoter region and was demonstrated to upregulate MMP13 expression levels. Furthermore, FOSL1 knockdown inhibited DSS-induced inflammation and barrier damage in HT29 cells via MMP13 downregulation. FOSL1 knockdown also ameliorated colonic injury, inflammation and barrier damage in the tissues of the UC mice model via MMP13 downregulation. Overall, FOSL1 knockdown was demonstrated to potentially ameliorate DSS-induced inflammatory injury and protect the intestinal barrier integrity in the UC mice model via MMP13 downregulation.

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Introduction

Ulcerative colitis (UC) is a type of inflammatory bowel disease (IBD), which is a group of chronic inflammatory disorders of the colonic mucosa (1). In recent years, the number of patients with UC has gradually increased in China and UC has become a common disease of the digestive system (2). The clinical features of UC include the development of colorectal inflammation and disruption of the normal colonic mucosal barrier (3). The tight junctions between intestinal epithelial cells serve an important role in maintaining intestinal mucosal permeability (4). However, intestinal infection, inflammation, mechanical injury and other factors induce abnormal intestinal mucosal barrier function. These factors increase mucosal permeability, which leads to the displacement of bacteria and antigens in the intestinal lumen to the lamina propria, which activates immune cells and thereby causes an abnormal immune reaction in the mucosa (5). Furthermore, inflammatory cytokines, inflammatory mediators and reactive oxygen radicals also mediate the abnormal expression of tight junction proteins (6). Therefore, the improvement of intestinal mucosal barrier function is a main focus of UC treatment and an important area in UC pathophysiological research.

Fos-like antigen 1 (FOSL1), a member of the Fos family, serves an important role in the progression and maintenance of certain transformed cancer cells, including in lung cancer, prostate cancer and colon cancer (7-10). Previous study demonstrated that FOSL1 expression levels are significantly elevated in patients with UC, especially in mild UC (11). However, the underlying molecular mechanisms are complex and remain to be elucidated. Furthermore, FOSL1 overexpression in intestinal mucosal epithelial cells increases the risk of IBD recurrence via the attenuation of the protective effect of the intestinal mucosa in IBD during remission, which results in the inhibition of the damage repair mechanism (12). FOSL1 is also one of the major transcription factors of the activator protein-1 (AP-1) family. Blocking the AP-1 transcription factor in mice models has been demonstrated to suppress dextran sodium sulfate (DSS)-induced colonic inflammation (13). It could therefore be hypothesized that FOSL1 may serve an

important role in UC. Therefore the present study aimed to understand the specific impact of FOSL1 in UC.

MMPs are protein hydrolases that degrade the extracellular matrix and are involved in tissue damage repair (14). A previous study reported that MMP13 mRNA expression levels are markedly elevated in mucosal samples from patients with IBD and are positively correlated with tissue inflammation levels (15,16). MMP13-mediated inflammation-related signaling pathways also have important implications for the integrity of the intestinal mucosal epithelial cell barrier (17). MMP13 can indirectly regulate the barrier function of intestinal mucosal epithelial cells via the activation of TNF-α, which increases the permeability of the intestinal epithelium (18). According to the JASPAR database, FOSL1 potentially binds to the MMP13 promoter and may regulate its expression levels. It can therefore be hypothesized that FOSL1 may interact with MMP13, which may affect inflammatory damage and intestinal barrier damage in UC.

In the present study, a DSS-induced *in vitro* cell model and an *in vivo* mice model of UC were created and the expression levels of FOSL1 and MMP13 in HT29 cells and UC mice intestinal tissues were examined. Subsequently, the association between FOSL1 and MMP13 was investigated. Furthermore, the present study explored the effect of FOSL1 knockdown on DSS-induced inflammation and barrier damage in HT29 cells and the UC mice model. The results of the present study provided a comprehensive account of the underlying mechanism of FOSL1 and have identified novel research areas for the development of effective therapeutic strategies for the treatment of UC.

Materials and methods

Cell culture and treatment. McCoy's 5a (modified) medium (American Type Culture Collection), containing a final concentration of 10% FBS (RWD Life Science Co., Ltd.), was used to culture the human colorectal cancer cell line HT29 cells (American Type Culture Collection) at 37˚C in 5% CO_2. For the establishment of the *in vitro* UC cell model, HT29 cells were cultured until 80% confluency and were subsequently treated with 2% DSS (MP Biomedicals, LLC) at 37˚C for 24 h.

Transfection. The FOSL1 overexpression vector (oe-FOSL1; 1 μg/ml), the MMP13 overexpression (oe) vector (oe-MMP13; 1 μg/ml), the negative control (oe-NC; 1 μg/ml), short hairpin RNA (shRNA/sh; 100 nm)-targeting FOSL1 (sh-FOSL1-1: forward, 5'-GCCTCTGACCTACCCTCAGTA-3' and reverse, 5'-TACTGAGGGTAGGTCAGAGGC-3'; sh-FOSL1-2: forward, 5'-AGTGGATGGTACAGCCTCATT-3' and reverse, 5'-AATGAGGCTGTACCATCCACT-3') and the corresponding negative control (sh-NC, 5'-CCGGCAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCTTCATCTTGTTGTTTTTG-3'), were purchased from Hunan Fenghui Biotechnology Co., Ltd. The vectors were transfected into HT29 cells seeded into 6-well plates at a density of $1x10^6$ cells/well using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) at 37˚C for 24 h. Reverse transcription-quantitative (RT-q) PCR and western blotting were used to examine the transfection efficiency of oe-FOSL1, sh-FOSL1-1/2 and oe-MMP13. Cells were collected for subsequent experiments after 24 h.

Animal experimental design. For the establishment of the *in vivo* UC mouse model, a total of 24 male C57BL/6 mice (age, 6-8 weeks; weight, 21-23 g; Beijing HFK Bioscience Co., Ltd.) were used. Mice were housed in specific pathogen-free conditions at 22-26˚C, under a 12/12 h light/dark cycle with 40-70% humidity and ad libitum access to food and water. Mice were randomly divided into four groups (n=6/group): mice in control group were fed as usual and given distilled water; mice in UC group were fed as usual but was given 5% DSS daily for 7 days for modeling; mice in UC+sh-FOSL1 group were injected with adeno-associated virus (AAV) shRNA-targeting FOSL1 (sh-FOSL1) via the tail vein, companied with 5% DSS administration; mice in UC+sh-FOSL1+oe-MMP13 group were injected with adeno-associated virus (AAV) shRNA-targeting FOSL1 (sh-FOSL1) and AAV targeting MMP13 overexpression (oe-MMP13) plasmids via the tail vein, accompanied with 5% DSS administration. By the end of experiment, the mice were euthanized via inhalation of isoflurane (5% for induction and 1.5-2% for maintenance; cat. no. HR135327; Hangzhou Hairui Chemical Co., Ltd.) prior to cervical dislocation. The animals were monitored every day and the humane endpoint of this experiment was as follows: Marked reduction in food or water intake, labored breathing, inability to stand and no response to external stimuli. No abnormal signs that signified the humane endpoints of the experiment were observed from any of the rats during the experiment. Mortality was verified by the lack of heartbeat and a cold body. All experimental procedures were approved by the Ethics Committee of the North China University of Science and Technology Affiliated Hospital (approval no. Lx201896). Colonic tissues were collected and the length was quantified. The mice were scored using the disease activity index (DAI) based on body weight, gross rectal bleeding and stool consistency (19). Serum and tissue samples were collected and stored at -80˚C for further analysis.

RT-qPCR. FOSL1 and MMP13 mRNA expression levels were determined using RT-qPCR. Briefly, total RNA was extracted from HT29 cells ($1x10^6$ cells) using the MolPure® Cell RNA kit (Shanghai Yeasen Biotechnology Co., Ltd.) according to the manufacturer's protocol. Complementary DNA was synthesized from the isolated RNA using the PrimeScript™ RT Reagent kit (Takara Bio, Inc.) according to the manufacturer's protocols. qPCR was performed using the One Step SYBR PrimeScript RT-PCR kit (Perfect Real Time; TakaRa Bio Inc.) and the ABI 7500 qPCR instrument (Applied Biosystems; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocols. The following thermocycling conditions were used: Initial denaturation at 95˚C for 10 min; 40 cycles of denaturation at 95˚C for 10 sec, annealing at 60˚C for 20 sec and elongation at 72˚C for 30 sec; and a final extension at 72˚C for 7 min. Relative mRNA expression levels were determined using the $2^{-\Delta\Delta Cq}$ method (20). The experiments have been replicated for 3 times. Primers sequences were: FOSL1, forward 5'-TGACCACACCCTCCCTAACTC-3' and reverse 5'-CTGCTGCTACTCTTGCGATGA-3'; MMP13; forward, 5'-AACATCCAAAAACGCCAGAC-3' and reverse

5'-GGAAGTTCTGGCCAAAATGA-3', GAPDH, forward 5'-CCATGGGGAAGGTGAAGGTC-3' and reverse 5'-AGTGATGGCATGGACTGTGG-3'.

Western blotting. Total protein was extracted from HT29 cells and mice colon tissues, from the different treatment groups, on ice using RIPA lysis buffer (Beijing Solarbio Science & Technology Co., Ltd.) for 15 min. Total protein concentration was determined using the BCA assay (Beijing Solarbio Science & Technology Co., Ltd.) according to the manufacturer's protocol. Total protein (30 μg protein/lane) was separated using SDS-PAGE on a 15% gel (Beijing Leagene Biotechnology Co., Ltd.). Separated proteins were transferred onto nitrocellulose membranes and the membranes were blocked using 5% skimmed milk at room temperature for 1 h. Subsequently, membranes were incubated with primary antibodies against the following: FOSL1 (1:1,000; cat. no. ab252421; Abcam), MMP13 (1:1,000; cat. no. ab51072; Abcam), occludin (1:1,000; cat. no. ab216327; Abcam), zona occludens-1 (ZO-1; 1:1,000; cat. no. ab276131; Abcam), claudin-2 (1:2,000; cat. no. ab211737; Abcam) and GAPDH (1:2,500; cat. no. ab9485, Abcam), overnight at 4˚C. Then the membranes were incubated with HRP-conjugated IgG secondary antibody (1:20,000; cat. no. ab205718; Abcam) for 1 h at room temperature. An ECL reagent (Beyotime Institute of Biotechnology) was used to visualize the separated proteins. Image Pro Plus software version 7.0 (Media Cybernetics, Inc.) was used to analyze the protein blots. GAPDH served as an internal control.

Dual-luciferase reporter assay. The wild-type (WT) or mutant (MUT) reporter plasmids of MMP13 were generated by inserting WT or MUT sequences into pGL3 luciferase reporter plasmids (Promega Corporation). HT29 cells were seeded into 24-well plates ($1x10^5$ cells/well) and incubated at 37˚C for 24 h. Subsequently, cells were co-transfected with reporter plasmids and oe-FOSL1/oe-NC using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). Following transfection for 48 h, the luciferase reporter activity was detected using the Dual-luciferase reporter assay (Promega Corporation) and normalized to *Renilla* luciferase activities.

Chromatin immunoprecipitation (ChIP) assay. The interaction between FOSL1 and the MMP13 promoter was assessed using the ChIP Assay kit (Beyotime Institute of Biotechnology). Cells were fixed with 4% formaldehyde at room temperature for 10 min for fixation. Subsequently, glycine solution was added and the cells were incubated at room temperature for 5 min. The culture medium was then discarded and the pre-chilled PBS was added to wash the cells. Following centrifugation at 4˚C at 716 x g for 5 min, SDS Lysis Buffer was added for 10 min in an ice bath to fully lyse the cells. After sonicating the samples (20 kHz; 4 pulses of 12 sec each, followed by 30 sec rest on ice between each pulse), 1.8 ml ChIP dilution buffer was added. Subsequently, 70 μl protein A/G agarose was added for 30 min at 4˚C. After centrifugation at 4,000 x g for 1 min at 4˚C, the supernatant was collected and incubated overnight at 4˚C with anti-FOSL1 (1:30; cat. no. ab252421; Abcam) or anti-IgG (1:100; cat. no. ab90285; Abcam). Following incubation, 60 μl protein A/G agarose was added for 60 min at 4˚C to precipitate the protein or corresponding complex recognized by the primary antibody. The resulting precipitates were detected via RT-qPCR according to the aforementioned protocol.

ELISA. The levels of the inflammatory cytokines, TNF-α, IL-1β and IL-6 in HT29 cells and UC mice serum were assessed using the corresponding ELISA kits (Mouse TNF-α ELISA KIT (cat. no. SEKM-0034), Human TNF-α ELISA KIT (cat. no. SEKM-0047), Mouse IL-1β ELISA KIT (cat. no. SEKM-0002), Human IL-1β ELISA KIT (cat. no. SEKM-0002), Mouse IL-6 ELISA KIT (cat. no. SEKM-0007), Human IL-6 ELISA KIT (cat. no. SEKM-0013)) from Beijing Solarbio Science & Technology Co., Ltd. according to the manufacturer's protocol.

Trans-epithelial electrical resistance (TEER) assay. The TEER assay was performed to identify HT29 cell monolayer permeability. Briefly, cells were seeded into a polyester Transwell plate (diameter, 12 mm; pore size, 0.4 μm; 24-well; Corning, Inc.). After 24 h, initial resistance readings of all co-cultures were obtained from an epithelial voltmeter ($EVOM_2$, World Precision Instruments, Inc.). The resistance of each cell monolayer was determined by the relative TEER value of each group, compared to the control.

Hematoxylin and eosin (H&E) staining. Colon tissues from the UC model mice were first washed with PBS, then fixed using 4% paraformaldehyde at room temperature for 24 h. Next, tissues were dehydrated in a gradient alcohol series, treated in xylene and were embedded in paraffin. Subsequently, these tissues were made into 3-4 μm paraffin sections. Following de-paraffinization, sections were stained using hematoxylin for 2 min at room temperature. The samples were rinsed under running water and were dehydrated in 95% alcohol for 30 sec prior to being stained with eosin for 2 min at room temperature. The sections were then washed, dehydrated and sealed with neutral resin sealing sheets. An Olympus light microscope (BX43; Olympus Corporation) was used to capture the images.

Statistical analysis. All data are presented as the mean ± SD. Data were analyzed using SPSS version 20.0 (IBM Corp.). Unpaired Student's t-test was used for statistical comparisons between two groups and one-way ANOVA followed by Tukey's post hoc test was used for statistical comparisons among more than two groups. $P<0.05$ was considered to indicate a statistically significant difference.

Results

FOSL1 and MMP13 expression levels are upregulated in DSS-induced HT29 cells. To examine the expression levels of FOSL1 and MMP13 in DSS-induced HT29 cells, an *in vitro* cell model of UC was constructed and RT-qPCR and western blotting were performed. The results demonstrated that there was an increase in the relative FOSL1 and MMP13 mRNA and protein expression levels in DSS-induced HT29 cells, compared with the control group (Fig. 1A and B). These results therefore indicated that DSS potentially induced high mRNA and protein expression levels of FOSL1 and MMP13 in HT29 cells.

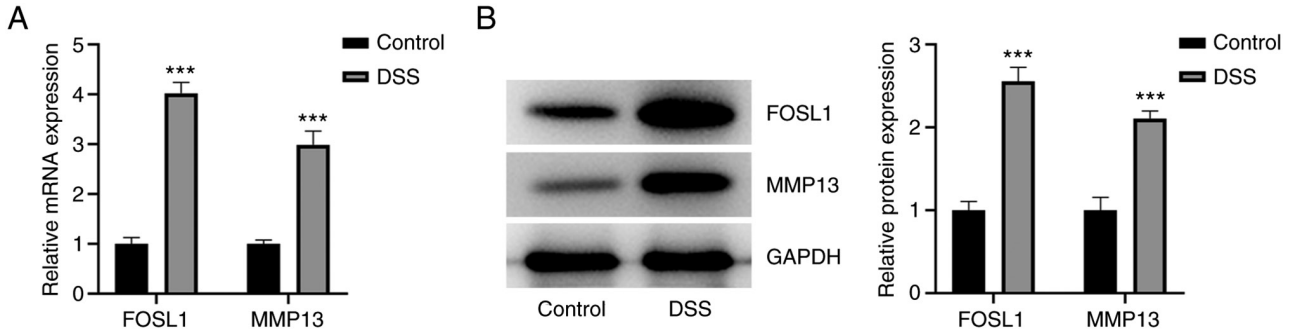

Figure 1. DSS-induced HT29 cells exhibited increased FOSL1 and MMP13 expression levels. (A) Reverse transcription-quantitative PCR and (B) western blotting were performed to determine the FOSL1 and MMP13 mRNA and protein expression levels, respectively, in HT29 cells. ***P<0.001 vs. control. DSS, dextran sodium sulfate; FOSL1, Fos-like antigen-1.

FOSL1 binds to the MMP13 promoter region and upregulates MMP13 expression levels. To determine the relationship between FOSL1 and MMP13, the binding site of FOSL1 and the MMP13 promoter was predicted using the JASPAR database. This analysis demonstrated that FOSL1 may bind to the MMP13 promoter as a transcription factor (Fig. 2A). Furthermore, the results demonstrated that FOSL1 mRNA and protein expression levels were increased following transfection with oe-FOSL1 compared with the oe-NC group. However, these expression levels were reduced following transfection with sh-FOSL1-1/2 compared with the sh-NC group (Fig. 2B and C). sh-FOSL1-2 was selected for use in subsequent experiments because of its higher efficiency. To further determine the association between FOSL1 and MMP13, the mRNA and protein expression levels of MMP13 were determined via RT-qPCR and western blotting, respectively, following transfection. The results demonstrated that FOSL1 overexpression resulted in elevated MMP13 mRNA and protein expression levels compared with the oe-NC group, whereas sh-FOSL1-2 resulted in decreased MMP13 mRNA and protein expression compared with the sh-NC group (Fig. 2D and E). Furthermore, the dual-luciferase reporter assay demonstrated that there was markedly elevated MMP13 promoter activity in the MMP13-wild-type (WT) + oe-FOSL1 group compared with the MMP13-WT + oe-NC group. However, there was no difference between the MMP13-mutant (MUT) + oe-FOSL1 and MMP13-MUT + oe-NC groups (Fig. 2F). The ChIP experiment also demonstrated that the relative enrichment abundance of anti-MMP13 at the target gene site on anti-FOSL1 was markedly higher compared with the NC IgG group (Fig. 2G). These results indicated that as a transcription factor, FOSL1 may bind to the MMP13 promoter region and therefore may have potentially upregulated MMP13 expression levels.

FOSL1 knockdown inhibits DSS-induced inflammation and barrier damage in HT29 cells via MMP13 downregulation. To clarify whether FOSL1 knockdown had an effect on DSS-induced HT29 cells MMP13 was overexpressed. The results indicated that MMP13 was highly expressed following its overexpression in DSS-induced HT29 cells compared with the oe-NC group (Fig. 3A and B). Furthermore, in DSS-induced HT29 cells there were increased levels of the inflammatory cytokines, TNF-α, IL-1β and IL-6, compared with the control (Fig. 3C-E). Following transfection with sh-FOSL1-2, TNF-α, IL-1β and IL-6 levels markedly decreased in DSS-induced HT29 cells compared with the DSS + sh-NC group, whereas MMP13 overexpression enhanced the levels of TNF-α, IL-1β and IL-6 compared with the DSS + sh-FOSL1-2 + oe-NC group. Subsequently, TEER was performed to detect cell monolayer permeability. The results demonstrated that DSS induced a marked decrease in cell monolayer permeability, whereas there was a significant increase in cell permeability following FOSL1 knockdown; however, MMP13 overexpression reversed the effect of FOSL1 knockdown (Fig. 3F). These results indicated that FOSL1 knockdown may have effectively protected the HT29 cell barrier from DSS-induced damage. It was also demonstrated that the levels of tight junction-associated proteins, occludin and ZO-1, in DSS-induced HT29 cells were markedly reduced in the DSS group compared with the control group. However, these levels were markedly elevated in the DSS + sh-FOSL1-2 group compared with the DSS + sh-NC group, whereas in the DSS + sh-FOSL1-2 + oe-MMP13 group these levels were reduced compared with the DSS + sh-FOSL1-2 + oe-NC group (Fig. 3G). In addition, the expression levels of claudin-2 in each group exhibited a completely different trend from that of occludin and ZO-1. These results indicated that FOSL1 knockdown potentially inhibited DSS-induced inflammation and barrier damage in HT29 cells via MMP13 downregulation.

FOSL1 knockdown ameliorates DSS-induced colonic injury, inflammation and barrier damage in UC mice via MMP13 downregulation. Subsequently it was investigated whether FOSL1 knockdown could ameliorate DSS-induced colonic injury in UC mice via the downregulation of MMP13. Therefore, a DSS-induced UC mouse model was constructed. The results demonstrated that there was a marked increase in the protein expression levels of FOSL1 and MMP13 in UC mice, compared with the control group. Injection with sh-FOSL1 greatly resulted in the reduction of FOSL1 and MMP13 expression in colon tissues of UC mice and oe-MMP13 elevated the protein expression of MMP13 but had no influence on FOSL1 expression (Fig. 4A). It was observed that the colons of UC mice were significantly shorter compared with the control group (Fig. 4B and C). However, the colons of UC mice injected with sh-FOSL1 were longer compared with UC mice, whereas co-injection with oe-MMP13 and sh-FOSL1

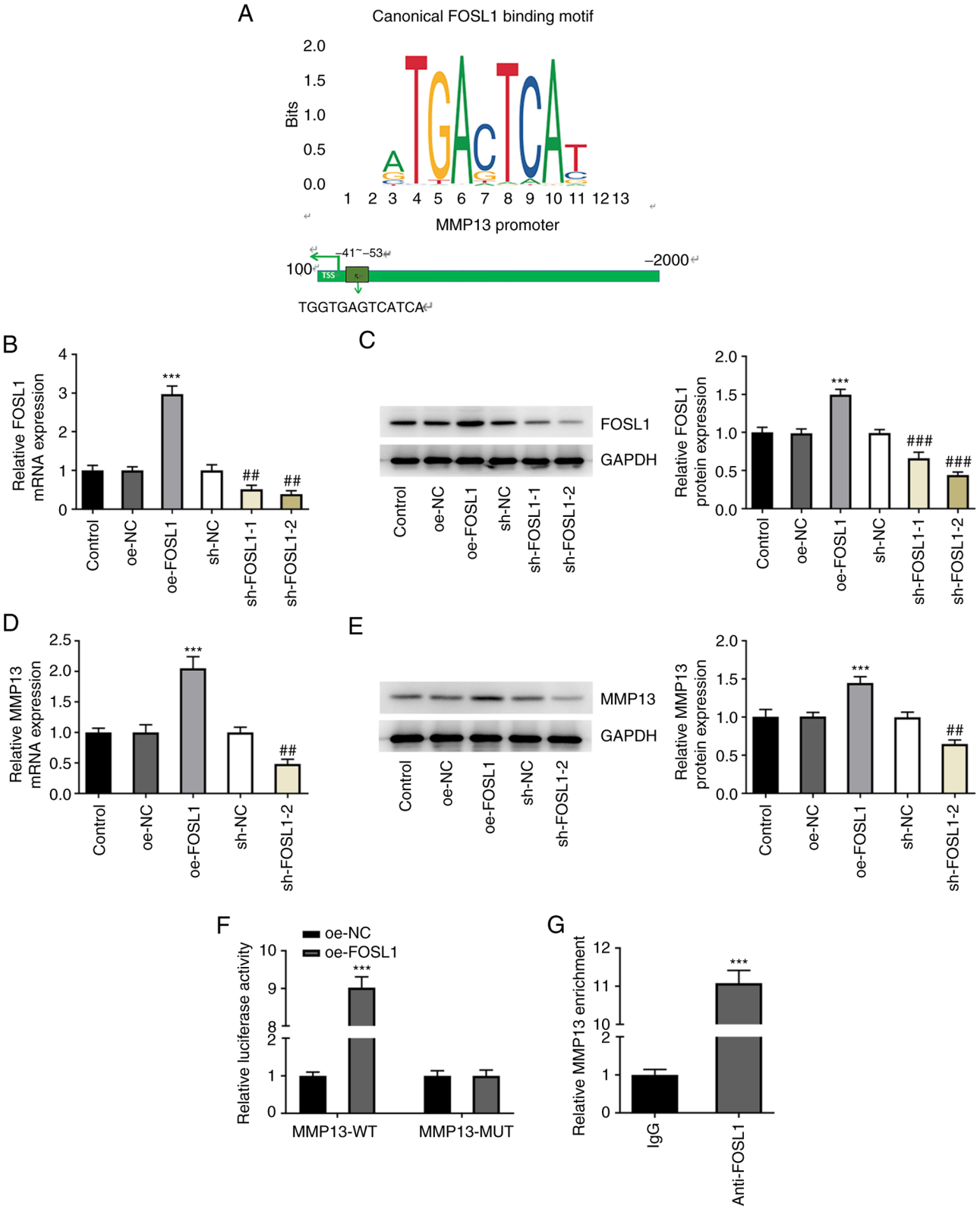

Figure 2. FOSL1 binds to the MMP13 promoter region and upregulates MMP13 expression levels. (A) The binding site of FOSL1 and the MMP13 promoter was predicted using the JASPAR database. FOSL1 overexpression and knockdown were detected using (B) RT-qPCR and (C) western blotting. ***P<0.001 vs. oe-NC; ##P<0.01, ###P<0.001 vs. sh-NC. MMP13 expression levels following FOSL1 overexpression and knockdown were detected via (D) RT-qPCR and (E) western blotting. ***P<0.001 vs. oe-NC; ##P<0.01 vs. sh-NC. (F) MMP13 promoter activity was assessed using the dual-luciferase reporter assay. ***P<0.001 vs. oe-NC. (G) FOSL1 binding to the MMP13 promoter was assessed via chromatin immunoprecipitation. ***P<0.001 vs. oe-NC. FOSL1, Fos-like antigen-1; RT-qPCR, reverse transcription-quantitative PCR; oe, overexpression; NC, negative control; sh, short hairpin RNA.

resulted in shorter colons of UC mice compared to injection with sh-FOSL1 alone. Furthermore, following H&E staining, the structure of the intestinal tissues in UC mice was markedly altered compared with the control (Fig. 4D), which indicated

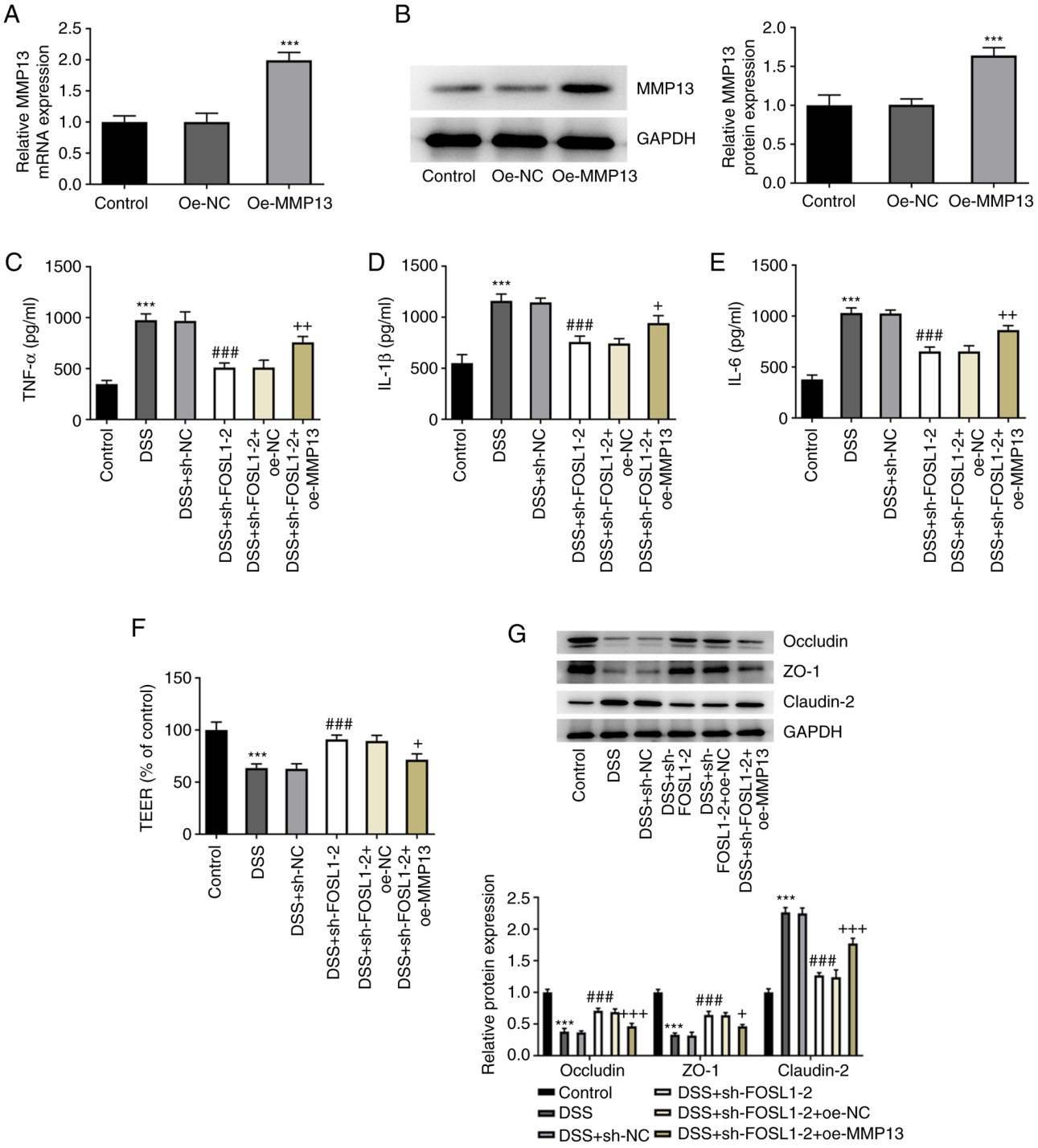

Figure 3. FOSL1 knockdown inhibits DSS-induced inflammation and barrier damage in HT29 cells via the downregulation of MMP13. MMP13 overexpression levels in HT29 cells were determined via (A) reverse transcription-quantitative PCR and (B) western blotting. ***P<0.001 vs. oe-NC. (C-E) Inflammatory factor levels in HT29 cells were detected via ELISA. ***P<0.001 vs. control; $^{###}$P<0.001 vs. DSS + sh-NC; $^{+}$P<0.05, $^{++}$P<0.01 vs. DSS + sh-FOSL1-2 + oe-NC. (F) Cell monolayer permeability was determined using the TEER assay. ***P<0.001 vs. control; $^{###}$P<0.001 vs. DSS + sh-NC; $^{+}$P<0.05 vs. DSS + sh-FOSL1-2 + oe-NC. (G) Tight junction protein expression levels in HT29 cells were determined via western blotting. ***P<0.001 vs. control; $^{###}$P<0.001 vs. DSS + sh-NC; $^{+}$P<0.05, $^{+++}$P<0.001 vs. DSS + sh-FOSL1-2 + oe-NC. FOSL1, Fos-like antigen-1; DSS, dextran sodium sulfate; oe, overexpression; NC, negative control; sh, short hairpin RNA; TEER, trans-epithelial electrical resistance assay.

that the colon tissues were diseased. However, the intestinal tissue structure of UC mice injected with sh-FOSL1 was markedly recovered, whereas MMP13 overexpression damaged the structure of the intestinal tissues of UC mice injected with sh-FOSL1. Furthermore, the DAI of the mice in the different groups was recorded. The results demonstrated that there was a marked increase in the DAI of UC mice compared with the control group (Fig. 4E). However, the UC mice injected with sh-FOSL1 exhibited a marked decrease in the DAI, but the overexpression of MMP13 increased the DAI. Subsequently, ELISA was performed and the results demonstrated increased levels of the inflammatory cytokines, TNF-α, IL-1β and IL-6 in the UC group, whereas decreased levels of TNF-α, IL-1β and IL-6 were demonstrated in the UC-sh-FOSL1 group.

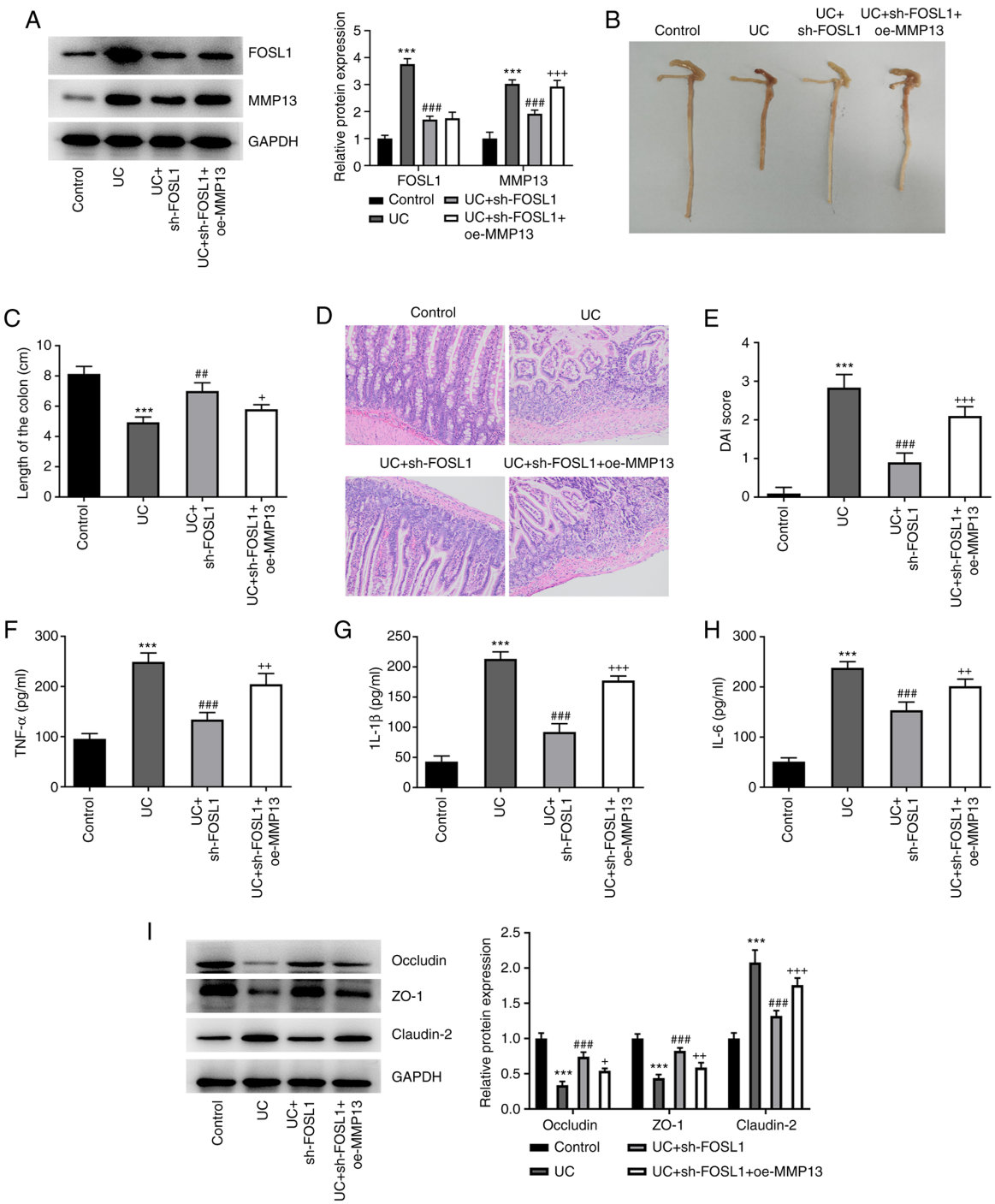

Figure 4. FOSL1 knockdown ameliorates DSS-induced colonic injury, inflammation and barrier damage in UC mice via the downregulation of MMP13. (A) FOSL1 and MMP13 protein expression levels in colonic tissues were determined via western blotting. ***P<0.001 vs. control; $^{###}$P<0.001 vs. UC; $^{+++}$P<0.001 vs. UC + sh-FOSL1. (B and C) Colon length in UC mice. ***P<0.001 vs. control; $^{##}$P<0.01 vs. UC; $^{+}$P<0.05 vs. UC + sh-FOSL1. (D) Colonic damage level was assessed using H&E staining. Magnification, x200. (E) Disease activity index. ***P<0.001 vs. control; $^{###}$P<0.001 vs. UC; $^{+++}$P<0.001 vs. UC + sh-FOSL1. (F-H) Serum inflammatory factors levels were determined via ELISA. ***P<0.001 vs. control; $^{###}$P<0.001 vs. UC; $^{++}$P<0.01, $^{+++}$P<0.001 vs. UC + sh-FOSL1. (I) Tight junction-associated protein expression levels in tissues were detected via western blotting. ***P<0.001 vs. control; $^{###}$P<0.001 vs. UC; $^{+}$P<0.05, $^{++}$P<0.01, $^{+++}$P<0.001 vs. UC + sh-FOSL1. FOSL1, Fos-like antigen-1; DSS, dextran sodium sulfate; UC, ulcerative colitis; sh, short hairpin RNA; NC, negative control; oe, overexpression.

In addition, elevated levels of TNF-α, IL-1β and IL-6 were demonstrated in the UC + sh-FOSL1 + oe-MMP13 group (Fig. 4F-H). Furthermore, the protein expression levels of occludin and ZO-1 were markedly decreased in the UC group, but were markedly increased in the UC + sh-FOSL1 group and slightly decreased in the UC + sh-FOSL1 + oe-MMP13 group (Fig. 4I). The expression levels of claudin-2 in each group exhibited a completely different trend from that of occludin and ZO-1. These results indicated that FOSL1 knockdown may have ameliorated DSS-induced colonic injury, inflammation and barrier damage in UC mice via the downregulation of MMP13.

Discussion

The incidence of UC in Asian populations has increased rapidly over the last few decades (21). The high incidence of UC is mainly due to inflammation and damage to the intestinal mucosal barrier (3). At present UC is an urgent global health problem and early prevention and treatment is critical (22). In the present study it was demonstrated that FOSL1 was a potentially key biological factor in UC, as determined by the following observations. First, the FOSL1 and MMP13 expression levels in DSS-induced HT29 cells and UC mice were markedly increased. Second, FOSL1 was demonstrated to bind to the MMP13 promoter region and consequently upregulated the MMP13 expression level. Third, FOSL1 knockdown inhibited DSS-induced inflammation and barrier damage via the downregulation of MMP13 expression levels in HT29 cells and UC mice.

FOSL1 is one of the major transcription factors of the AP-1 family and is upregulated in numerous malignancies, including prostate cancer, breast cancer and colon cancer (23-25). A previous study reported that FOSL1 was elevated in patients with mild UC (11). In the present study it was demonstrated that FOSL1 was highly expressed in DSS-induced HT29 cells and the serum of UC mice colon tissues. In addition, MMP-13 has previously been demonstrated to be a major protease in the pathogenesis of IBD-associated mucosal ulcers (26). A significant increase in MMP13 mRNA expression levels has also previously been reported in IBD biopsy specimens (15). The results of the present study demonstrated a significant three-fold increase in the expression levels of MMP13 in DSS-induced HT29 cells and the serum of UC mice colon tissues. Together, these data indicated that FOSL1 and MMP13 may serve key roles in UC. Furthermore, the promoters of most human MMP genes contain an AP-1 binding site at ~-70 bp and AP-1 has been broadly known to modulate the expression of MMP in numerous cell types (27). In the present study, the potential relationship between FOSL1 and MMP13 was predicted using the JASPAR database. It was demonstrated that the expression of MMP13 was increased following FOSL1 overexpression but decreased following FOSL1 knockdown. Furthermore, higher promoter region activity and relative MMP13 enrichment levels also indicated that FOSL1 interacted with the MMP13 promoter region, which upregulated MMP13 expression levels in DSS-induced HT29 cells and the serum and tissues of UC mice. In addition, the present study investigated colonic morphology and determined the DAI of UC mice based on their body weight, fecal condition and occult blood. It was reported that DSS induced a shorter colonic length, a severe level of colonic damage and resulted in a higher DAI. However, these conditions were alleviated by FOSL1 knockdown but were partially reversed by MMP13 overexpression. Therefore the results suggested that FOSL1 knockdown may have protected the colon of UC mice from DSS-induced injury via the downregulation of MMP13.

A dysregulated inflammatory response serves a key role in the development of UC (1). It has previously been reported that cells immersed in a chronic inflammatory cytokine environment can overproduce pro-inflammatory cytokines, such as TNF-α and IL-6 (28). TNF-α is elevated in the blood and mucosa of patients with UC (29). Furthermore, IL-6 transduction signaling stimulates inflammatory cells and stromal cells to induce colonic inflammation (1). The results of the present study demonstrated that DSS induced high levels of the pro-inflammatory cytokines, TNF-α, IL-1β and IL-6 in HT29 cells and UC mice. In addition, it has previously been reported that the increased secretion of IL-6 promotes the deacetylation of FOSL1 in colorectal cancer cell lines, which leads to increased FOSL1 expression (30). AP-1 regulates numerous pro-inflammatory cytokine genes, including TNF-α, IL-1 and IL-6, which perform a key role during chronic inflammation (31). It can therefore be hypothesized that reduced FOSL1 may affect the expression of pro-inflammatory cytokines. In the present study the knockdown of FOSL1 reduced the levels of TNF-α, IL-1β and IL-6 in DSS-induced HT29 cells and the serum of UC mice, which was consistent with the aforementioned report (32). However, this effect was partially reversed by MMP13 overexpression. These results further indicated that FOSL1 knockdown may have ameliorated DSS-induced inflammation in HT29 cells and the serum of UC mice via the downregulation of MMP13.

The tight junctions between intestinal epithelial cells serve an important role in maintaining intestinal mucosal permeability (4). However, in UC, damage to the epithelial barrier leads to increased permeability, which may be due to defects in the regulation of tight junctions (32). Furthermore, inflammatory cytokines can also mediate the abnormal expression of tight junction proteins due to the ability of TNF-α to abnormalize the function of ZO-1. Subsequently, this result in the disruption of the structure of the tight junction-associated protein claudin-1, which in turn disrupts other tight junction proteins and leads to the development of IBD (6). In the present study, it was demonstrated that cell monolayer permeability and the levels of tight junction-associated protein occludin and ZO-1 were markedly decreased, whereas the level of claudin-2 was markedly elevated in DSS-induced HT29 cells and the tissues of UC mice. In addition, the activation of AP-1 reduces ZO-1 expression in Caco-2 intestinal epithelial cells via the transcriptional repression of the ZO-1 promoter (33). However, the reduction of AP-1 may increase the expression levels of tight junction-associated proteins. In the present study, the increased expression levels of occludin and ZO-1 and decreased expression levels of claudin-2 also demonstrated that FOSL1 knockdown may alleviate barrier damage in DSS-induced HT29 cells and the tissues of UC mice. However, MMP13 overexpression partially reversed these effects. These results indicated that FOSL1 knockdown may have ameliorated DSS-induced barrier damage in HT29 cells and the tissues of UC mice via the downregulation of MMP13.

However, limitations still exist in the present study. Here, HT29 cell line, a colorectal cancer cell line, was applied for constructing colitis *in vitro*; however, the use of other non-cancerous cells to simulate colitis *in vitro* is deserved to be explored in future work. In addition, the clinical significance of FOSL1 in colitis needs to be verified urgently.

In conclusion, this present study assessed the impacts of FOSL1 on UC. The results demonstrated that FOSL1 knockdown may downregulate MMP13 expression levels to improve inflammatory damage and protect intestinal barrier integrity in DSS-induced HT29 cells and UC mice. The present study

has therefore provided a novel approach for the diagnosis and treatment of UC.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

LM designed the experiments. LM, XZ, CZ, BH and HZ conducted the experiments. LM, XZ and CZ analyzed and interpreted the data. LM and XZ drafted and revised the manuscript. LM and XZ confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

All experimental procedures were approved by the Ethics Committee of the North China University of Science and Technology Affiliated Hospital (approval no. Lx201896).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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