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Genetic Deletion of Fbw7 in the mouse intestinal epithelium aggravated dextran sodium sulfate–induced colitis by modulating the inflammatory response of NF-κB pathway

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ABSTRACT

Fbw7 is a type of E3 ubiquitin ligase that targets various proteins for degradation and has been found to have a high expression level in progenitor cells. Deletion of Fbw7 in the intestine results in the accumulation of progenitor cells. Moreover, Fbw7 loss increases the susceptibility of colorectal cancer. However, the involvement of Fbw7 in the progress and development of inflammatory bowel disease (IBD) is still controversial. To identify the function of Fbw7 on dextran sodium sulfate (DSS)-induced colonic inflammation, we generated Fbw7$^{\Delta G}$ mice, lacking Fbw7 specifically in intestinal epithelium. Colitis was induced in male Fbw7 $^{\Delta G}$ and wild-type (WT) mice (both age and body weight matched) by treating with 3% DSS in drinking water. We demonstrate that deletion of Fbw7 in the mouse intestinal epithelium aggravates DSS–induced colitis, showing inflammatory response and reduced survival rate. Furthermore, we found that Fbw7 loss caused activation of NF-κB signaling. Thus, FBW7 plays a protective role in acute intestinal inflammation by modulating the inflammatory response of NF-κB pathway.

1. Introduction

IBD, primarily including Crohn's disease and ulcerative colitis, is a group of chronic, potentially disabling diseases of the intestinal tract [1], which affect millions of people worldwide. The main clinical manifestations of IBD include weight loss, diarrhea and bloody stools, as well as colonic mucosal ulceration and recurrent abdominal pain [2,3]. The incidence of IBD is rising worldwide, especially in developed countries. However, many IBD treatments often cause additional side effects such as systemic immunosuppression. Thus, patients cannot receive long-term therapy, causing an urgent need to develop a more effective and safer therapeutic for IBD. Although the mechanism underlying IBD is not completely known, evidence shows that mucosal barrier dysfunction, largely caused by hyper-activation of effector immune cells, has a key role in IBD.

The active immune cells in chronic mucosal inflammation produce high levels of TNF-α, IL-6 and IL-1β, known as pro-inflammatory cytokines, causing damage to colonic tissue and barrier functions [4]. Nuclear factor-kappa B (NF-κB), which is markedly activated in IBD patients, has been identified to play a key role in mucosal inflammation regulation [5,6]. A large number of inflammatory mediators have been found to be induced by NF-κB and NF-κB is shown to be crucial in the transcription, which is related to the different immune responses. Pro-inflammatory cytokines such as TNF-α and IL-1β can inhibit the phosphorylation as well as degradation of IκB proteins and cause the abnormal expression of NF-κB. To study IBD, researchers generally use Dextran sulfate sodium (DSS) to induce colitis in animal models such as mice and rabbits, because the colitis model induced by DSS has similar symptoms to human IBD, which is defined by crypt damage, epithelial ulceration and mucosal edema [7–9]. Abnormal expression of NF-κB is activated in DSS-induced colitis model and then inflammatory mediators such as TNF-α are induced by NF-κB, causing damage to colonic tissue and barrier functions [10,11].

Fbw7 (F-box/WD repeat–containing protein 7) is a type of E3 ubiquitin ligase complex and has also been known as Fbxw7,

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hSel10, CDC4 and Ago [12]. Fbw7 has been reported to target various proteins, functioning in differentiation and proliferation, for degradation, including c-Jun, c-Myc, Notch and Cyclin E1 [13–16]. Because of its degradation effect on these oncogenic proteins, Fbw7 acts as a tumor suppressor in several types of cancer. The human FBW7 gene resides on chromosome 4q32 and produces three mRNAs, which encode three protein isoforms, Fbw7α, Fbw7β and Fbw7γ, under their own transcriptional control. The three Fbw7 isoforms have respective cellular localization: Fbw7α localizes to the nucleus, Fbw7β is mainly expressed in the cytoplasm and Fbw7γ localizes to the nucleolus [17].

The mechanism behind the function of Fbw7 as a tumor suppressor has been extensively studied. However, there are few studies on its function in other kinds of disease. To study the physiological function of Fbw7 in intestine, ROCIO et al. [18], generated Fbw7 knock-out mice which has a deletion of Fbw7 especially in the intestinal epithelia and then the research group evaluated the effect on homeostasis of intestine in the absence of Fbw7. Impaired differentiation of goblet cells and progenitor cells accumulation have been found when Fbw7 deletion in the intestine, indicating that Fbw7 regulates intestinal biology and may have effect on other intestinal diseases. Recently, studies have shown that FBW7α attenuates inflammatory signaling by down-regulating an inflammatory response gene C/EBPδ and its target gene Toll-like receptor 4 (Tlr4), identifying a novel role for FBW7 as a suppressor of inflammatory gene expression [19]. Based on those findings, our group thought that FBW7 may have effects on IBD. Therefore, we generated Fbw7 $^{\Delta G}$ mice to investigate the anti-inflammatory effects of FBW7 on a DSS-induced colitis mouse model. Our results show that deletion of FBW7 in the intestine promotes colitis induced by DSS through activating the NF-κB signaling and that FBW7 may be a new therapeutic target for IBD.

2. Materials and methods

2.1. Animals

We generated Fbw7$^{\Delta G}$ mice, lacking Fbw7 specifically in intestinal epithelium by crossing C57BL/6 mice which carry the floxed FBW7 gene (Fbw7$^{fl/fl}$) with C57BL/6 mice which carry the Cre recombinase and villin promoter (Vil/Cre) as previously described [18]. Mice were raised in cages with sterile water and food. The temperature is controlled at 21 °C and light is on 08:30–20:30. Male Fbw7$^{\Delta G}$ mice at the age of 8–9 weeks and body weight of 22.5–23.5 g were used in all the experiments. WT C57BL/6 mice, both age- and sex-matched, were used as control.

2.2. Induction of colitis

For acute colitis induction, mice were given 5 days of 3% (w/v) DSS solution in drinking water and DSS solution was replaced every 3 days. Weight loss, diarrhea and hemafecia were also recorded daily. For assessment of disease activity and immune-histochemical characterization, colon tissues were collected from Fbw7$^{\Delta G}$ and WT mice on the day 0, 5 and 7. The collected colon was gently washed with PBS (pH = 7.4) and the length was measured and recorded.

2.3. Assessment of disease activity index and inflammatory score

DAI was used to determine the severity and define response or remission of IBD and was the sum of weight loss index (0–4), stool consistency index (0–4), and fecal bleeding index (0–4), described in detail by Cooper, H. S [20] and are shown in table 1. Inflammatory score is a reflection of severity of inflammatory damage to colon and the detail assessment was shown in table 2.

2.4. Myeloperoxidase (MPO) activity

Colonic MPO activity was measured with

We use an MPO biochemical kit (Servicebio Technology CO. LTD, Wuhan, China) to detect the level of MPO activity of intestine epithelium. The intestinal epithelium were isolated on procedures as described [20].

2.5. Cytokine analysis by ELISA and quantitative RT-PCR

For analysis of cytokines in colon epithelial cells, we used ELISA kits (IL-6, IL-10, TNF-α; eBioscience) to determine the levels of cytokines in the colon. For experiments of qRT-PCR, Total RNA was extract from intestine epithelium using RNAiso Plus (Takara, Japan). Total RNA (500 ng) was reverse transcribed to cDNA, using SYBR Green PCR Light Cycler H. Denaturation temperature is 97 °C for 10 s. Annealing temperature is 56 °C for 25 s over 39 cycles. The primer sequences are shown in table 3.

2.6. Protein extraction and western blot analysis

20 μg protein samples were subjected to 12% SDS-PAGE gel electrophoresis and transferred to NC membrane, using a transfer apparatus. 2.5% milk solution was used to block the NC membranes for 1 h before incubating with antibodies for proteins of interest at 4 °C overnight. The NC membrane then was incubated with secondary antibody for another 1 h. Clarity Western ECL Substrate (Bio-Rad) was used to detect the protein–antibody complexes and ImageJ software was used to qualify the results.

2.7. Histology, immunohistochemistry, and immunofluorescence analysis

Paraffin sections of colons from Fbw7$^{\Delta G}$ and WT mice were cut into 5-μm sections and stained with HE, according to protocols. Then, we examined, analyzed and scored the samples under a light microscope as described [21,22]. For histopathology analysis, tissues were fixed in 3% paraformaldehyde solution for at least 10 h, then dehydrated and embedded in paraffin. For immunohistochemical staining, we dewaxed the section with xylene, and used graded alcohol to rehydrate. Then, we boiled the section in Na citrate buffer (0.01 M, pH 6) for 10 min. The sections were incubated at 37 °C for 30 min with secondary antibody conjugated with horseradish peroxidase. Diaminobenzidine was used to show color and followed by counterstaining with hematoxylin. For immunofluorescent staining, the sections were incubated with secondary antibody at 37 °C for 1 h.

2.8. Statistical analysis

Unpaired Student's *t*-test was used to compare the two mean values and one-way analysis was used to compare the 3 more groups. All data were considered significant at a value of $P < 0.05$.

3. Results

3.1. Clinical activity and survival analysis of DSS-treated Fbw7$^{\Delta G}$ and DSS-treated WT mice

To investigate the function of Fbw7 in the DSS-induced acute colitis, we generated Fbw7$^{\Delta G}$ mice, lacking Fbw7 especially in the intestinal tissue. Western blot analysis on protein isolated from different tissues demonstrated efficient fbw7 deletion in the intestinal epithelium in the Fbw7$^{\Delta G}$ mice. However, in other organs such as brain, lung and kidney, there was no significant difference