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# Sam68 modulates apoptosis of intestinal epithelial cells via mediating NF-κB activation in ulcerative colitis

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ABSTRACT

Sam68 (Src-associated substrate during mitosis of 68 KDa), also known as KHDRBS1 (KH domain containing, RNA binding, signal transduction associated 1), belongs to the prototypic member of the signal transduction activator of RNA (STAR) family of RNA-binding proteins. Sam68 is implicated in various cellular processes including RNA metabolism, apoptosis, signal transduction. Previous researches demonstrated that Sam68 regulated nuclear transcription factor kappa B (NF-κB) to induce inflammation. However, the expression and biological functions of Sam68 in ulcerative colitis (UC) are not clear. In this study, we reported for the first time that Sam68 was up-regulated in intestinal epithelial cells (IECs) of patients with UC. In DSS-induced mouse colitis model, we observed the overexpression of Sam68 accompanied with increased levels of IEC apoptotic markers (active caspase-3 and cleaved PARP) and NF-κB activation indicators (p-p65 and p-IκB) in colitis IECs. Co-localization of Sam68 with active caspase-3 (and p-p65) in IECs of the DSS-induced colitis group further indicated the possible involvement of NF-κB-mediated IEC apoptosis. Applying TNF-α-treated HT-29 cells as an in vitro IEC inflammation model, we confirmed the positive correlation amomg Sam68, NF-κB activation and caspase-dependent apoptosis. Immunofluorescence and immunoprecipitation assay identified nuclear translocation and physical interaction of Sam68 and NF-κB subunits in TNF-α-treated HT-29 cells. Besides, depletion of Sam68 by RNA interference greatly alleviated NF-κB activation and apoptosis in TNF-α-treated HT-29 cells. Taken together, our results indicated that Sam68 modulates apoptosis of intestinal epithelial cells via mediating NF-κB activation in UC.



## 1. Introduction

Inflammatory bowel diseases (IBD) have two major forms, ulcerative colitis (UC) and Crohn's disease (CD). Ulcerative colitis is one of chronic inflammatory disorders of human gastrointestinal tract, characterized by diffuse mucosal inflammation extended proximally from rectum to a varying degree, with clinical symptoms such as diarrhea, abdominal pain, bloody stools, and weight loss (Xavier and Podolsky, 2007; Conrad et al., 2014). A variety of epidemiological studies state that UC is commonly observed in westernized and industrialized countries (Geremia et al., 2014), and the incidence of UC in China has greatly increased recently (Ng et al., 2015). As no curative therapy currently exists, some concerns have been raised to effective treatment in patients with UC nowadays (Dignass et al., 2012). Although exact etiology and pathology of UC are not completely understood, certain studies have shown that immunological dysregulation, genetic predisposition, and environmental factors all contribute to the pathogenesis of UC, and the intestinal epithelial homeostasis is the frontline among these factors (Ordas et al., 2012). Epithelial homeostasis acts as a first line of the defense against a variety of harmful substances including bacteria and luminal antigens in the guts. In UC, persistently increased expression of mucosal inflammatory cytokines and dense leukocyte infiltration lead to epithelial homeostasis destruction, including IEC apoptosis and subsequent crypt hyper-proliferation, which contribute to widespread tissue ulceration and epithelial hyperplasia (Koch and Nusrat, 2012). It is the evidence that elevated IEC apoptosis induced by inflammation takes part in the damage of epithelial homeostasis.

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However, the molecular mechanisms that involve in the regulation of IEC apoptosis in UC remain unclear.

Sam68 (Src-associated substrate during mitosis of 68 KDa), also known as KHDRBS1 (KH domain containing, RNA binding, signal transduction associated 1) is the prototypic member of the STAR (signal transduction activator of RNA) family of RNA-binding proteins, which regulate RNA metabolism, apoptosis, signal transduction (Sanchez-Jimenez and Sanchez-Margalet, 2013). In view of being highly restricted cells of epithelial origin, Sam68 is expressed in several tissues including breast, kidney, cervix, prostate, colon and rectum, with particularly high expression in the gastrointestinal tract (Li et al., 2012; Morishita et al., 2011). Sam68 is implicated in various pathophysiologic processes, including inflammatory disorders and cancer (Paronetto et al., 2007; Song et al., 2010). Sam68 belongs to the STAR family of RNA-binding proteins, which contain a GSG domain, and can interact with both RNA targets and other proteins (Elliott and Rajan, 2010). For instance, Sam68 can interact with signaling proteins such as Src, Grb2, Fyn, BRK and PI3K (Quintana-Portillo et al., 2012). Additionally, Sam68 interacts with NF-κB to promote the expression of CD25 in activated T lymphocytes (Fu et al., 2013). Moreover, interaction between Sam68 with NF-κB results in activation of NF-κB to induce apoptosis in articular chondrocytes during osteoarthritis (Xu et al., 2015). Recently, clinical trials demonstrated that the expression and nuclear translocation of Sam68 can be induced by inflammatory cytokines. Moreover, nuclear translocation of Sam68 participates in progression of human colorectal cancer (Liao et al., 2013). Based on above studies, we put forward the presumption that Sam68 constitutively activates NF-κB to regulate IEC apoptosis.

Collectively, since NF-κB signaling and apoptosis is highly activated in UC pathology and Sam68 acts as a modulator of NF-κB signaling and apoptosis, we asked the question whether Sam68 stimulates NF-κB signaling, apoptosis and ultimately accelerates catabolic events in UC. The aim of this study was to analyze whether and how Sam68 affects NF-κB signaling and IEC apoptosis in UC patients and experimental UC model.

## 2. Materials and methods

### 2.1. Mucosal biopsy specimens

The study was performed at the Department of Gastroenterology of the Affiliated Hospital of Nantong University from September 2014 to June 2015 under a protocol approved by its ethical committee. Mucosal biopsy specimens were prospectively collected from inflamed and non-inflamed areas of UC patients with clinically and macroscopically active UC (n = 40), while control samples were obtained from the normal areas of healthy subjects (n = 40). Biopsy specimens were immediately fixed in formalin and embedded in paraffin until use for immunohistochemistry analysis. Written informed consent was obtained before specimen collection.

### 2.2. Animals and experimental colitis

Animal experiments were performed in accordance with National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (National Research Council, 1996, USA) and were approved by the Chinese National Committee to Use of Experimental Animals for Medical Purposes, Jiangsu Branch. We obtained seven- to eight-week-old female C57BL/6 mice (n = 24) weighing 18–20 g from the Experimental Animal Center of Nantong University. Induction of acute, recovery and chronic dextran sulfate sodium (DSS) colitis was performed as described previously, and depicted in Fig. 2a (Koch et al., 2013; Nava et al., 2010; Koch et al., 2011). In brief, 2.5% or 4% DSS wt/vol (molecular weight, 36,000–50,000; MP Biomedicals) was dissolved in tap water and given ad libitum. Animals were monitored for changes in body weight, stool consistency, and presence of blood in the stool. Mice were sacrificed after 7 days (acute phase), 10 days (recovery phase), or 4 weeks (chronic phase).

### 2.3. Assessment of colitis activity

To examine the severity of colitis, we evaluated disease activity index (DAI), which was determined by scoring changes in animal weight, gross bleeding, occult blood, and stool consistency. We used five grades of weight loss (0, no loss or weight gain; 1, 1–5% loss; 2, 5–10% loss; 3, 10–20% loss; 4, 20% loss), three grades of stool consistency (0, normal; 2, loose; and 4, diarrhea), and three grades of occult blood (0, negative; 2, occult blood-positive; and 4, gross bleeding). After determination of the DAI, mice were euthanizing, the entire colon was removed from the cecum to the anus. Subsequently, colonic tissue was fixed in 4% buffered formalin, embedded in paraffin and stained with hematoxylin and eosin. According to a well-established scoring system described previously, histological score on microscopic cross sections was assigned (Neurath et al., 1995).

### 2.4. Cell culture and stimulation

The human colon epithelial cell line HT-29 was purchased from Cell library, China Academy of Science. HT-29 cells were grown in 1640 (GibCoBRL, Grand Island, NY, USA) medium containing 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin (GibCo BRL, Grand Island, NY, USA). Cultures were incubated at 37 °C in a 95% air/5% $CO_2$ atmosphere. Cells were treated with different concentrations (0.1, 1, 10, 100 ng/ml) of TNF-α (Sigma-Aldrich, USA). Cultured HT-29 cells were collected, washed with phosphate buffer solution (PBS) and suspended in hypotonic buffer to achieve nuclear extracts. The cultured cells were homogenized, meanwhile nuclei were pelleted. Then we removed the cytoplasmic extracts and resuspended nuclei in a low-salt buffer. A high-salt buffer was added to release soluble proteins from the nuclei, and the nuclei were removed by centrifugation. The nuclear extracts were dialyzed into a moderate salt solution.

### 2.5. Immunohistochemical studies

Mice colon samples were freshly isolated and frozen in O.C.T (Sakura Finetek, USA) or fixed in 10% neutral buffered formalin and embedded in paraffin wax. Immunohistochemical studies were performed using paraffin-embedded sections (4.5 μm). All sections were deparaffinized, rehydrated, and thereafter, the sections were processed in 10 mM citrate buffer (pH 6.0) and heated to 121 °C in an autoclave for 20 min to retrieve the antigen. After rinsing in PBS, sections were first treated with 3% hydrogen peroxide to block endogenous peroxidase activity, and then blocked by 1.5% normal goat serum for 15 min. For analysis for Sam68, sections were incubated 2 h at room temperature (RT) with primary antibody against Sam68 (1:500). All slides were processed using the peroxidase anti-peroxidase method (DAKO, Hamburg, Germany). After rinsing in PBS, peroxidase reaction was visualized by incubating the sections with the liquid mixture DAB. After rinsing in water, the sections were counterstained with hematoxylin, dehydrated, and cover slipped.

### 2.6. Western blot analysis

For Western blot analysis, colon tissues were dissected and flash-frozen at −80 °C. To prepare the lysates, frozen samples were weighed and minced on ice. The samples were homogenized in