Pathology - Research and Practice xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Activating transcription factor 3 promotes intestinal epithelial cell apoptosis in Crohn's disease

Liugen Gu[a,*], Zhenming Ge[a], Yamin Wang[a], Meiqin Shen[a], Ping Zhao[b]

[a] Department of Gastroenterology, The Second Affiliated Hospital of Nantong University, Nantong 226001, Jiangsu, China
[b] Department of Clinical Laboratory, The Second Affiliated Hospital of Nantong University, Nantong 226001, Jiangsu, China

ARTICLE INFO

Keywords:
Activating transcription factor 3
Crohn's disease
Intestinal epithelial cell
Apoptosis
p53
Bax

ABSTRACT

Intestinal epithelial cell (IEC) apoptosis plays a vital role in the pathogenesis of Crohn's disease (CD), which is an inflammatory bowel disease (IBD). Activating transcription factor 3 (ATF3) modulates apoptosis under stress via regulating the p53 pathway. However, the expression and function of ATF3 in CD are unclear. In the present study, ATF3, p53, and p53 target gene Bax expression increased in CD patients; a mouse 2, 4, 6-trinitrobenzenesulfonic acid (TNBS)-induced CD model; and a TNF-α-treated HT29 cell colitis model. ATF3 knockdown effectively decreased TNF-α-induced p53 and Bax expression, as well as inhibited the apoptosis of HT29 cells. Additionally, ATF3 enhanced the stability and transcription activity of p53 via interacting with p53. In summary, these data indicated that ATF3 might promote IEC apoptosis in CD via up-regulating the stability and transcription activity of p53, implying a novel molecular target for CD therapy.

1. Introduction

Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), is one of the most common inflammatory diseases in China [1,2]. Apoptosis causes intestinal epithelial cell (IEC) shedding, which in turn leads to barrier loss [3]. Abnormal apoptosis detected in the intestinal epithelium of IBD patients [4] is considered one of the major courses that accelerates IBD. Several animal studies further confirm the central role of IEC apoptosis in the pathogenesis of CD. For instance, the conditional signal transducer and activator of transcription 3 (STAT3) knockout of intestinal epithelial cell (IECs) mice are highly susceptible to experimental colitis, with important defects in epithelial restitution and enhanced apoptosis [5]. Aberrant intestinal epithelial cell (IEC) apoptosis impairs the mucosal barrier, which leads to intestinal hyper-permeability, invasion of luminal antigens and commensal microflora, and triggers the production of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α). TNF-α further induces IEC apoptosis, and this vicious feedback eventually results in the clinical signs and symptoms of IBD [6,7].

Activating transcription factor 3 (ATF3), a member of the ATF/CREB family of transcription factors [8], is maintained at a low level in normal and quiescent cells. As a highly versatile stress sensor, ATF3 is induced by a variety of stresses including DNA damage, oxidative stress and endoplasmic reticulum stress [9]. Therefore, ATF3 acts as an adaptive response gene participating in a variety of cellular processes including immune response [10], atherogenesis [11], cell cycle [12], glucose homeostasis [13], and apoptosis [14]. With regard to apoptosis, ATF3 has double pro-apoptotic or anti-apoptotic roles depending on cell type and physiologic circumstances. ATF3 plays tumor suppressing roles in different cancer types, including colon cancer [15] and esophageal squamous cell carcinomas (ESCC) [16]. In contrast, ATF3 functions as a tumor promoter in hepatocytes [17] and breast cancer [18]. However, the relationship between ATF3 and IEC apoptosis in CD is unknown.

ATF3 contains a central leucine zipper domain (Zip) that is well characterized as a mediator of protein–protein interaction [19]. ATF3 binds to p53 via this domain, and as a consequence, p53 ubiquitination catalyzed by E3 ubiquitin ligase murine double minute 2 (MDM2) [20,21] is blocked, leading to up-regulation of p53 tumor suppressor activity, independent of ATF3 transcriptional activity [22]. Exposure to cellular stress triggers transcription factor p53 to induce cell growth arrest [23] or apoptosis [24]. The intrinsic apoptotic pathway is dominated by the B cell lymphoma-2 (Bcl-2) family of proteins, which govern the release of cytochrome c from the mitochondria [25]. BCL2-associated X (Bax) is the first member of this group shown to be induced by p53 [26]. It is revealed that p53 expression is up-regulated in non-cancerous tissues of CD patients [27]. Bax expression also dramatically increases in rat dextran sulfate sodium (DSS)-induced colitis model [28].

Here, we show that ATF3 might promote IEC apoptosis in CD via up-

* Corresponding author.
E-mail address: guliugen@ntu.edu.cn (L. Gu).

https://doi.org/10.1016/j.prp.2018.04.013
Received 28 January 2018; Received in revised form 6 April 2018; Accepted 17 April 2018

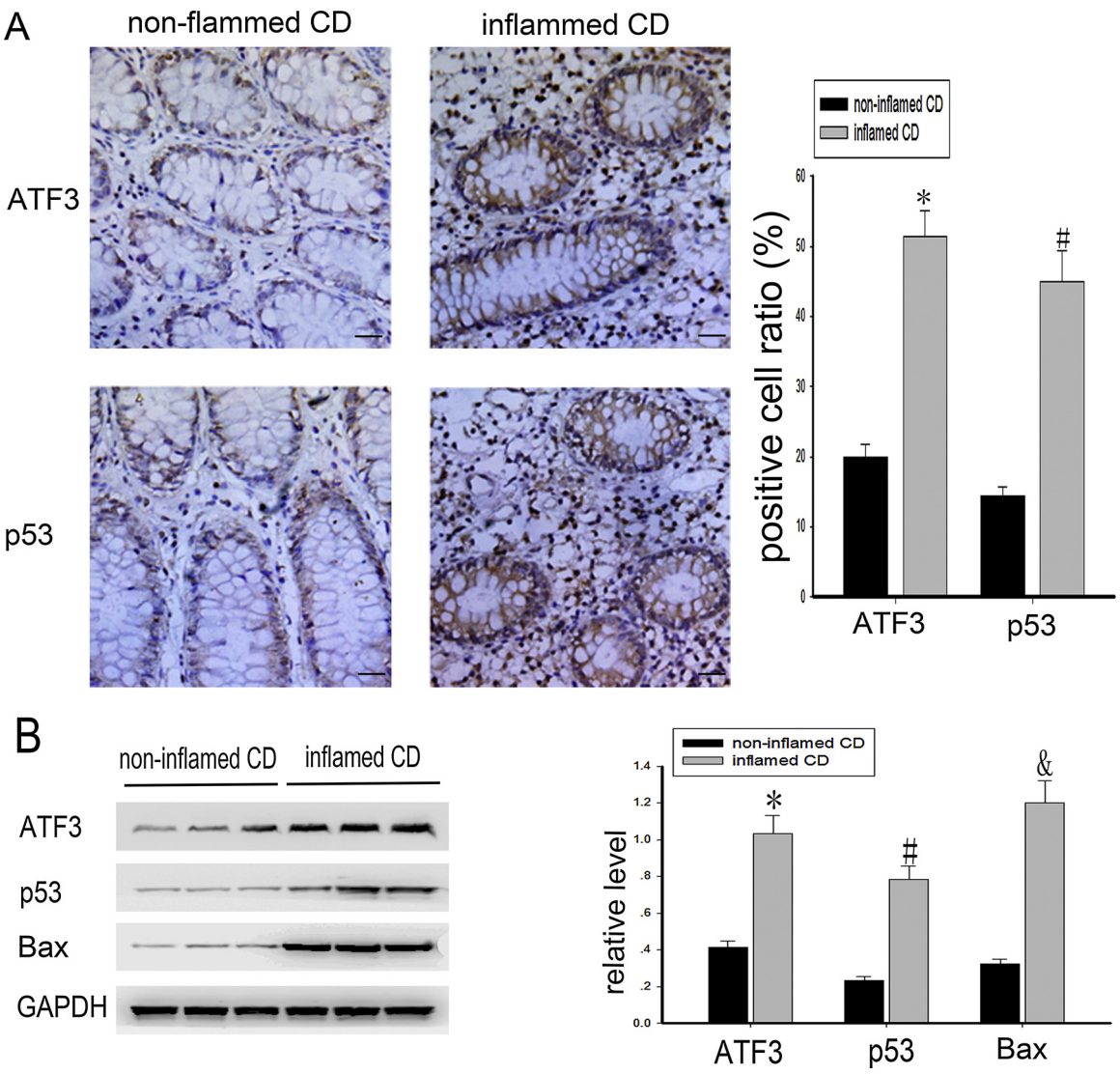

Fig. 1. ATF3 is up-regulated in the inflamed region of intestinal tissues from CD patients. (A) IHC analysis of ATF3 and p53 in mucosal biopsies from inflamed (n = 10) and non-inflamed tissues (n = 10). Scale bar = 100 μm. The bar graph indicates the positive cell ratio of AFT3 and p53. $^{*,\#}P < 0.05$ vs non-inflamed tissues. (B) ATF3, p53 and Bax expression in non-inflamed and inflamed colon tissues from healthy control and CD patients, respectively, were detected by western blot. GAPDH was used as a loading control. The bar chart shows quantification of ATF3, p53 and Bax protein level relative to GAPDH. n = 3, $^{*,\#,\&}P < 0.05$ vs non-inflamed tissues.

regulating the stability and transcription activity of p53 using CD patient colon tissues; a mouse 2,4,6-trinitrobenzenesulphonic acid (TNBS)-induced colitis model; and an HT29 cell inflammatory model. Our results present a novel mechanism of IEC apoptosis in CD.

2. Materials and methods

2.1. Human intestinal tissues

The intestinal tissue specimens from the terminal ileum and rectum were obtained from patients with newly diagnosed CD (n = 10) and healthy subjects with non-inflammatory conditions of the gastrointestinal tract (n = 10) under endoscopy at the Second Affiliated Hospital of Nantong University from 2014 to 2017. The CD patients had diagnoses based on standard criteria, including clinical presentation as well as endoscopic, radiologic, and/or pathologic confirmation [29]. CD patients who had infectious colitis or colorectal cancer or who had received anti-inflammation drug therapy within 6 months were excluded. Some biopsy specimens were used for western blot, and other biopsies were immediately fixed in formalin and embedded in paraffin until use for immunohistochemistry analysis. Written informed consent was obtained before specimen collection.

2.2. Mouse colitis model

All animal care and surgical procedures were performed according to Guide for the Care and Use of Laboratory Animals promulgated by National Research Council in 1996 and supported by the Chinese National Committee for Use of Experimental Animals for Medical Purposes, Jiangsu Branch. BALB/c mice (8–10 weeks, weight 18–20 g) were obtained from the Department of Experimental Animal Center, Nantong University. Chemical colitis was induced by 2,4,6-trinitrobenzenesulfonic acid (TNBS; Sigma Chemical Co., St. Louis, MO, USA). As previously described [30], mice were fasted for 24 h. Then, they were anesthetized through intraperitoneal injection of sodium pentobarbital (0.3% solution). A 3.5 F catheter was carefully inserted through the anus into the colon, and the tip was 4 cm proximal to the anal verge. To induce colitis, 0.1 ml of 2.5% (w/v) TNBS solution in 50% ethanol was injected slowly into the lumen of the colon via a catheter fitted to a 1 ml syringe. In the EtOH (ethanol) group, mice