RTCB deficiency triggers colitis in mice by influencing the NF-κB and Wnt/β-catenin signaling pathways

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RTCB deficiency triggers colitis in mice by influencing the NF-κB and Wnt/β-catenin signaling pathways

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Running title: RTCB deficiency triggers colitis via NF-κB/Wnt/β-catenin
Abstract

RNA terminal phosphorylase B (RTCB) has been shown to play a significant role in multiple physiological processes. However, the specific role of RTCB in the mouse colon remains unclear. In this study, we employed a conditional knockout mouse model to investigate the effects of RTCB depletion on the colon and potential molecular mechanisms. We assessed the efficiency and phenotype of \textit{Rtcb} knockout using PCR, Western blot, histological staining, and immunohistochemistry. Compared with the control mice, the \textit{Rtcb} knockout mice exhibited compromised colonic barrier integrity and prominent inflammatory cell infiltration. In the colonic tissues of \textit{Rtcb} knockout mice, the protein levels of TNF-\(\alpha\), IL-8, and p-p65 were increased, whereas the levels of IKK\(\beta\) and I\(\kappa\)B\(\alpha\) were decreased. Moreover, the level of GSK3\(\beta\) was increased, whereas the levels of Wnt3a, \(\beta\)-catenin, and LGR5 were decreased. Collectively, our findings unveil a close association between RTCB and colonic tissue homeostasis and demonstrate that RTCB deficiency can lead to dysregulation of both the NF-\(\kappa\)B and Wnt/\(\beta\)-catenin signaling pathways in colonic cells.
Keywords: RTCB; colitis; NF-κB; Wnt/β-catenin; LGR5

Introduction

RNA terminal phosphate cyclase B (RTCB), also known as FAAP, HSPC117, C22orf28, and D10Wsu52e, is a ligase that specifically connects the 2’,3’-cyclic phosphate and 5’-hydroxyl terminus of RNA[1, 2]. RTCB is a highly conserved protein with a unique structure. It is present in archaea, bacteria, and animals but absent in plants and fungi[3]. In archaea and mammals, RTCB appears to serve as a catalyst in tRNA splicing[2, 4]. In eukaryotes, RTCB is also involved in the unfolded protein response (UPR) by influencing the splicing of Xbp1 mRNA[5].

RTCB has been shown to play a significant role in multiple physiological processes. In embryonic development, inhibition of RTCB in mouse embryos resulted in embryonic death and placental dysplasia[6]. In reproductive function, while worms can grow to adulthood but fail to generate oocytes after knockdown of Rtcb [7], mice have premature ovarian failure after conditional knockout of Rtcp[8], and RTCB is specifically highly expressed in the initial segment of the mouse epididymis[9]. In immune function, specific knockout of Rtcp in mouse B cells led to abnormal cell proliferation and differentiation and disrupted the structure of the endoplasmic reticulum (ER), ultimately affecting the secretion of some antibodies[5]. In neuroprotection, RTCB could ameliorate damage to dopaminergic neurons in a worm
model of Parkinson’s disease[10]. A previous study showed that RTCB is widely expressed in intestinal tissues[11]; however, its function is unknown.

Colitis is an inflammatory disease of colonic tissues induced by various factors. Inflammation extends from the rectum to the proximal colon from onset, causing persistent superficial mucosal inflammation of varying degrees [12]. Inflammatory bowel disease (IBD) includes ulcerative colitis (UC) and Crohn’s disease (CD). UC is most common in industrialized countries, and its incidence has been increasing in Asia[13, 14]. The total incidence and prevalence of UC are reported to be 0.0012%–0.0203% and 0.0076%–0.245% per year, respectively[15]. The differences in incidence among races are more related to environment, diet, and lifestyle. There is no significant sex difference in UC patients[16]. The onset time of UC has a bimodal distribution, with the first peak at 20–30 years old and the second peak at 50–80 years old[17]. The MAPK, NF-κB and Wnt/β-catenin signaling pathways play pivotal roles in the pathogenesis of colitis and its associated disorders[18, 19]. Alterations in these signaling pathways are commonly concurrent with the onset of colitis[20, 21].

Ubiquitin is a small protein that is very conserved in eukaryotes[22]. It has been shown that the promoter of ubiquitin C (UBC) is highly active in conferring expression of exogenous genes in various cell lines after transient transfection of appropriate expression vectors[23]. Most importantly, the UBC promoter retains high activities in tissues where some other promoter or enhancer sequences are inactive[24]. Cre-ERT2 is a fusion protein composed of Cre recombinase and a mutant form of the estrogen
receptor that is selectively activated only in the presence of tamoxifen[25] Therefore, in UBC-CreERT2 transgenic mice, interest genes controlled by the Cre cleavage site can be knocked out in adulthood under tamoxifen induction.

The aim of this study was to explore the function of RTCB and its molecular basis in the colon. Therefore, we constructed a conditional Rtcb knockout mouse model using the UBC-CreERT2 tool mouse and observed lesions in the colon tissues after Rtcb knockout. Interestingly, the colitis caused by RTCB deficiency may not depend on the XBP1-UPR pathway but on the NF-κB and Wnt/β-catenin signaling pathways.

Materials and Methods

Generation of Rtcb<sup>fl/fl</sup>;UBC-CreERT2 mice

Loxp sites were inserted on both sides of the fourth exon of the Rtcb gene to generate Rtcb<sup>fl/fl</sup> homozygous mice. Rtcb<sup>fl/fl</sup> mice and UBC-CreERT2 mice were bred to generate Rtcb<sup>fl/w</sup>;UBC-CreERT2 heterozygous mice, which were then backcrossed with Rtcb<sup>fl/fl</sup> mice to generate Rtcb<sup>fl/fl</sup>;UBC-CreERT2 mice and control F2 Rtcb<sup>fl/fl</sup> mice. To activate CreERT2 for Rtcb knockout, these mice were intraperitoneally injected with 20 mg/mL tamoxifen (TAM; Sigma) (75 mg/kg) for five consecutive days. The physiological conditions of the mice were monitored. The experiments were conducted immediately after the mice were deceased.
All mice (C57BL/6J; Shandong Normal University, Jinan, China) used were adult males at 6 weeks after birth. The mice were raised in the animal house of Shandong Normal University and fed freely. The environment was a 12-hour light-dark cycle system (light from 7:00 to 19:00) with a temperature of 21–24°C and humidity of approximately 55%. The mouse feed was provided by Jinan Xingkang Biotechnology (Jinan, China). This study was approved by the Animal Ethics Committee of Shandong Normal University under admissible number AEECSDNU 2021026.

Genotyping

We generated $Rtcb^{f/f};UBC$-CreERT2 transgenic mice allowing for conditional deletion of $Rtcb$ in adult mice. $Rtcb^{f/f}$ mice were crossed with $UBC$-CreERT2 mice to obtain $Rtcb^{f/f};UBC$-CreERT2 mice in the offspring (Supplementary Figure S1A). To determine the genotype of the mice, the primers loxP1, loxP2, loxP3, Frt3, Frt4, Frt5, and Frt6 were designed based on the loxP sites (Supplementary Figure S1B). To detect the presence of UBC-CreERT2, the primers Universal-Cre-F and Universal-Cre-R were designed. To verify the efficiency of knockout, the primers loxP5 and Frt2 were designed. Genotyping of the mice was performed using PCR analysis, and the genotype of $Rtcb^{f/f};UBC$-CreERT2 transgenic mice was determined based on the size of the PCR product bands (Supplementary Figure S1C). The PCR procedure was as follows: predenaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 45 s, denaturation at 60°C for 45 s, and extension at 72°C for 30 s, followed by incubation at 72°C for 8 min and retention at 10°C. The sequences of the primers and the sizes of
their products are shown in Table 1.

**Western blot**

Colon tissues from TAM-treated mice were isolated and lysed in RIPA lysis buffer (150 mM NaCl, 1% NP-40, 0.5% deoxycholic acid, 0.1% SDS, 50 mM Tris, pH 7.4, and 1% PMSF). The lysates were then sonicated and centrifuged. Protein concentrations were quantified using a BCA Protein Concentration Determination Kit (AR0146; Boster). Equal amounts of protein samples were loaded for 10%, 12.5% and 15% SDS-PAGE and transferred onto polyvinylidene fluoride membranes. The membranes were blocked with 8% milk and incubated with primary antibodies at 4°C overnight and then with the appropriate enzyme-labelled secondary antibodies at room temperature for 1 h. The signals were developed using an Omni-ECL™ Femto Light Chemiluminescence Kit (SQ201; EpiZyme). The images were captured with a Tanon-5200 imaging system (Tanon, Shanghai, China).

**Histology and immunohistochemistry**

For histological analysis, the colon tissues were fixed with PFA and Bouin’s solution, embedded in paraffin, cut into 5 μm sections, and finally stained with hematoxylin and eosin (H&E; Solarbio; Beijing, China).

For immunohistochemical analysis, the paraffin sections were deparaffinized and rehydrated using standard methods. The slides were treated as follows: placed in Tris-EDTA (pH 9.0) solution, heated at 95°C for 10 min cooled at room temperature for 20
min, soaked in 3% H$_2$O$_2$ for 15 min, blocked with 1% BSA for 1 h, incubated with the primary antibody rabbit anti-E-cadherin (24E10; Cell Signaling) at 4°C overnight, incubated with the secondary antibody for 1 h, stained with the DAB Chromogen solution (ZLI-9018; ZSGB-BIO), the Alcian Blue solution (G1027-100ML; Servicebio) for 15 min, and the Nuclear Fast Red solution (G1035-100ML; Servicebio) for 5 min, and finally observed under an E100 light microscope (Nikon, Tokyo, Japan).

**Statistical Analysis**

The Western blot results were analysed for grayscale using ImageJ software. The data obtained were analysed using Student’s $t$ test in GraphPad Prism 8.0 statistical software. $P < 0.05$ represented a statistically significant difference.

**Results**

1. TAM treatment resulted in weight loss and animal death of $Rtcp^{f/f};UBC$-CreERT2 mice

To induce $Rtcp$ knockout, adult $Rtcp^{f/f}$ and $Rtcp^{f/f};UBC$-CreERT2 mice were intraperitoneally injected with TAM for 5 consecutive days. After TAM treatment, the mice were kept under normal conditions for 7 days. Their body weights were recorded daily (the weight on day 1 of injection was set as 100%). The $Rtcp^{f/f};UBC$-CreERT2 mice maintained a stable weight during the 5 days of TAM injection but suffered
significant weight loss thereafter, whereas the \( \text{Rtcb}^{\text{fr}} \) mice maintained a stable weight throughout (Figure 1A). Furthermore, the \( \text{Rtcb}^{\text{fr}};\text{UBC-CreERT2} \) mice died after TAM treatment, whereas the \( \text{Rtcb}^{\text{fr}} \) mice survived (Figure 1B).

2. TAMs successfully induced \( \text{Rtcb} \) knockout in \( \text{Rtcb}^{\text{fr}};\text{UBC-CreERT2} \) mice

To check whether the \( \text{Rtcb} \) gene was knocked out in the transgenic mice treated with TAM, we extracted genomic DNA from various tissues, including heart, liver, lung, kidney, and colon, of the \( \text{Rtcb}^{\text{fr}} \) and \( \text{Rtcb}^{\text{fr}};\text{UBC-CreERT2} \) mice and performed PCR analysis with specific primers (Table 3). In the samples obtained from the TAM-treated \( \text{Rtcb}^{\text{fr}} \) mice, only one DNA fragment of 1237 bp was detected. However, in the samples obtained from the TAM-treated \( \text{Rtcb}^{\text{fr}};\text{UBC-CreERT2} \) mice, two DNA fragments of 1406 bp and 624 bp were detected (Figure 2B). Furthermore, \( \text{Rtcb} \) knockout was confirmed at the protein level (Figure 2C,D). This demonstrated that TAM treatment successfully induced \( \text{Rtcb} \) knockout in multiple tissues of \( \text{Rtcb}^{\text{fr}};\text{UBC-CreERT2} \) mice.

3. RTCB deficiency led to colonic barrier damage in mice

Histological analysis was performed to examine the impact of RTCB deficiency on the colon. We dissected the TAM-treated mice and observed a shortened colon length accompanied by minor bleeding in the \( \text{Rtcb}^{\text{fr}};\text{UBC-CreERT2} \) mice but not the \( \text{Rtcb}^{\text{fr}} \) mice (Figure 3A). H&E staining revealed that after \( \text{Rtcb} \) knockout, colonic tissues exhibited a significant loss of goblet cells and an occasional loss of epithelial
cells in the mucosa, slight dilation and deformation of intestinal glands in the lamina propria, and scattered infiltration of inflammatory cells (Figure 3B).

Intestinal barrier homeostasis is closely associated with adherens junctions and tight junctions between intestinal epithelial cells (IECs). The formation of adherens junctions depends on the interaction between cadherins and catenins, with E-cadherin being a crucial molecule[26]. Then, we examined the integrity of the colonic barrier and the expression of E-cadherin. The results showed that RTCB deficiency resulted in a destruction of epithelial cell adhesion and a reduction in goblet cells and acidic mucus substances, indicating that the colonic barrier was damaged (Figure 3C,D). These data indicated that RTCB involved in structure and intestinal epithelial maintain of colon.

4. RTCB deficiency results in colonic inflammation in mice

To investigate whether the damage to the colonic barrier was caused by increased apoptosis, we assessed the expression of cleaved caspase-3 (CC-3) in the colon tissues of Rtcb<sup>−/−</sup>;UBC-CreERT2 mice (Figure 4A). The decrease of CC3 indicated that RTCB deficiency caused a decrease rather than an increase in apoptosis. Furthermore, to examine whether RTCB deficiency increased inflammation in colon tissues, we measured the protein expression of IL-8 and TNF-α, two markers of colonic inflammation, in the colon tissues of Rtcb<sup>−/−</sup>;UBC-CreERT2 mice. The significant increase of these two cytokines (Figure 4C–F) showed that RTCB deficiency could induce colonic inflammation in mice. These data indicated that RTCB deficiency resulted in colonic inflammation in mice rather than apoptosis.
5. XBP1 deficiency did not induce colonic inflammation

Xbp1 mRNA is the only known substrate of RTCB. However, we found that Rtcb knockout did not significantly affect the expression of the XBP1-S protein (Figure 5A,B). Additionally, to explore the effects of XBP1 deficiency on the colon, we generated Xbp1<sup>f/f</sup>;UBC-CreERT2 mice. The survival rate and body weights of the mice were monitored during TAM treatment. All mice were alive, and their body weights remained relatively stable (Figure 5C,D). Then, we validated the Xbp1 knockout at the protein level (Figure 5E,F). After conducting histological analysis, we observed neither colon shortening nor hemorrhage in the Xbp1<sup>f/f</sup>;UBC-CreERT2 mice. Moreover, there were no discernible pathological changes or damage to the colonic barrier (Figure 5G–J). These data indicated that RTCB deficiency could not lead to such severe colitis through the XBP1-dependent signalling pathway.

6. NF-κB signaling pathway was activated in the colonic tissues of Rtcb Knockout Mice

RTCB deficiency resulted in increased expression of IL-8 and TNF-α (Figure 4C,E), which are downstream signaling molecules of the NF-κB pathway that is typically activated during inflammatory responses. Thus, we examined the expression of IKKβ, IκBα and p-p65, the key molecules in the NF-κB pathway. The increase in p-p65 and the decrease in IKKβ and IκBα (Figure 6A–E) indicated that the NF-κB
pathway was activated in the colonic tissues of $Rtcb^{0/};UBC$-CreERT2 mice.

7. Wnt/β-Catenin signaling pathway was inhibited in the colonic tissues of $Rtcb$ knockout mice

RTCB deficiency led to impaired epithelial adhesion in colonic tissues (Figure 3), and β-catenin is one of the key molecules involved in adherens junctions and a downstream component of the canonical Wnt signaling pathway. Thus, we examined the expression of Wnt3a, GSK3β and β-catenin, the key molecules in the Wnt/β-catenin pathway. The increase in GSK3β and the decrease in Wnt3a and β-catenin (Figure 7A–F) indicated that the Wnt/β-catenin pathway was inhibited in the colonic tissues of $Rtcb^{0/};UBC$-CreERT2 mice.

Intestinal stem cells (ISCs) play a crucial role in intestinal damage repair and barrier maintenance. Leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5) is a marker of colonic stem cells expressed at the crypt base and regulated by β-catenin. Thus, we also examined the expression of LGR5. The decrease in LGR5 (Figure 7G,H) indicated a loss of colonic stem cells in the $Rtcb^{0/};UBC$-CreERT2 mice.

Discussion

In this study, we successfully generated $Rtcb^{0/};UBC$-CreERT2 mice for conditional knockout of $Rtcb$, which can avoid embryonic lethality. We observed body
weight loss, short-term mortality, and severe pathological changes in colon tissues upon TAM-induced \textit{Rtcb} knockout in adult mice.

In HeLa cells, knockdown of \textit{Rtcb} using RNA interference (RNAi) did not affect the splicing of \textit{Xbp1} mRNA\cite{27}. This is due to the presence of Archease, which appears to be an important auxiliary factor of RTCB in both tRNA and \textit{Xbp1} mRNA splicing. Even a small amount of RTCB after knockdown can catalyze \textit{Xbp1} mRNA splicing with the help of Archease\cite{28}. Significant inhibition of \textit{Xbp1} occurred upon simultaneous knockdown of RTCB and Archease\cite{29}.

It has been reported that although XBPI deficiency in IECs causes severe inflammation in the small intestine, no pathological change was found in the colon \cite{30}. To investigate whether RTCB deficiency caused colonic pathology through the mediation of XBPI, we also examined colonic changes in \textit{Xbp1} knockout mice. No colitis was observed in the adult mice with XBPI deficiency. The IRE1α-XBP1 signaling pathway is a major component of the UPR against ER stress that is associated with various diseases. The pathway is targeted by many drugs, including activators such as tunicamycin and inhibitors such as STF-083010\cite{31}. We also examined the effects of activating or inhibiting the IRE1α-XBP1 pathway on the mouse male reproductive system by injecting tunicamycin or STF-083010, respectively. During these experiments, no mortality or significant body weight loss was observed (data not shown). Therefore, it appeared that the IRE1α-XBP1 signaling pathway may not be involved in the colonic inflammation and mortality caused by \textit{Rtcb} knockout.
During IBD, cytokine production and signal transduction become dysregulated in intestinal epithelial cells, macrophages, and lymphocytes, with NF-κB being one of the key regulatory factors. In IBD patients, NF-κB is not only highly expressed but also maintained in an active state in mucosal macrophages and IECs, and the degree of NF-κB activation is significantly positively correlated with the severity of intestinal inflammation[32]. In mucosal macrophages, along with the increased expression of NF-κB, there is an increased secretion of TNF-α, IL-1, and IL-6, suggesting that NF-κB can induce proinflammatory cytokines that may account for mucosal damage in IBD [33]. Notably, there is a positive feedback mechanism where NF-κB-induced TNF-α can enhance NF-κB activation[34]. Moreover, NF-κB controls not only genes with classical proinflammatory functions but also those involved in cell protection. In IECs, NF-κB has a protective role in acute intestinal inflammation[35]. Thus, NF-κB signaling may have different effects depending on the set of genes mobilized according to the context[36]. In addition, it has been shown that dextran sulfate sodium (DSS) induces mouse colitis mainly by causing apoptosis and damage to IECs. In the DSS mouse model of colitis, the NF-κB target genes that possess antiapoptotic and cell-protective functions are downregulated, which may aggravate the inflammatory response[35, 37].

In this study, we examined the protein levels of two downstream proinflammatory cytokines (TNF-α and IL-8) and two key components (IKKβ and IκBα) of the NF-κB signaling pathway in the colonic tissues of Rteb knockout mice. The significant upregulation of TNF-α and IL-8 and downregulation of IKKβ and IκBα indicated the
activation of the NF-κB pathway in the colitis induced by RTCB deficiency. Then, it is hypothesized that the disruption of colonic epithelial adhesion due to RTCB deficiency prevents the intestinal barrier from exerting its physiological function of resisting pathogens, leading to an increase in proinflammatory cytokines in colonic tissues and the onset of colonic inflammation. Simultaneously, NF-κB signaling is enhanced through positive feedback from TNF-α.

The Wnt signaling pathway plays a crucial regulatory role in ISCs [38]. Inhibition of Wnt signaling in the intestines has a severe impact on epithelial cell proliferation, resulting in damage to the intestinal barrier and eventually a period of inflammation in the small intestine and colon[39]. Reducing the secretion of Wnt from colonic epithelial cells led to impaired cell regenerative capacity and exacerbated mucosal damage[40]. In addition, removing β-catenin and TCF4 from the intestinal epithelia severely disrupted crypt formation, leading to mouse death[41]. In ISCs, the Wnt/β-catenin signaling pathway is closely related to various signaling pathways and is modulated by proinflammatory cytokines such as TNF-α and IFN-γ. During colitis, TNF-α causes intestinal epithelial barrier dysfunction and cell apoptosis. It can activate β-catenin through the AKT signaling pathway to induce the expression of Wnt target genes in crypt base columnar cells (CBCs), the main population of ISCs [42]. After DSS treatment, loss of TNF receptors in colonic epithelial cells induced colitis in mice, leading to weakened Wnt signaling and impaired tissue damage repair. IFN-γ can induce the expression of the Wnt antagonist Dickkopf-related protein 1 (DDK1) through STAT3, thereby weakening Wnt signaling[43]. Furthermore, IFN-γ can
decrease the number of LGR5-positive cells, active stem cells, and Paneth cells, exacerbating colitis damage[44].

In this study, we examined the protein levels of three key molecules (Wnt3a, GSK3β, and β-catenin) of the Wnt/β-catenin signaling pathway in the colonic tissues of Rtcb knockout mice. The significant decrease in the levels of Wnt3a and β-catenin and increase in the level of GSK3β indicated the inhibition of the Wnt/β-catenin pathway in the colitis induced by RTCB deficiency. CBCs express LGR5, the R-spondin receptor that drives the continuous renewal of the intestinal epithelial barrier and gives rise to all intestinal epithelial cell lineages[45]. It has been reported that high levels of β-catenin in colorectal cancer upregulate LGR5 expression, suggesting that LGR5 may be a downstream factor of β-catenin contributing to the occurrence of colorectal cancer[46]. Activation of the Wnt signaling pathway is associated with increased expression of LGR5, which may lead to malignant transformation of IECs[47]. Moreover, during the activation of the Wnt/β-catenin signaling pathway, inactivation of LGR5 in the small intestine did not affect the proliferation rate of epithelial cells, indicating the essential role of LGR5 in rapid epithelial cell proliferation [48]. Additionally, the loss of ISC function and reduced LGR5 positivity led to rapid crypt death and impaired tissue regeneration. In this study, we detected a significant decrease in LGR5 expression in colonic tissues after Rtcb knockout, indicating loss of colonic stem cells.
In conclusion, our study shows that RTCB deficiency can lead to colonic barrier damage and severe colitis in mice. The NF-κB and Wnt/β-catenin signaling pathways might play a key role in this pathological process. These findings may have important implications for understanding the pathogenesis of colitis.
327 Funding

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331 Conflict of Interest

The authors declare that they have no conflict of interest.

334 References


9 Ding NZ, He M, He CQ, Hu JS, Teng J, Chen J. Expression and regulation of FAAP in the mouse epididymis. Endocrine 2010, 38: 188-193


15 Danese S, Fiocchi C. Ulcerative colitis. The New England journal of medicine 2011, 365:


18 He Y, Chen L, Chen K, Sun Y. Immunohistochemical analysis of HNF4A and β-catenin expression to predict low-grade dysplasia in the colitis-neoplastic sequence. Acta biochimica et biophysica sinica


21 Moparthi L, Koch S. Wnt signaling in intestinal inflammation. Differentiation; research in biological diversity 2019, 108: 24-32


23 Wulff BS, O'Hare MM, Boel E, Theill LE, Schwartz TW. Partial processing of the neuropeptide Y precursor in transfected CHO cells. FEBS letters 1990, 261: 101-105


26 Hartsock A, Nelson WJ. Adherens and tight junctions: structure, function and connections to the actin cytoskeleton. Biochimica et biophysica acta 2008, 1778: 660-669

27 Iwawaki T, Tokuda M. Function of yeast and amphioxus tRNA ligase in IRE1alpha-
dependent XBP1 mRNA splicing. Biochemical and biophysical research communications 2011, 413: 527-531


34 Holtmann MH, Neurath MF. Differential TNF-signaling in chronic inflammatory disorders.

35 Current molecular medicine 2004, 4: 439-444


38 Barker N. Adult intestinal stem cells: critical drivers of epithelial homeostasis and regeneration. Nature reviews Molecular cell biology 2014, 15: 19-33


Li W, Henderson LJ, Major EO, Al-Harthi L. IFN-gamma mediates enhancement of HIV replication in astrocytes by inducing an antagonist of the beta-catenin pathway (DKK1) in a STAT 3-dependent manner. Journal of immunology (Baltimore, Md : 1950) 2011, 186: 6771-


Figure Legends:
Figure 1. Growth changes in mice after TAM treatment  (A) Body weight changes of the *Rtcb*/* and *Rtcb*/*;UBC-CreERT2* mice after TAM treatment (*n*=3). (B) Survival rates of the *Rtcb*/* and *Rtcb*/*;UBC-CreERT2* mice after TAM treatment (*n*=9).

Figure 2. Experimental design and efficiency evaluation of TAM treatment  (A) Experimental design. Mice were injected intraperitoneally with TAM for 5 consecutive days (D1 to D5). Body weight was measured daily until D9. Tissue collection was performed on D9. (B) PCR analysis of genomic DNA from different tissues of the TAM-treated *Rtcb*/* and *Rtcb*/*;UBC-CreERT2* mice using loxP5 and Frt2 primers (*n*=3). (C, D) Western blot analysis of RTCB in the colon tissues of TAM-treated *Rtcb*/* (control) and *Rtcb*/*;UBC-CreERT2* mice (*n*=3). All values are expressed as the mean ± SEM; *P < 0.05.

Figure 3. Histological analysis of the mouse colons after TAM treatment on D9  (A) Comparison of colon length between the TAM-treated *Rtcb*/* and *Rtcb*/*;UBC-CreERT2* mice (*n*=3). (B) Representative H&E staining of colon sections from the TAM-treated *Rtcb*/* and *Rtcb*/*;UBC-CreERT2* mice (*n*=3). (C) Representative immunohistochemical staining of E-cadherin in colon sections from TAM-treated *Rtcb*/* and *Rtcb*/*;UBC-CreERT2* mice (*n*=3). (D) Representative immunohistochemical staining of E-cadherin and Alcian blue (AB) in colon sections from TAM-treated *Rtcb*/* and *Rtcb*/*;UBC-CreERT2* mice (*n*=3). All scale bars: 50 μm.

Figure 4. Analysis of colonic inflammation in mice after TAM treatment on D9  Western blot analyses of cleaved caspase 3 (A, B), IL-8 (C, D), and TNF-α (E, F) in
the colon tissues of TAM-treated Rtcβf/f (control) and Rtcβf/f; UBC-CreERT2 mice (n=3). All values are expressed as the mean ± SEM; *P < 0.05; **P < 0.01; ***P < 0.001.

Figure 5. Analysis of the IRE1α-XBP1 signaling pathway in colon tissues after TAM treatment on D9 (A, B) Western blot analysis of XBP1-S in the colon tissues of TAM-treated Rtcβf/f (control) and Rtcβf/f; UBC-CreERT2 mice (n=3). (C) Survival rate of the Xbp1f/f and Xbp1f/f; UBC-CreERT2 mice after TAM treatment (n=3). (D) Body weight changes of the Xbp1f/f and Xbp1f/f; UBC-CreERT2 mice after TAM treatment (n=3). (E, F) Western blot analysis of XBP1-S expression in the colon tissues of TAM-treated Xbp1f/f (control) and Xbp1f/f; UBC-CreERT2 mice (n=3). (G) Comparison of colon length between TAM-treated Xbp1f/f and Xbp1f/f; UBC-CreERT2 mice (n=3). (H) Representative H&E staining of colon sections from TAM-treated Xbp1f/f and Xbp1f/f; UBC-CreERT2 mice (n=3). Scale bar: 50 μm. (I) Representative immunohistochemical staining of E-cadherin in colon sections from TAM-treated Xbp1f/f and Xbp1f/f; UBC-CreERT2 mice (n=3). Scale bar: 50 μm. (J) Representative immunohistochemical staining of E-cadherin and Alcian blue in colon sections from TAM-treated Xbp1f/f and Xbp1f/f; UBC-CreERT2 mice (n=3). Scale bar: 50 μm. All values are expressed as the mean ± SEM; *P < 0.05; ns, no significance.

Figure 6. Analysis of the NF-κB signaling pathway in colon tissues after TAM treatment on D9 Western blot analyses of IKKβ (A, B), IκBα (C, D), and p-p65 (E) in the colon tissues of TAM-treated Rtcβf/f (control) and Rtcβf/f; UBC-CreERT2 mice (n=3). All values are expressed as the mean ± SEM; *P < 0.05; **P < 0.01.
Figure 7. Analysis of the Wnt/β-catenin signaling pathway in colon tissues after TAM treatment on D9. Western blot analyses of Wnt3a (A, B), GSK3β (C, D), β-catenin (E, F), and LGR5 (G, H) in the colon tissues of TAM-treated Rtcpff (control) and Rtcpff;UBC-CreERT2 mice (n=3). All values are expressed as the mean ± SEM; *P < 0.05; **P < 0.01.
A

B

C

D

138x102mm (300 x 300 DPI)
A.

B.

C.

D.

165x103mm (300 x 300 DPI)
A

Cleaved Caspase 3

β-actin

B

C

IL-8

β-actin

D

E

TNF-α

β-actin

178x85mm (300 x 300 DPI)
A

IKKβ
β-actin

87 kDa
42 kDa

C

IkBα
β-actin

36 kDa
42 kDa

E

p-p65
β-actin

65 kDa
42 kDa

B

IKKβ/β-actin

D

IkBα/β-actin

**

79x74mm (300 x 300 DPI)
A  B  C  D  E  F  G  H

173x67mm (300 x 300 DPI)
Table 1. The sequences of the primers used for genotyping

<table>
<thead>
<tr>
<th>Primer pair</th>
<th>Sequence (5’→3’)</th>
<th>Fragment length</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>WT</td>
</tr>
<tr>
<td>loxP1/loxP2</td>
<td>loxP1: TTTGCTGTGCTTCATGTCTC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>loxP2: ATGGCGAGCTCAGACCATAAC</td>
<td>0 bp</td>
</tr>
<tr>
<td>loxP2/loxP3</td>
<td>loxP2: ATGGCGAGCTCAGACCATAAC</td>
<td>0 bp</td>
</tr>
<tr>
<td></td>
<td>loxP3: ATCAGTGGGTCACTCAGAAGCTG</td>
<td></td>
</tr>
<tr>
<td>Frt3/Frt4</td>
<td>Frt3: GTCTCTGTGGATCAGACATAGC</td>
<td>304 bp</td>
</tr>
<tr>
<td></td>
<td>Frt4: ACAAGCTCTCCTGAGTTCTATG</td>
<td></td>
</tr>
<tr>
<td>Frt5/Frt6</td>
<td>Frt5: TAGCAGCCCTCTGTCGTGATAC</td>
<td>493 bp</td>
</tr>
<tr>
<td></td>
<td>Frt6: GGGTGGCCTCTATGCTTAGTACCT</td>
<td></td>
</tr>
<tr>
<td>Universal-Cre-F/R</td>
<td>F: ATTTGCTGTGCTATTACCGTTCG</td>
<td>0 bp</td>
</tr>
<tr>
<td></td>
<td>R: CAGCATCATTGCTACTTTGGTC</td>
<td></td>
</tr>
<tr>
<td>loxP5/Frt2</td>
<td>loxP5: TCAATCCAGTCTCACCACA</td>
<td>1237 bp</td>
</tr>
<tr>
<td></td>
<td>Frt2: CTAAGCAAGCATGTCTGTAG</td>
<td></td>
</tr>
</tbody>
</table>
Supplementary Figure S1. Generation and genotyping of *Rtcb*\textsuperscript{ff};UBC-CreERT2 mice  
(A) Strategy for TAM-induced *Rtcb* gene deletion using UBC-CreERT2 mice. 
(B) PCR analysis of *Rtcb* gene using specific primers. (C) Genotyping of *Rtcb*\textsuperscript{ff};UBC-CreERT2 mice by PCR. M represents a 2000 bp marker. Lane 1: Detection of loxP1 and loxP2 primers, resulting in a 370 bp target fragment. Lane 2: Detection of loxP2 and loxP3 primers, resulting in a 468 bp target fragment. Lane 3: Detection of Frt3 and Frt4 primers, resulting in a 494 bp target fragment. Lane 4: Detection of Frt5 and Frt6 primers, resulting in a 683 bp target fragment. Lane 5: Detection of Universal-Cre-F and Universal-Cre-R primers, resulting in a 309 bp target fragment.
RTCB is the only known RNA ligase widely present in multiple tissues of mice. Here, we show that knockout of \( \text{Rtcb} \) in mice can disrupt the colonic barrier and cause spontaneous colitis, with both NF-\( \kappa \)B and Wnt/\( \beta \)-catenin signaling pathways being affected.

1. \( \text{Rtcb} \) knockout impaired colonic barrier integrity and exacerbated inflammation in mice.
2. \( \text{Rtcb} \) knockout increased inflammation independent of its substrate \( Xbp1 \) mRNA.
3. \( \text{Rtcb} \) knockout activated the NF-\( \kappa \)B signaling pathway.
4. \( \text{Rtcb} \) knockout inhibited the Wnt/\( \beta \)-catenin signaling pathway.