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ARTICLE

TAOK1 negatively regulates IL-17-mediated signaling and inflammation

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Interleukin 17 (IL-17) is an important cytokine that can induce tissue inflammation and is involved in the pathogenesis of numerous autoimmune diseases. However, the regulation of its signaling transduction has not been well described. In this study, we report that thousand and one kinase 1 (TAOK1) functions as a negative regulator of IL-17-mediated signal transduction and inflammation. TAOK1 knockdown promotes IL-17-induced cytokine and chemokine expression and the activation of mitogen-activated protein kinases and nuclear factor-κB. We further demonstrate that TAOK1 interacts with IL-17 receptor A (IL-17RA) independent of its kinase activity, and TAOK1 dose-dependently prevents the formation of the IL-17R-Act1 (nuclear factor activator 1, also known as tumor necrosis factor receptor-associated factor 3 interacting protein 2) complex. Consistent with this, TAOK1 deficiency exacerbates colitis in the 2,4,6-trinitrobenzenesulfonic acid)-induced experimental model of inflammatory bowel disease, likely by its promotion of the IL-17-mediated signaling pathway. TAOK1 expression is decreased in the colons of ulcerative colitis patients. In conclusion, these findings suggest that TAOK1 is involved in the development of IL-17-related autoimmune disorders.
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Keywords: Act1; IL-17; IL-17RA; inflammation; TAOK1

INTRODUCTION

The interleukin 17 (IL-17) family consists of six molecules, namely, IL-17A to IL-17F. IL-17 (also named IL-17A) is a fundamental member and the most investigated cytokine in this family.[1] IL-17A is a hallmark cytokine of T helper type 17 (Th17) cells and can be produced by many innate immune cells,[2] including γδT, iNKT and NK cells.[3–5] As a proinflammatory cytokine, IL-17 exerts its effects by promoting the expression of matrix metalloproteinases and proinflammatory genes such as IL-6, CXC-chemokine ligand 1 (CXCL1), CXCL2, CC-chemokine ligand 20 (CCL20) and colony-stimulating factor 3 (CSF3), which can result in neutrophil infiltration and inflammation in tissue.[1] Dysregulation of IL-17 signaling may lead to autoimmune diseases such as inflammatory bowel disease (IBD) and Sjögren's syndrome.[6,7]

The IL-17 receptor (IL-17R) family has been described as consisting of five subunits, from IL-17 receptor A (IL-17RA) to IL-17RE. IL-17 binds a receptor complex of IL-17RA and IL-17RC subunits. Targeting IL-17RA can block IL-17-mediated proinflammatory pathways.[6,8] The binding of IL-17 to the IL-17RA–IL-17RC complex results in recruitment of the ubiquitin ligase adaptor protein Act1. Act1 further recruits tumor necrosis factor receptor-associated factor 6 (TRAF6) to induce the activation of nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK) pathways and the upregulation of many proinflammatory cytokines and chemokines.[1,9] The IL-17R/Act1 complex also recruits TRAF2 and TRAF5 to prolong the half-life of CXCL1.[10] In contrast, TRAF3 has been reported to play a negative regulatory role in IL-17 signaling by

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interfering with the formation of the IL-17R–Act1–TRAF6 complex.[10,11] Due to a broad role in driving inflammatory responses, IL-17 signaling should be under tight control to avoid harmful inflammation and autoimmune diseases. However, in contrast to studies on Th17 cells, there are far fewer studies focused on IL-17 downstream signaling,[12] particularly on whether and how IL-17 downstream signaling affects the development of autoimmune diseases.

Thousand and one kinase 1 (TAOK1), also known as prostate-derived sterile 20 (Ste20)-like kinase 2, TAO1 (thousand and one amino-acid protein 1) or MARKK, is a member of the MAP kinase kinase kinases and belongs to the germinal-center kinase-like class of sterile 20 (Ste20)-like kinases.[13,14] TAOK1 can function as a regulator of microtubule dynamics and mitotic progression through interaction with a spindle checkpoint component.[15,16] A recent study has reported that TAOK1 is a direct kinase for large tumor suppressor 1/2 of the Hippo pathway.[17] Furthermore, TAOK1 is involved in the activation of P38 and c-Jun NH2-terminal kinase (JNK) in response to DNA damage and stress stimuli.[18,19] These reports provide insight into the potential role of TAOK1 in the MAPK signaling pathway.

In this study, we report that TAOK1 functions as a negative regulator in IL-17-mediated signaling and inflammation. TAOK1 inhibits the IL-17-induced expression of proinflammatory cytokines and chemokines. Furthermore, TAOK1 inhibits the IL-17-triggered activation of p38, JNK, extracellular signal-regulated kinase (ERK1/2) and p65 in HeLa cells. TAOK1 interacts with IL-17RA and inhibits the formation of the IL-17RA–Act1 complex in a kinase activity-independent manner, which results in the negative regulation of the IL-17-mediated inflammatory response. In addition, the expression of TAOK1 in the colon mucosal epithelia of ulcerative colitis (UC) patients is decreased. TAOK1-deficient mice are more sensitive to 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced IBD, suggesting that TAOK1 prevents TNBS-induced IBD.

Table 1 Basic information of control and UC patients from Xinhua hospital

	Normal control, n = *29*	*UC*, n = *31*
Age (years)		
⩽30	0	2
30–50	3	10
⩾50	26	19
Gender		
Male	13	18
Female	16	13
Location		
Colon	29	31

Basic information of human samples about non-IBD control and UC patients from Xinhua hospital. Twenty-nine non-IBD control colon sections were obtained from the resection edges of tumor biopsies that appeared to be healthy at the histological level. Thirty-one human UC colon sections were obtained from colonoscopies. The diagnosis of UC was based on clinical, endoscopic, radiologic and histologic criteria.

MATERIALS AND METHODS

Mice

TAOK1$^{fl/fl}$ mice were obtained from Cyagen Biosciences Inc. (Suzhou, China). The mice were crossed with Pvillin-Cre transgenic mice to generate TAOK1 conditional knockout (KO) mice (Pvillin-cre$^{+/-}$ *TAOK1*$^{fl/fl}$) for the specific deletion of TAOK1 in intestinal epithelial cells (IECs). The mice were maintained and bred under pathogen-free conditions. All animal experimental procedures were carried out in accordance with protocols approved by the Scientific Investigation Board of Zhejiang University (Hangzhou, China).

Human samples

Paraffin-embedded human colon sections from UC patients were obtained from the Second Affiliated Hospital of Zhejiang Chinese Medical University (also called Xinhua Hospital). Control colon samples were obtained from the resection edges of tumor biopsies and appeared to be normal at the histological level. Detailed information from all patients, including sex, age and location, is shown in Table 1.

Reagents and plasmids

TNBS was purchased from Sigma (St Louis, MO, USA). Recombinant IL-17A (human) was purchased from Peprotech (Rocky Hill, NJ, USA). A human IL-6 ELISA Kit was obtained from eBioscience (San Diego, CA, USA). Phospho-antibodies against NF-κB p65, ERK, JNK and p38 and corresponding antibodies against total proteins were obtained from Cell Signaling Technology (Danvers, MA, USA). Anti-TRAF6 and anti-β actin were purchased from Santa Cruz (Santa Cruz, CA, USA). Anti-TAOK1 and anti-IL-17RA were purchased from Abcam Biotechnology (Cambridge, UK). Anti-HA, anti-DDK (Flag) and anti-Myc were obtained from Origene Technologies (Rockville, MD, USA). Horse radish peroxidase-coupled secondary antibodies were purchased from Cell Signaling Technology. pCMV6-TAOK1 and pCMV6-Act1 vectors expressing human TAOK1 and Act1 were purchased from Origene. Truncated TAOK1 was amplified from cDNA expressing full-length TAOK1 and cloned into the pCMV6-Myc expression vector (Clontech Laboratories, Mountain View, CA, USA) to construct Myc-tagged TAOK1 expression plasmids. HA-IL-17RA was provided by Xiaojian Wang (Institute of Immunology, Zhejiang University).

Cell culture, plasmid transfection and siRNA silencing

HeLa and HEK293T cells were obtained from American Type Culture Collection (ATCC, Rockville, MD, USA). HeLa cells were cultured in 1640 supplemented with 10% fetal bocine serum (FBS). HEK293T cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% FBS. The plasmids were transfected into HeLa and HEK293 cells with jetPEI (Polyplus, S.Brant. Illkirch, France) according to the manufacturer's protocols.[20] Control Non-target siRNA and TAOK1 on-target siRNA were obtained from Dharmacon (Thermo Fisher Scientific, Waltham, MA, USA). siRNA were transfected into HeLa cells with Lipofectamine RNAiMAX

Table 2 Primers for real-time PCR

Human gapdh, forward primer	5′-ATTCCACCCATGGCAAATTC-3′
Human gapdh, reverse primer	5′-GGATCTCGCTCCTGCAAGATG-3′
Human il-6, forward primer	5′-ATGAACTCCTTCTCCACAAGCGC-3′
Human il-6, reverse primer	5′-GGGAAGGCAGCAGGCAACAC-3′
Human cxcl1, forward primer	5′-AGTCATAGCCACACTCAAGAATGG-3′
Human cxcl1, reverse primer	5′- GATGCAGGATTGAGGCAAGC-3′
Human cxcl2, forward primer	5′-CTCAAGAATGGGCAGAAAGC-3′
Human cxcl2, reverse primer	5′-AAACACATTAGGCGCAATCC-3′
Human ccl20, forward primer	5′-GCGCAAATCCAAAACAGACT-3′
Human ccl20, reverse primer	5′-CAAGTCCAGTGAGGCACAAA-3'
Mouse actin, forward primer	5′- AGTGTGACGTTGACATCCGT-3′
Mouse actin, forward primer	5′-GCAGCTCAGTAACAGTCCGC-3′
Mouse il-6, forward primer	5′-TAGTCCTTCCTACCCCAATTTCC-3′
Mouse il-6, forward primer	5′-TTGGTCCTTAGCCACTCCTTC-3′
Mouse il-1β, forward primer	5′-CCAAAAGATGAAGGGCTGCT-3′
Mouse il-1β, forward primer	5′-ACAGAGGATGGGCTCTTCT-3′
Mouse TNFα, forward primer	5′-AAGCCTGTAGCC CACGTCGTA-3′
Mouse TNFα, forward primer	5′-GGCACCACTAGTTGGTTGTCTTTG-3′
Mouse cxcl1, forward primer	5′-CGCTTCTCTGTGCAGCGCTGCTGCT-3′
Mouse cxcl1, forward primer	5′-AAGCCTCGCGACCATTCTTGAGTC-3′
Mouse cxcl2, forward primer	5′-CCTGGTTCAGAAAATCATCCA-3′
Mouse cxcl2, forward primer	5′-CTTCCGTTGAGGGACAGC-3′
Mouse ccl20, forward primer	5′-AACTGGGTGAAAAGGGCTGT-3′
Mouse ccl20, forward primer	5′-GTCCAATTCCATCCCAAAAA-3′

(Thermo Fisher Scientific, Waltham, MA, USA) according to the standard protocol.[21]

Quantitative real-time PCR and ELISA

Total RNA was extracted with an RNA extraction kit, and cDNAs were synthesized using a cDNA synthesis kit (Takara) according to the manufacturer's instructions.[22] Real-time PCR was performed with a SYBR PCR kit (Takara) on an ABI PRISM® 7900 Sequence Detection System (Applied Biosystems). The primers used are shown in Table 2. The data were normalized to the gapdh or β-actin expression level in each sample. The production of IL-6 in the culture supernatants was detected using an ELISA Kit (eBioscience).

Co-immunoprecipitation and immunoblot analysis

Co-immunoprecipitation and immunoblotting were carried out as previously described.[23] Briefly, cells were lysed with cell lysis buffer supplemented with a cocktail of proteinase inhibitors (Cell Signaling Technology). Protein concentrations in the extracts were measured with the BCA assay (Thermo Fisher Scientific). Equal amounts of supernatants were incubated with specific antibodies and protein A/G Sepharose (sc-2003, Santa Cruz) for immunoprecipitation or loaded for SDS-polyacrylamide gel electrophoresis. Immunoblotting was performed using the indicated antibodies.

TNBS-induced colitis and histological analysis

The mice were presensitized with 1% (wt/vol) TNBS that was diluted in acetone and olive oil and applied to the abdomen of mice. Seven days later, the mice were rectally injected with TNBS (200 mg/kg) in 50% ethanol through a catheter inserted into the colon, as previously described.[24] Mice were sacrificed 4 days after the TNBS injection.

Mouse colon tissue was fixed in 10% formalin and embedded in paraffin. Cut tissues were stained with hematoxylin and eosin (H&E). Histological scores were assessed blindly using criteria that have been previously described.[25,26] Briefly, 0 = no inflammation, 1 = moderate mucosal inflammation without erosion or ulceration, 2 = increased mucosal inflammation with erosion, 3 = increased mucosal inflammation with ulceration (<1 mm), and 4 = increased mucosal inflammation with ulceration (>1 mm).

Disease activity index

The DAI score was measured by adding three separate scores, namely, body weight loss, presence of blood in stool, and the severity of diarrhea, as previously described.[25] Briefly, body weight loss score (0, none; 1, 1–5%; 2, 6–10%; 3, 11–20%; and 4, >20%), presence of blood in stool (0, no blood; 1, positive hemoccult; 2, visible blood traces in stool; 3, visible blood in stool and anal region; and 4, gross bleeding), and severity of diarrhea (0, normal stool; 1, soft stool; 2, soft and pasty stool; 3, soft and pasty stool that adhered to the anus; and 4, liquid stool). The DAI score was determined blindly during the colitis experiment.

Statistical analysis

The results are expressed as the mean ± standard deviation (s.d.). The significance of the difference between 2 groups was determined using Student's *t*-test analysis. The specimen analysis of UC patients was assessed using Pearson's chi-squared tests with a 95% confidence interval (CI). A value $P<0.05$ was regarded as statistically significant.

RESULTS

TAOK1 knockdown promotes IL-17-induced inflammation

To evaluate the potential role of TAOK1 in IL-17 signaling, we analyzed the effect of TAOK1 knockdown on IL-17-induced cytokine and chemokine production in HeLa cells. TAOK1 protein expression was significantly decreased in HeLa cells transfected with small interfering RNA (siRNA) specifically targeting TAOK1 (Figure 1a). As shown in Figure 1b, TAOK1 knockdown remarkably upregulated the IL-17 induction of IL-6 protein secretion by HeLa cells. Moreover, in addition to increasing the IL-6 mRNA expression, IL-17 treatment markedly induced chemokine mRNA expression in HeLa cells, such as CXCL1, CXCL2, CCL20 and CSF3, and the induction of these cytokines and chemokines was substantially upregulated in TAOK1 knockdown cells (Figure 1c). These results indicate that TAOK1 serves as a negative regulator of IL-17-induced proinflammatory cytokine and chemokine production in HeLa cells.

TAOK1 inhibits IL-17-induced NF-κB and MAPK activation

Upon IL-17 stimulation, the IL-17RA/RC complex recruits Act1 to trigger downstream signaling pathways, including the activation of MAPKs and NF-κB.[9,27] To investigate the mechanism by which TAOK1 negatively regulates IL-17-mediated cytokine and chemokine production, we observed

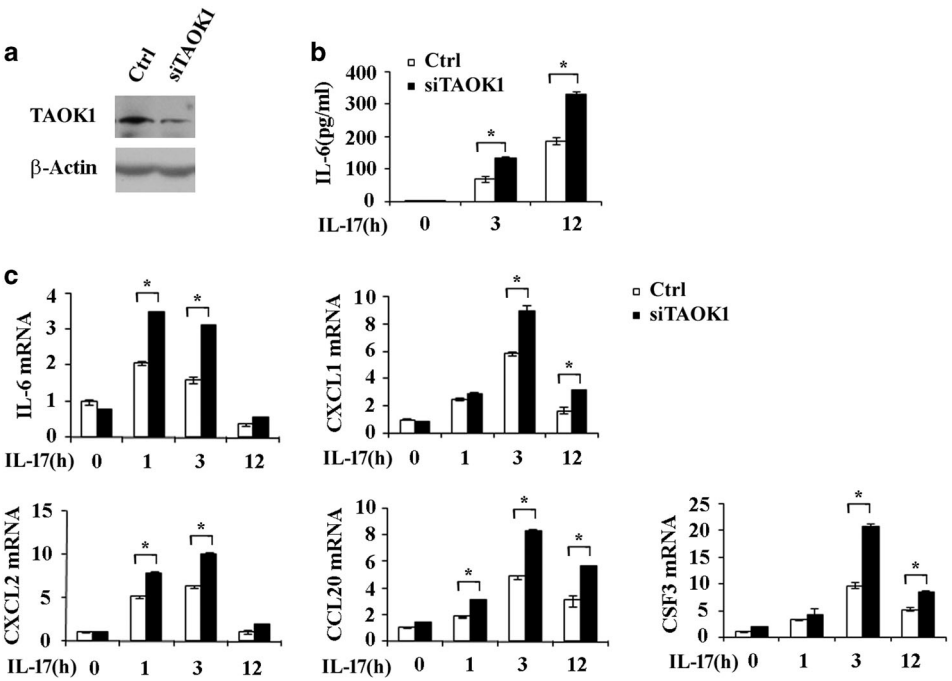

Figure 1 TAOK1 knockdown enhances IL-17-induced cytokine and chemokine production in HeLa cells. (**a**) Immunoblot analysis of TAOK1 expression levels in HeLa cells transfected with 30 nM of control siRNA (Ctrl) or TAOK1 siRNA (siTAOK1) for 36 h. (**b**) ELISA of IL-6 levels in culture supernatants of TAOK1-silenced HeLa cells treated with IL-17 (50 ng/ml) for the indicated times. (**c**) qPCR analysis of IL-6, CXCL1, CXCL2, CCL20 and CSF3 mRNA levels in TAOK1-silenced HeLa cells treated with IL-17 (50 ng/ml) for the indicated times. Similar results were obtained in three independent experiments.*$P<0.05$, **$P<0.01$, compared with control. ELISA, enzyme-linked immunosorbent assay; IL, interleukin; sirNA, small interfering RNA; TAOK1, thousand and one kinase 1.

the effects of TAOK1 knockdown and overexpression on IL-17 triggered NF-κB and MAPK activation in HeLa cells. As shown in Figure 2a, phosphorylation of the NF-κB P65 subunit and MAPKs was significantly enhanced in response to IL-17 treatment in TAOK1 knockdown HeLa cells compared to control cells. Consistent with the results of TAOK1 knockdown, phosphorylation of NF-κB and MAPKs was inhibited in TAOK1-overexpressing HeLa cells compared to control cells (Figure 2b). Collectively, these results demonstrate that TAOK1 negatively regulates IL-17-induced cytokine and chemokine production, possibly by regulating the interaction or activation of adaptors upstream of NF-κB and MAPKs. We examined the effect of TAOK1 on TRAF6 expression, an important upstream adaptor of NF-κB and MAPKs in the IL-17 signaling pathway. However, TAOK1 knockdown did not significantly affect TRAF6 expression in both unstimulated and IL-17-stimulated HeLa cells (Figure 2a).

TAOK1 associates with IL-17RA

To further investigate the underlying mechanisms by which TAOK1 inhibits IL-17 triggered signaling, HeLa cells overexpressing Myc-tagged TAOK1 were lysed to detect the target proteins of TAOK1 in the IL-17 signaling pathway. Main upstream signal regulators of IL-17 signaling, such as IL-17RA, Act1, TRAF6, TRAF2 and TRAF5, were detected in the immunoprecipitated complexes using an antibody against the Myc tag.[6,28] The results showed that TAOK1 physically interacted with endogenous IL-17RA in resting HeLa cells, and the interaction was enhanced in response to IL-17 treatment (Figure 3a). However, the association of TAOK1 with Act1, TRAF6, TRAF2 and TRAF5 was undetected. TAOK1 contains an N-terminal catalytic domain (CAT), a central substrate binding domain, a spacer and a tail domain. To investigate whether TAOK1 interacts with IL-17RA via its kinase catalytic activity, we constructed a kinase-inactive mutant TAOK1 K57A plasmid and N-terminal CAT-deleted (TAOK1-C) plasmid (Figure 3b). Consistent with the results of the wild-type TAOK1, both TAOK1 K57A and TAOK1-C interacted with IL-17RA in HEK293T cells (Figure 3c). Furthermore, similar to the results of the wild-type TAOK1, overexpression of TAOK1 K57A and TAOK1-C in HeLa cells downregulated IL-17-induced phosphorylation of NF-κB and MAPKs (Figure 3d). Taken together, these data suggest that TAOK1 inhibits the IL-17 signaling pathway in a kinase activity- and CAT-independent manner.

TAOK1 inhibits the interaction between IL-17RA and Act1

Upon binding with IL-17, the IL-17RA/IL-17RC heterodimer recruits TRAF6 via the adaptor protein Act1 to activate downstream NF-κB and MAPK signaling and induce the production of proinflammatory cytokines and chemokines.[1] Considering that the association of TAOK1 with IL-17RA was enhanced in response to IL-17 treatment in HeLa cells, we investigated whether TAOK1 inhibits IL-17-mediated signaling via interfering with the interaction between IL-17RA and Act1. HEK293 cells were co-transfected with Flag-Act1, HA-IL-17RA and varying

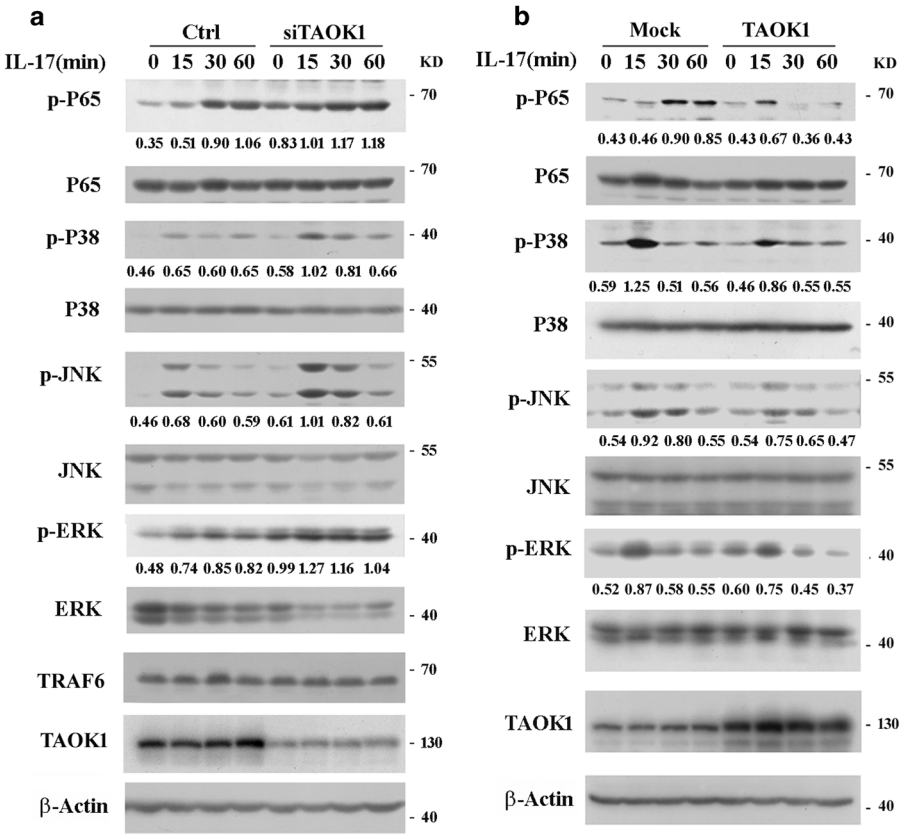

Figure 2 TAOK1 inhibits IL-17-induced NF-κB and MAPK activation. HeLa cells transfected with 30 nM control siRNA (Ctrl) or TAOK1 siRNA (**a**) or transfected with plasmids encoding Myc-TAOK1 (**b**) were treated with IL-17 (50 ng/ml) for the indicated time. Phosphorylation and total NF-κB P65, P38, JNK and ERK and expression levels of TRAF6, TAOK1 and β-actin were detected by western blot. Numbers below lanes (top) indicate densitometry of the protein relative to β-actin expression in that same lane (below). Data are from one of three experiments with similar results. ERK, extracellular signal-regulated kinase; IL, interleukin; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-κB; siRNA, small interfering RNA; TAOK1, thousand and one kinase 1; TRAF6, tumor necrosis factor receptor-associated factor 6.

doses of Myc-TAOK1 and harvested 24 h after transfection for immunoblot analysis with an antibody to the HA or Flag tag. As shown in Figure 4a, co-immunoprecipitation analysis confirmed the interaction between ectopically expressed TAOK1 and IL--17RA, and TAOK1 dose-dependently inhibited the association between IL-17RA and Act1, as determined using an antibody to the HA tag. Similar results were obtained in a separate experiment following the same plasmid transfection and detection with an antibody to the Flag tag (Figure 4b). These results suggest that TAOK1 might inhibit IL-17 signaling by downregulating the formation of the IL-17RA and Act1 complex.

TAOK1 inhibits colitis

IL-17R signaling has been reported to promote the development of human IBD and augment IBD in the TNBS-induced mouse IBD model.[24,29,30] We collected a cohort of UC patient biopsies and normal control subject biopsies from the Xinhua Hospital of Zhejiang Province, China, and used the anti-TAOK1 antibody to detect TAOK1 expression in the colonic biopsies. Only 3.2% of the UC patient biopsies (1/31) exhibited strong TAOK1 staining, whereas more than 60% of the control subjects (62.1%, 18/29) showed strong TAOK1 staining ($P<0.005$) (Figure 5a). Immunohistochemical analysis showed that the TAOK1 protein expression level in IECs was markedly decreased in patients with UC compared with normal control subjects (Figure 5b). These results collectively indicate that TAOK1 might be involved in the development of human colitis.

We further investigated the role of TAOK1 in the TNBS-induced mouse IBD model. Pvillin-cre$^{+/-}$ *TAOK1*$^{fl/fl}$ (*TAOK1 cKO*) mice and control Pvillin-cre$^{-/-}$ *TAOK1*$^{fl/fl}$ (wild-type (WT)) littermates were treated with TNBS as described previously.[24] Immunohistochemical analysis showed that TAOK1 protein expression was significantly decreased in the colonic epithelia of *TAOK1 cKO* mice (Figure 6a). Compared with WT mice, *TAOK1 cKO* mice exhibited higher sensitivity to TNBS-induced colitis, which was demonstrated by greater weight loss (Figure 6b) and a higher disease activity index (DAI) (Figure 6c). *TAOK1 cKO* mice showed more severe colon shortening than WT mice (Figures 6d and e). Histological analysis of the intestine showed that WT and *TAOK1 cKO* mice did not have obvious pathological signs under basal conditions. However, TNBS treatment revealed more severe epithelial damage and crypt disruption in *TAOK1 cKO* mice compared to WT mice (Figures 6f and g). Consistent with these results, the

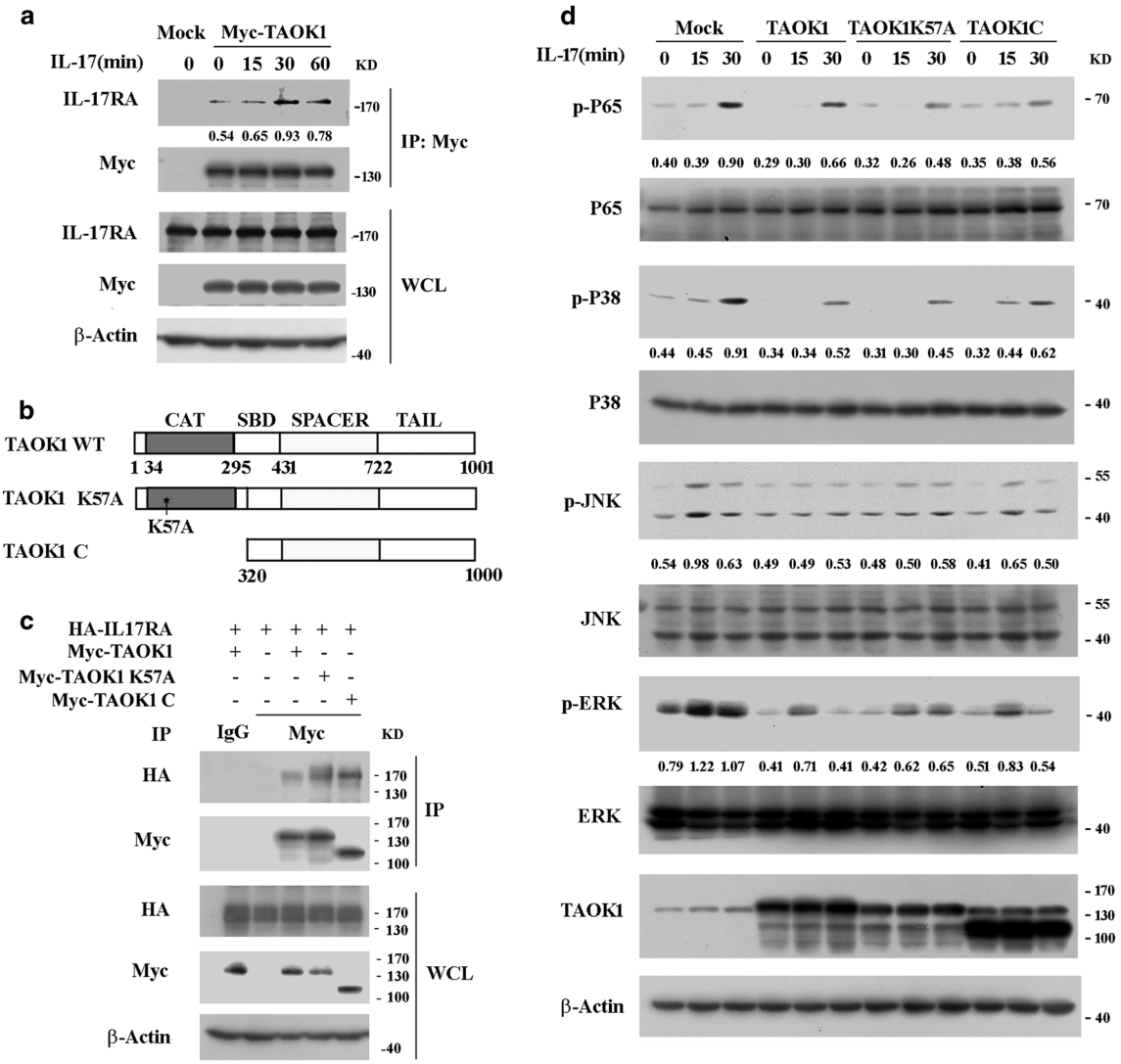

Figure 3 TAOK1 interacts with IL-17RA. (**a**) HeLa cells were transfected with Myc-TAOK1 plasmid for 24 h and then stimulated with IL-17 (50 ng/ml) for the indicated time. Immunoblot analysis of endogenous IL-17RA immunoprecipitated with an antibody to the Myc tag. Numbers between the two blots indicate the densitometry of IL-17RA relative to Myc in the immunoprecipitates. (**b**) Schematic structures of TAOK1 and the derivatives used are shown. (**c**) HEK293T cells were transfected with HA-IL-17RA expressing plasmid with Myc-TAOK1, Myc-TAOK1 K57A or Myc-TAOK1-C plasmids. Cells were harvested 24 h after transfection for immunoblot analysis of HA and Myc and immunoprecipitated with an antibody to the Myc tag. (**d**) HeLa cells transfected with plasmids encoding Myc-TAOK1, Myc-TAOK1 K57A or Myc-TAOK1-C were treated with IL-17 (50 ng/ml), and the whole cell lysates were detected with the indicated antibodies. The numbers below the lanes (top) indicate the densitometry of the presented protein relative to β-actin expression in the same lane (below). Similar results were obtained from three independent experiments. IL, interleukin; TAOK1, thousand and one kinase 1.

amount of TNBS-induced mucosal proinflammatory cytokine and chemokine (IL-6, CXCL1 and CXCL2) expression in the colons of *TAOK1 cKO* mice was substantially higher than that of WT mice (Figure 6h). These data demonstrate that the lack of TAOK1 in the intestine contributes to TNBS-induced colonic damage and colitis.

DISCUSSION

IL-17 plays critical roles in the pathogenesis of various inflammatory responses and autoimmune disorders. Thus, precise regulation of IL-17-triggered signaling is needed to avoid harmful immunopathology. In this study, we examined the effect of TAOK1 on the IL-17 triggered inflammatory response. We showed that TAOK1 negatively regulates IL-17 signaling by inhibiting the formation of the IL-17RA and Act1 complex. IL-17 binds to the IL-17RA–IL-17RC heterodimer and induces IL-17R–Act1–TRAF6 complex formation, leading to cytokine and chemokine production. Owing to the contribution of excessive IL-17 to many inflammatory and autoimmune diseases, the development of new IL-17-based treatments that target steps in the signaling pathway is an attractive therapeutic strategy.

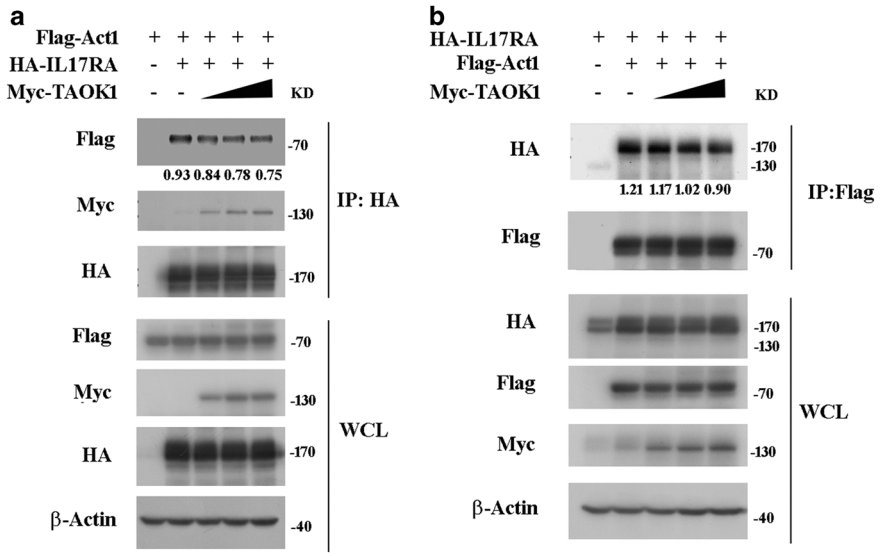

Figure 4 TAOK1 inhibits the interaction between IL-17RA and Act1. HEK293 cells were transfected with plasmids encoding Flag-Act1, HA-IL-17RA and varying doses of a plasmid encoding Myc-TAOK1 (0.5, 1.0 and 1.5 μg). Cells were harvested 24 h after transfection for immunoblot analysis, as indicated, with antibody to the HA tag (**a**) or Flag tag (**b**). Numbers between the two blots indicate the densitometry of the flag or HA relative to that of HA (**a**) or Flag (**b**) in the immunoprecipitates (IPs). Data are from one of three experiments with similar results. IL, interleukin; TAOK1, thousand and one kinase 1; WCL, whole cell lysate.

a

TAOK1 expression in patients with UC

TAOK1					
		TAOK1 expression			
Group types	Total no. studied	- %	+ %	++ %	+++ %
Normal	29	2 (6.9%)	4 (13.8%)	5 (17.2%)	18 (62.1%)
UC ***	31	18 (58.1%)	3 (9.7%)	9 (29.0%)	1 (3.2%)

Note: Correlations were analyzed using Pearson's χ^2 test.
P<0.005 compared with normal tissues

b

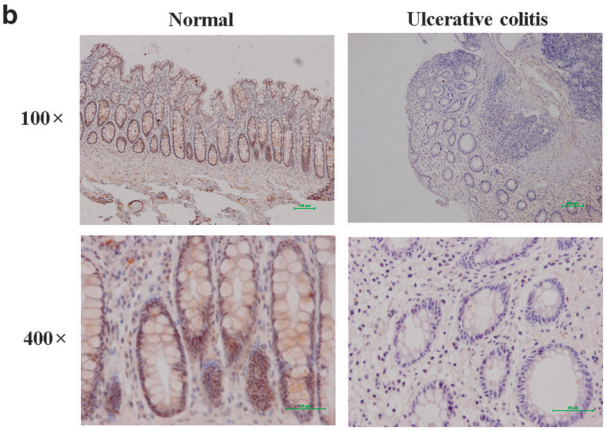

Figure 5 Decreased TAOK1 expression in human UC. (**a**) Statistical correlation analysis between TAOK1 expression and the severity of colitis in patients with UC. (**b**) Representative immunohistochemical staining of human colon sections from normal control and patients with UC using an anti-TAOK1 antibody. Scale bar 100 μm (upper), 50 μm (lower). TAOK1, thousand and one kinase 1; UC, ulcerative colitis.

TAOK1 can restrict cell proliferation by directly phosphorylating T195 to regulate Hippo-Salvador-Warts pathway signaling in developing imaginal epithelia.[31] As substrates of MST3, TAO kinases can regulate neuron dendritic filopodia and spine synapse development.[32] However, the role of TAOK1 in inflammation and other specific signaling pathways is still poorly understood. In this study, we identified TAOK1 as a negative regulator in IL-17-mediated inflammation *in vitro* and *in vivo*. TAOK1 knockdown significantly upregulated inflammatory gene expression induced by IL-17 treatment. The association of TAOK1 with endogenous IL-17RA was detected in resting cells, and the interaction was enhanced upon IL-17 treatment. Interestingly, overexpressed TAOK1, the kinase-inactive mutant K57A and the CAT-deleted plasmid TAOK1-C interacted with IL-17RA. IL-17 is involved in the pathogenesis of IBD.[33,34] In addition to enhancing the IL-17-triggered response in *vitro*, TAOK1 deficiency also resulted in exacerbated inflammatory responses in TNBS-induced colitis in *vivo*.

Act1,[33,34] TRAF3,[10] TRAF6,[34] TRAF2 and TRAF5[35] have been demonstrated to be involved in the regulation of IL-17-mediated NF-κB and MAPK activation. In this study, we found that TAOK1 significantly inhibited the activation of NF-κB and MAPKs upon IL-17 stimulation, suggesting that TAOK1 is a negative regulator of IL-17R proximal signaling. Co-immunoprecipitation experiments demonstrated that TAOK1 interacted with IL-17RA and that the interaction was enhanced upon IL-17 treatment. Further results showed that TAOK1 negatively regulated IL-17-mediated signaling by inhibiting the formation of the IL-17RA–Act1 complex. TRAF3 has been reported to play a negative role in IL-17R-induced signaling.

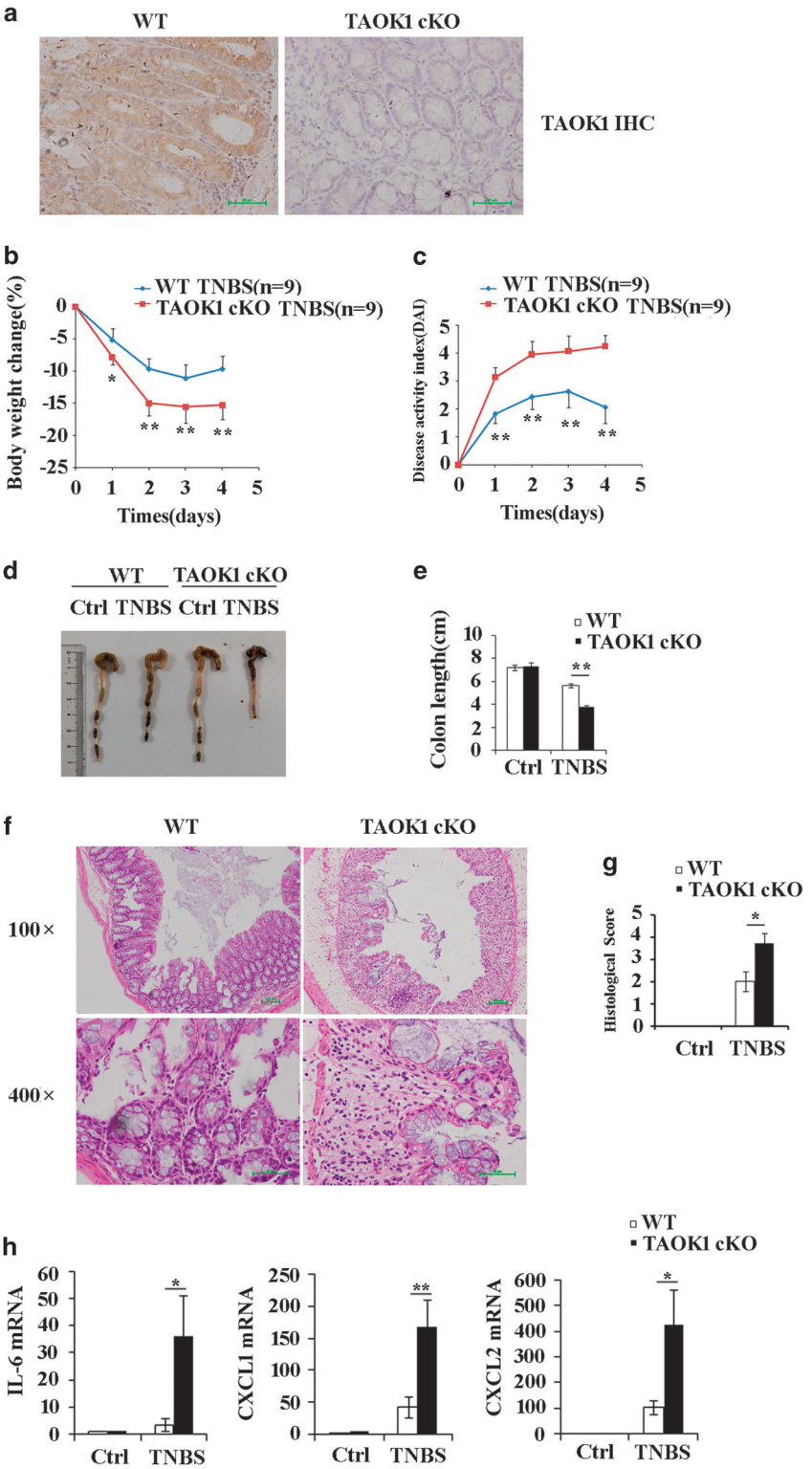

Figure 6 TAOK1 knockout (*TAOK1 cKO*) mice are more sensitive to TNBS-induced experimental colitis. *TAOK1 cKO* mice and control WT mice were treated intra-rectally with 50% TNBS (200 mg/kg) to induce acute colitis. (**a**) Immunohistochemical analysis of TAOK1 protein expression in colonic tissue from WT and *TAOK1 cKO* mice. Body weight change (**b**) and disease activity index (**c**) were measured daily for a total of 4 days. (**d**) Gross morphology images of the colon from WT and *TAOK1 cKO* mice sacrificed on day 4 and (**e**) colon length were surveyed. (**f**) H&E staining of colonic cross sections. Scale bar, 100 μm (upper), 50 μm (lower). (**g**) Semiquantitative histological scores were assigned as described in the Materials and methods section. $^{*}P<0.05$ versus WT TNBS. WT ($n=5$) and *TAOK1 cKO* ($n=5$). (**h**) Relative mRNA cytokine levels in the colon ($n=7$/group). $^{*}P<0.05$, $^{**}P<0.01$, compared with WT TNBS. cKO, conditional knockout; H&E, hematoxylin and eosin; mRNA, messenger RNA; TAOK1, thousand and one kinase 1; TNBS, 2,4,6-trinitrobenzenesulfonic acid; UC, ulcerative colitis; WT, wild type.

The binding of TRAF3 to IL-17R interfered with IL-17RA–Act1–TRAF6 complex formation, resulting in inhibition of downstream signaling.[10] The effect of TRAF3 on TAOK1-regulated IL-17 signaling needs to be further studied.

Our findings show a previously unreported, novel function of TAOK1 in IL-17 signaling that is independent of its kinase activity. TAOK1 interacts with IL-17R and interferes with the formation of the IL-17RA and Act1 complex, which is required for downstream NF-κB and MAPK activation, leading to decreased proinflammatory factor production. TAOK1-deficient mice are more sensitive to TNBS-induced colitis, and TAOK1 expression was decreased in the colon tissue of UC patients. Our results demonstrate that TAOK1 is a negative regulator of IL-17 signaling and that it may have therapeutic utility as a target for the treatment of IL-17-associated inflammatory disorders.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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