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Toll-like receptor 3 signaling attenuated colitis-associated cancer development in mice

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Inflammatory bowel disease is associated with a high risk of colitis-associated cancer (CAC). We evaluated the role of TLR3 in CAC using a murine model. Wild-type (WT) and TLR3-knockout ($TLR3^{-/-}$) mice received azoxymethane (AOM) 12.5 mg/kg intraperitoneally on day zero, followed by three cycles of 2% dextran sulfate sodium (DSS) for five days and free water for two weeks. We evaluated clinical indices, such as weight change, colon length, histological severity of colitis, and tumor number. We performed immunofluorescence assays for phospho-IκB kinase and β-catenin in colon tissues. To elucidate the antitumorigenic mechanism of TLR3 signaling, we injected poly(I: C) or phosphate-buffered saline intraperitoneally into an AOM/DSS-induced tumorigenesis model in WT mice. We also evaluate the direct antitumor effect of TLR signaling in AOM-treated WT and $TLR3^{-/-}$ mice without DSS. TLR3 deficiency increased tumor burden and colitis severity in the colon tissue than in the WT mice. β-catenin immunoreactivity was higher in $TLR3^{-/-}$ mice, while phospho-IκB kinase expression was similar. TLR3 activation by poly(I: C) did not reduce tumor burden in WT mice, but long-term AOM administration without DSS significantly increased tumor burden in $TLR3^{-/-}$ mice. TLR3 signaling attenuates CAC development, suggesting it may be a target for preventing CAC in inflammatory bowel disease.

Inflammatory bowel disease (IBD), which includes Crohn's disease and ulcerative colitis, is a chronic immune-mediated inflammatory disorder of the gastrointestinal tract, characterized by a relapsing-remitting course. Patients with IBD have an increased risk of developing colorectal cancer and a poor prognosis[1–3]. Colorectal cancer in patients with IBD, that is, colitis-associated cancer (CAC), develops through the "inflammation-dysplasia-carcinoma" sequence as a result of repeated injuries and healing of the intestinal epithelium[4]. Therefore, immune system dysregulation may be critical for CAC development in patients with IBD.

Toll-like receptors (TLRs) are essential for the initiation of the immune system and the induction of proinflammatory cytokines, and TLR3 signaling regulates innate and adaptive immune systems by the recognition of viral double-stranded RNA[5–7]. Activation of TLR3 signaling by polyinosinic-polycytidylic acid (poly[I: C]), a synthetic TLR3 agonist, has been reported to dramatically attenuate dextran sulfate sodium (DSS)-induced acute colitis in wild-type (WT) mice[8]. Zhao HW et al. showed that poly(I: C) may protect against DSS-induced acute colitis by maintaining epithelial integrity and regulating innate immune responses[9].

Therefore, TLRs may be important for the development of colorectal cancer[10]. TLRs have recently been reported to be expressed in various cancer cells and are activated in the tumor microenvironment[11–15]. Activation of TLRs stimulates immune cells and induces apoptosis, resulting in antitumorigenic effects. In contrast, their pro-tumorigenic effect was expressed as the activation of immunosuppressive cells, with the consequent promotion of angiogenesis[11,14,16]. Recent reports have suggested that the neoplastic process may sabotage TLR signaling pathways to favor cancer progression. TLRs on tumor cells facilitate their evasion of immune surveillance by suppressing T-cell proliferation and natural killer cell activity. These studies suggest that TLR signaling in tumor cells is associated with cancer progression[11,14].

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TLR expression in cancer has been reported to have both pro-tumorigenic and antitumorigenic effects[13,16–19]. Two previous studies reported that TLR2 had pro- or anti-tumoral effects in colon cancer[2021]. In addition, TLR4 stimulation in a colon cancer model is known to induce nitric oxide and IL-6 production by tumor cells, reduce the proliferation of T cells and the cytotoxic function of NK cells, and confer resistance to CTLs in tumor cells[22]. However, little information is available on the role of TLR3 signaling in the development of colitis-associated colorectal cancer. Therefore, we investigated the role of TLR3 in colorectal cancer.

Results

TLR3-deficiency increases the tumor burden of the colon in a murine model of CAC

To investigate the effect of TLR3 on the tumorigenesis of CAC, we applied the azoxymethane (AOM)/DSS-induced CAC model to WT and TLR3$^{-/-}$ mice. On day zero, AOM was administered to WT and TLR3$^{-/-}$mice and subsequently DSS was administered for five days on week 1, week 4 and week 7. TLR3$^{-/-}$ mice showed significant weight loss during the DSS cycle. In addition, after administration of normal water for two weeks, the degree of weight recovery in TLR3$^{-/-}$ mice was less than that in WT mice (Fig. 1A). After sacrifice, TLR3$^{-/-}$ mice exposed to AOM/DSS exhibited a higher tumor burden than that of WT mice. Tumors were also larger in TLR3$^{-/-}$ mice than in WT mice and were located mainly from the middle of the colon to the rectum (Fig. 1B, C). In addition, TLR3$^{-/-}$ mice exhibited increased severity of colitis compared with that of WT mice, as well as immune cell infiltration in the lamina propria (Fig. 1D, E). There was no significant difference of colon length between two groups (Supplementary Fig. 1A).

Subsequently, we performed PCR analysis to investigate the changes in gene expression associated with the deletion of TLR3 (Fig. 1F). We focused on key proinflammatory cytokines and immune-related genes involved in CAC development to assess the impact of TLR3 deficiency. The PCR data revealed significant alterations in the expression levels of certain cytokines in TLR3$^{-/-}$ mice compared with those in WT mice. Specifically, the expression of tumor necrosis factor-alpha (TNF-α) was markedly upregulated in TLR3$^{-/-}$ mice, indicating an augmented proinflammatory response in the absence of TLR3. Additionally, the expression of interferon-gamma (IFN-γ) showed a substantial increase in TLR3$^{-/-}$ mice, suggesting an augmented immune response. We also examined the expression of interleukin-1 beta and interleukin-6 (IL-6), which are key mediators of inflammation, but neither showed significant differences. Overall, PCR data demonstrated that TLR3 deficiency in the AOM/DSS model upregulated proinflammatory cytokines and immune-related genes, suggesting a potential role of TLR3 in regulating the colonic immune microenvironment and developing CAC.

Immunofluorescence assays were performed to evaluate the histopathological effects of inflammation and tumorigenesis in WT and TLR3$^{-/-}$mice using NF-κB, IKK and β-catenin antibodies (Fig. 2A). There was no significant difference in IKK expression between the tumor sites in WT and TLR3$^{-/-}$ mice. However, the β-catenin and NF-κBexpression was significantly amplified in the tumor site of TLR3$^{-/-}$mice (Fig. 2B). Additional IHC staining for ZO-1 and ELANE was performed to assess intestinal integrity, permeability, and inflammation, but no significant differences were observed between the two groups (Supplementary Fig. 2A).

Poly(I: C), a synthetic TLR3 agonist, had no significant effect on colon tumorigenesis in the murine AOM/DSS model

Previously, the administration of poly(I: C), a synthetic TLR3 agonist, showed protective effects against DSS-induced murine colitis[9]. Therefore, we investigated the role of TLR3 signaling in colon tumorigenesis using an AOM/DSS mouse model to elucidate the inflammation-related colon tumorigenesis by TLR3 signaling. As shown in Fig. 3, there were no significant differences in weight loss or tumor development between the two groups (Fig. 3A, B, C). In addition, the severity of colitis or colon length did not change after AOM/DSS administration (Fig. 3D, E and Supplementary Fig. 1B). Additional IHC staining for ZO-1 and ELANE showed no significant differences were noted between the two groups (Supplementary Fig. 2B).

TLR3 might express tumor-protective effect by direct antitumor activity

Based on previous results, we hypothesized that TLR3 signaling is associated with antitumor effects, regardless of its TLR3-mediated anti-inflammatory effects. To test this hypothesis, we performed an additional experiment with long-term AOM treatment without colitis induction by DSS administration. Both WT and TLR3$^{-/-}$ mice were administered AOM injections weekly for 12 weeks. TLR3$^{-/-}$ mice had significantly lower body weights than WT mice (Fig. 4A). However, the tumor burden was notably higher in TLR3$^{-/-}$ mice than in WT mice (Fig. 4B, C). There was no significant difference in colitis severity or colon length between the two groups (Fig. 4D, E and Supplementary Fig. 1C).

β-catenin and NF-κB expression in the tumor site was significantly higher in the TLR3$^{-/-}$mice than in WT mice. However, IKK expression did not differ between the two groups (Fig. 5A, B). These findings indicate that TLR3 signaling plays a crucial role in colon tumorigenesis via direct antitumor rather than anti-inflammatory mechanisms. Additionally, ZO-1 and ELANE expression were found to be no significant differences between the two groups (Supplementary Fig. 2C).

Discussions

Studies have demonstrated that TLR3 exerts a protective effect against DSS-induced acute colitis; however, its role in CAC remains unexplored. In the AOM/DSS model, the genetic deletion of TLR3 led to an increased tumor burden compared with that in WT mice. The expression of IKK in colon tissue did not differ between TLR3 knockout and WT mice, but β-catenin showed higher expression in TLR3 knockout mice. Additionally, TLR3 knockout mice exhibited an elevated tumor burden induced by AOM alone. These findings suggest that TLR3 signaling may ameliorate the development of CAC through direct antitumor activity rather than the anti-

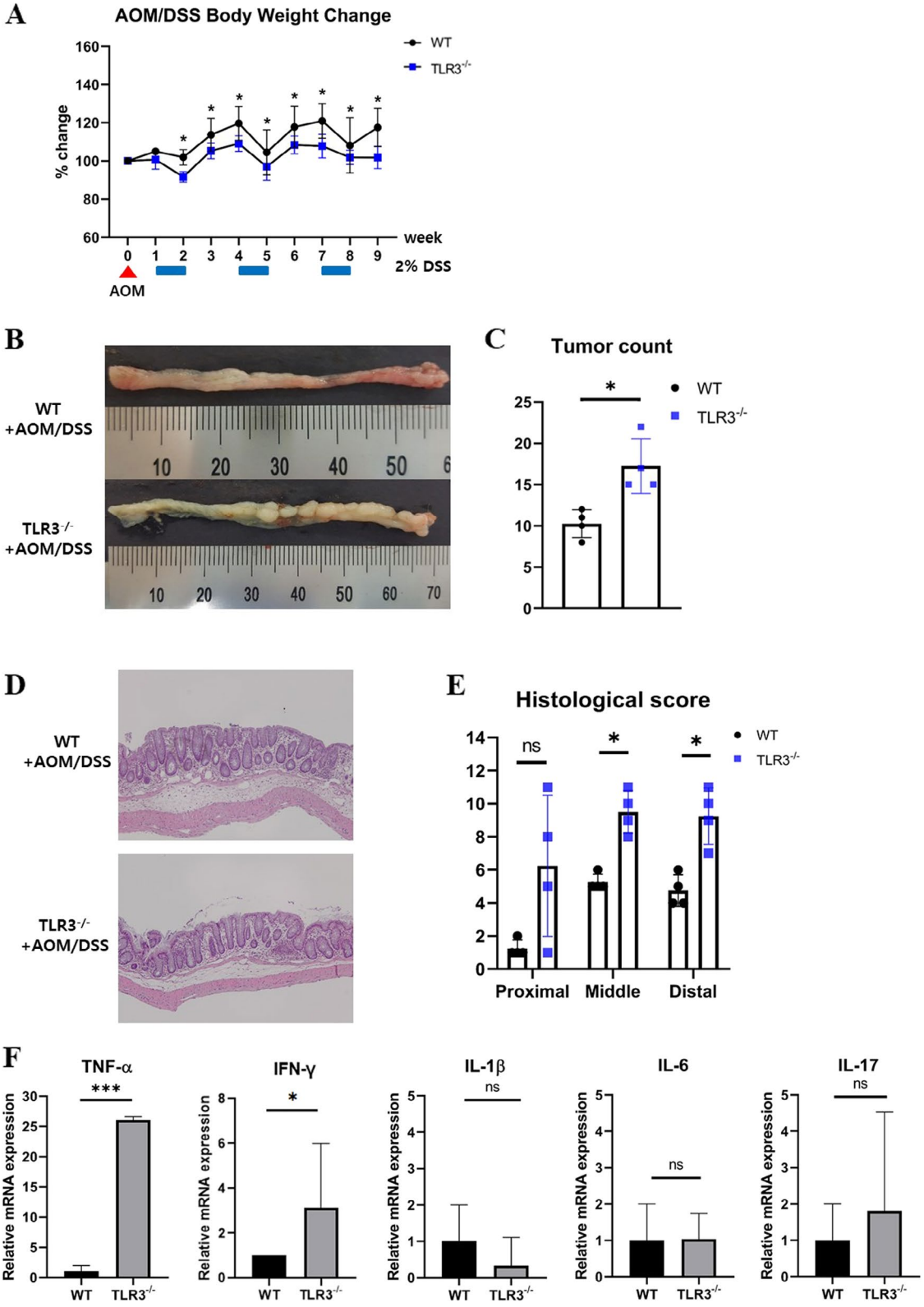

Fig. 1. Clinical and histological changes in WT and TLR3$^{-/-}$ mice after AOM/DSS treatment. (**A**) Body weight change of AOM/DSS-treated WT and TLR3$^{-/-}$ mice. TLR3$^{-/-}$ mice showed marked weight loss during the DSS cycle compared with WT. (**B**, **C**) Gross evaluation of tumor burden. The number of tumors was significantly higher in AOM/DSS-treated TLR3$^{-/-}$ mice than in WT mice. (**D**, **E**) Histological score of colon tissue. The histopathologic score of TLR3$^{-/-}$ mice was higher in all three sections of colon tissue than in WT mice. (**F**) Quantification of inflammatory cytokines from mouse colon lamina propria mononuclear cells (LPMC) by PCR. The level of TNF-α and IFN-γ were significantly elevated in TLR3$^{-/-}$ mice compared with that in WT mice. The symbols: $^{***}p < 0.001$, $^{**}p < 0.01$ and $^{*}p < 0.05$.

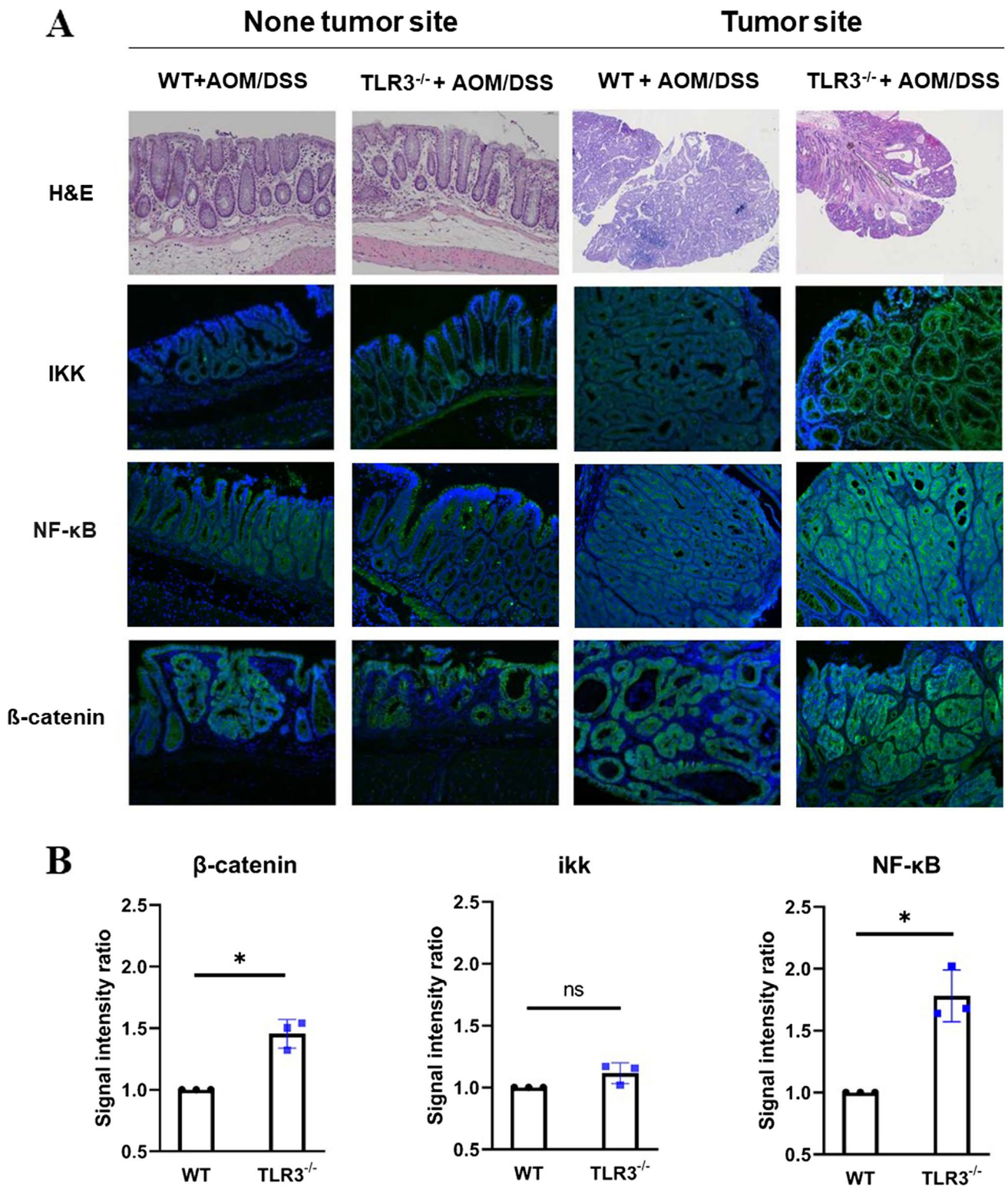

Fig. 2. Histological images from non-tumor site and tumor site in AOM/DSS-treated WT and TLR3$^{-/-}$ mice. (**A**) H&E and immunofluorescence assays of AOM/DSS-treated WT and TLR3$^{-/-}$ mice. Overall inflammation on both non-tumor and tumor sites was more severe in TLR3$^{-/-}$ mice compared with that in the WT mice. (**B**) Fluorescence intensity of IKK, *NF-κB* and β-catenin. The expression of β-catenin was significantly more amplified in tumor site of TLR3$^{-/-}$ mice than in the WT mice. However, there was no significant difference of the expression IKK in tumor site between WT and TLR3$^{-/-}$ mice. The symbol: $^{*}p < 0.05$.

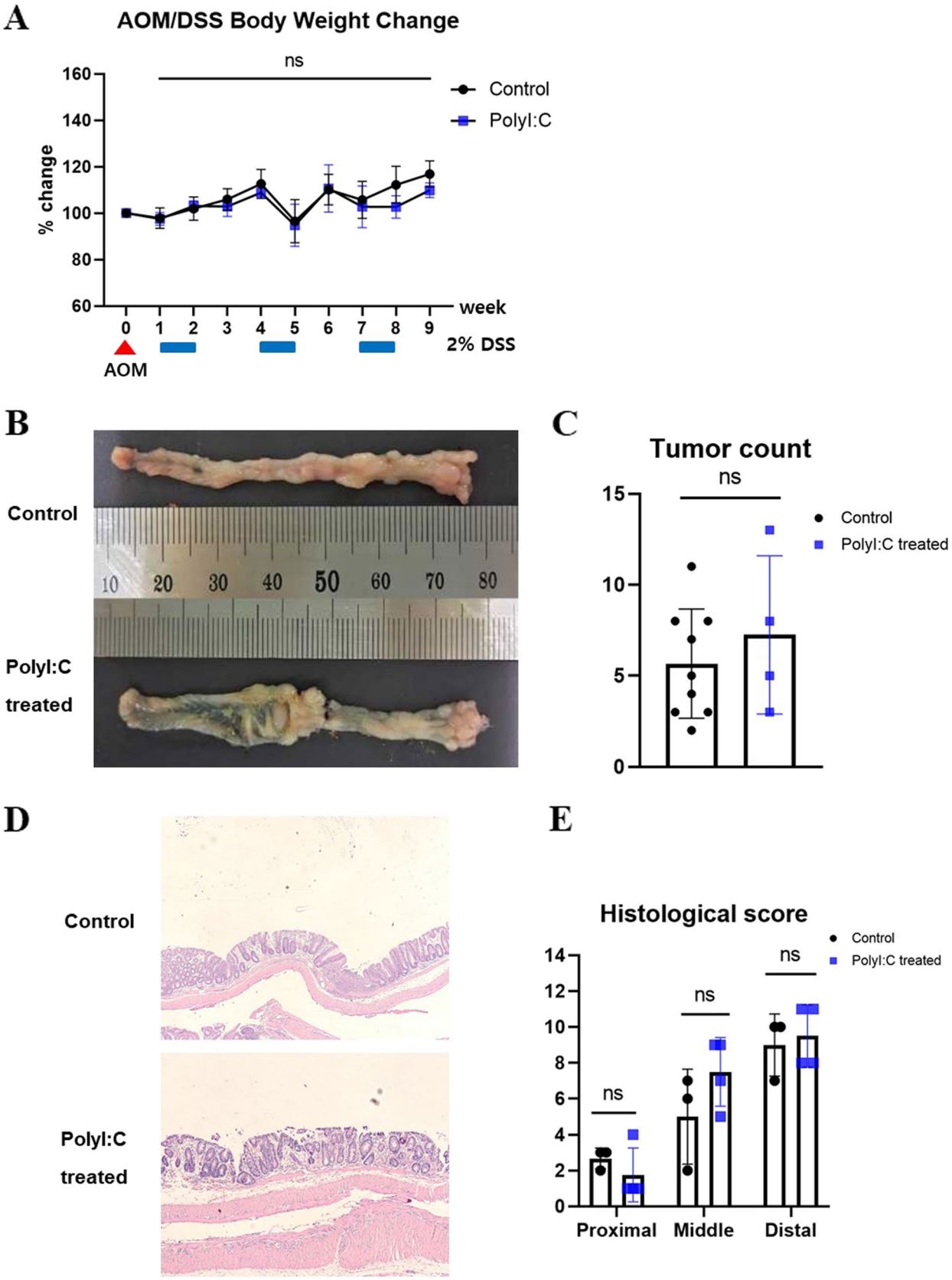

Fig. 3. Clinical and histological changes in AOM/DSS-treated WT mice with or without Poly(I: C) injection. (**A**) There was no significant difference of body weight change between Poly(I: C) injected and control groups. (**B**, **C**) Gross evaluation of tumor burden did not show significant difference between the two groups. (**D**, **E**) There was no significant difference in histologic score between the two groups.

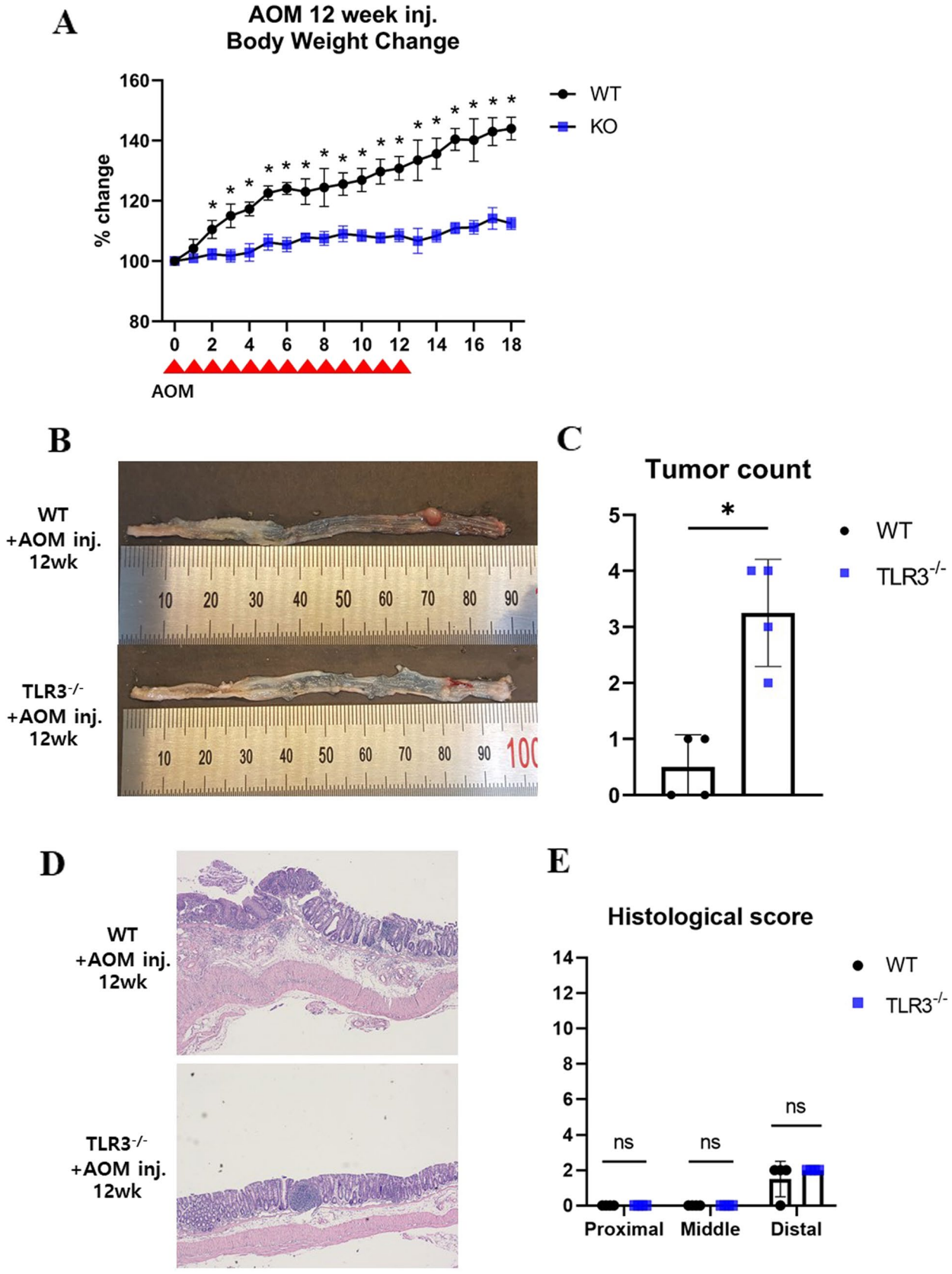

Fig. 4. Clinical and histological changes in WT and TLR3$^{-/-}$ mice after AOM treatment. (**A**) TLR3$^{-/-}$ mice showed significantly decreased weight gain over 12 weeks compared with that of WT mice. (**B**, **C**) Gross evaluation of tumor burden. The number of tumors showed no significant difference between WT and TLR3$^{-/-}$ mice. (**D**, **E**) There was no significant difference of histological score from all three sections of colon tissue in WT and TLR3$^{-/-}$ mice. The symbol: $^{*}p < 0.05$.

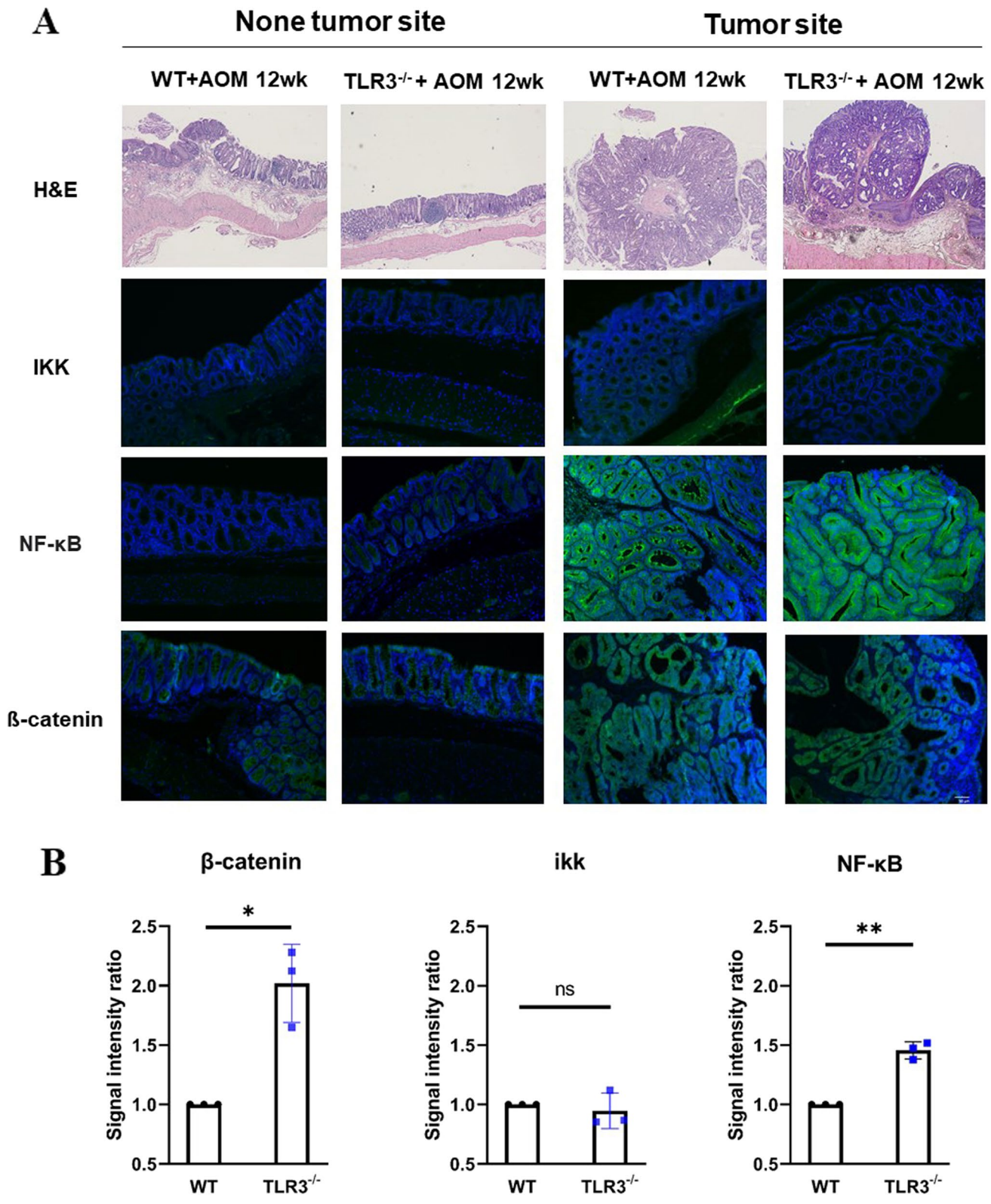

Fig. 5. Histological images from non-tumor site and tumor site in AOM-treated WT and TLR3$^{-/-}$ mice. (**A**) H&E and immunofluorescence assays of AOM-treated WT and TLR3$^{-/-}$ mice. Overall inflammation on both non-tumor and tumor sites showed no significant difference in WT and TLR3$^{-/-}$ mice. (**B**) Fluorescence intensity of IKK, *NF-κB* and β-catenin. The expression of β-catenin was significantly more amplified in tumor site of TLR3$^{-/-}$ mice than WT mice. However, there was no significant difference of the expression IKK in tumor site between WT and TLR3$^{-/-}$ mice. The symbols: $^{**}p < 0.01$ and $^{*}p < 0.05$.

inflammatory effect of colitis. To the best of our knowledge, this is the first study to demonstrate the role of TLR3 in CAC.

TLR signaling is crucial for both the innate and adaptive immune responses. Upon stimulation by various pathogen-associated molecular patterns and damage-associated molecular patterns, TLR signaling activates antigen-presenting cells and induces cytokines[23,24]. Its role in immunoregulation has been extensively studied in inflammatory diseases, such as IBD, and recent research has shed light on the cancer-related immune responses of TLR signaling. Among these pathways, TLR3 signaling plays a vital role in regulating innate and adaptive immune systems by recognizing viral double-stranded RNA. This recognition triggers the activation of nuclear factor-κB and interferon regulatory factor 3 transcription factors through binding to the Toll/Interleukin-1 receptor domain-containing adaptor-inducing IFN-β (TRIF) adaptor protein[25]. Consequently, proinflammatory cytokines, chemokines, and type I IFNs are expressed, initiating antiviral responses[26–28]. Additionally, the TLR3 signaling pathway induces apoptosis in infected cells to eliminate them[29,30]. Overall, TLR3 signaling shows potential as a target for managing colon inflammation and tumorigenesis, as it enhances anticancer immune responses by activating both innate and adaptive immunity[31,32].

TLR3 has been found to have an antitumorigenic effect consistently[13,17–19]. Bruno et al. suggested that TLR3 was expressed in several breast cancer cell lines and could induce apoptosis in those cells[19]. Qun Jiang et al. showed that TLR3 is widely expressed in various human and murine tumor cell lines, suggesting that TLR3 activation may be important in tumor biology[33]. For colorectal cancer, Nojiri et al. found that poly(I: C) transfection of SW480 cells activated TLR3 and induced apoptosis in colorectal cancer and Yoshida et al. suggested that TLR3 may inhibit colorectal cancer growth and metastasis via CCL2, CCL5, and IL-8 chemokines pathways[34,35]. However, the role of TLR3 in CAC remains obscure. Therefore, we focused on the role of TLR3 in CAC development. TLR3 mice showed an antitumor effect against CAC, consistent with previous studies, suggesting that TLR3 signaling may be a target for preventing CAC.

CAC is a type of colorectal cancer that develops due to chronic inflammation of the colon and is commonly associated with IBD[33]. Chronic intestinal inflammation results in the activation of signaling pathways, including the TLR signaling pathway, which leads to the production of inflammatory cytokines, such as TNF-α, IL-17, IL-23, IFN-γ, and IL-6, and the activation of nuclear factor-κB signaling resulting in dysplasia and cancer[33]. However, in sporadic colorectal cancer, mutations in adenomatous polyposis coli (APC) or β-catenin typically precede the formation of adenomas and the progression to colorectal carcinoma, indicating different pathways of tumorigenesis between CAC and non-CAC colorectal cancers. Nevertheless, there are considerable similarities between the mechanisms underlying the development of colorectal cancer and CAC[36]. In the present study, $TLR^{-/-}$ mice showed prominent intestinal inflammation and proinflammatory cytokine expression, such as TNF-α and IFN-γ, after the treatment of DSS. However, immunofluorescence analysis of colon tissue demonstrated no difference in IKK expression but increased immunoreactivity for β-catenin in $TLR3^{-/-}$ mice, particularly in the cell nucleus of tumor tissue. To test this hypothesis, we performed an in vivo study using a model of repeated AOM injection without DSS treatment. Repeated AOM injections induced prominent colon tumorigenesis in $TLR3^{-/-}$ mice compared with that in WT mice. Based on these results, TLR3 signaling attenuates CAC development by attenuating intestinal inflammation and direct antitumor activity. Further mechanistic studies are required to elucidate the precise control mechanisms of TLR3 signaling in CAC development.

In conclusion, TLR3 plays an essential role in the development of CAC and potentiates the antitumor effect directly rather than having an anti-inflammatory effect. These findings support the use of TLR3 as a preventive and therapeutic candidate for CAC in patients with IBD.

Methods

Mice

WT (C57BL/6; Orient Bio) and TLR3-deficient ($TLR3^{-/-}$) male mice (7–8 weeks old) were used. All mice were raised under specific pathogen-free conditions with a standard temperature, humidity, and 12 h light/dark cycle. Mice with more than 20% weight loss from baseline body weight were excluded from the experiments. The number of mice used in each experiment and the final number of mice analyzed, excluding those that died during the experiments, are as follows: In the AOM/DSS experiment, 5 mice per group (WT and $TLR3^{-/-}$) were allocated, with 4 mice per group ultimately analyzed. In the Poly(I: C) treatment experiment, 10 mice per group were allocated, with 9 WT and 4 $TLR3^{-/-}$mice included in the final analysis. In the long-term AOM treatment experiment, 5 mice per group were allocated, with 4 mice per group ultimately analyzed.

AOM/DSS-induced inflammatory reactions and colitis-associated tumorigenesis in mice

WT and $TLR3^{-/-}$mice were administered a single intraperitoneal injection of AOM (Sigma Aldrich) at 12.5 mg/kg on day zero, followed by three cycles of 2% DSS (MP Biomedical) in drinking water for five days on week 1, week 4 and week 7. The body weight was measured daily during the study period. Mice were euthanized by isoflurane inhalation, and colon tissues were obtained. Colon length, severity of colitis by histological score, and tumor burden were measured.

The colons were collected and longitudinally dissected. The gross tumor burden in the murine colon was evaluated using captured images. The number and size of tumors in the colon were assessed and analyzed using the ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Assessment of poly(I: C) on AOM/DSS-induced inflammatory reactions and colitis-associated tumorigenesis in WT mice

Poly(I: C) (InvivoGen) was intraperitoneally injected (20 μg/mouse in 100 μl PBS) on day 7 after AOM injection on day 0 in the AOM/DSS-induced tumorigenesis model of WT mice. Prior to administration of poly(I: C), it

was reconstituted in saline at 2 mg/mL and then heated to 50℃ in a water bath. Subsequently, the poly(I: C) solution was cooled to room temperature and administered to mice. The body weight was measured daily during the study period. Mice were euthanized by isoflurane inhalation, and colon tissues were obtained. Colon length, severity of colitis by histological score, and tumor burden were measured.

Assessment of the colon tumorigenesis by repeated AOM administration without DSS treatment in mice

WT and TLR3$^{-/-}$ mice were intraperitoneally injected with AOM 12.5 mg/kg weekly for 12 weeks. The body weight was measured daily during the study period. Mice were euthanized by isoflurane inhalation, and colon tissues were obtained. Colon length, severity of colitis by histological score, and tumor burden were measured.

Histological and immunofluorescence assays

Colon samples were fixed in 4% buffered formalin and embedded in paraffin. The colon sections were stained with hematoxylin and eosin (H&E) and antibodies specific for phospho-IκB kinase IKK (1:100, abcam), β-catenin (1:400, abcam), NF-kB (1:100, Santa Cruz), neutrophil elastase ELANE (1:200, Santa Cruz), and ZO-1 (1:200, Abcam), which were appropriately diluted with antibody dilution buffer (TBS-T with 0.5% BSA). Following primary antibody staining, a secondary antibody (Alexa 488, 1:400, Abcam) was diluted and applied using an antibody dilution buffer. The histological assessment of colitis severity was scored using H&E-stained slides as follows: crypt damage (0–4), extent of inflammation (0–3), severity of inflammation (0–4), and percentage of involvement (0–4). Each slide was evaluated based on a total score of 15 and the average value was statistically evaluated. Fluorescence density of IKK, β-catenin, NF-kB, neutrophil elastase ELANE, and ZO-1 were analyzed using the image of slides capture from a fluorescence microscope (Cellena-S, Logosbio), Image J and Aivia™ (EAS-Leica) software.

Real-time PCR

Real-time reverse transcription polymerase chain reaction was performed as described previously[37]. Briefly, lamina propria mononuclear cells were isolated from mouse colon tissue using the standard lamina propria mononuclear cells isolation protocol with TRIzol (Gibco BRL), collagenase (Sigma-Aldrich), and Percoll (Cytiva). One microgram of total RNA was reverse transcribed and amplified using an SYBR Green PCR master mix and the LightCycler® 480 Real-Time PCR System (Roche). Amplification was performed in triplicate and the results were normalized to the expression level of β-actin. The primers used for target genes were as follows: TNF-α (Forward: 5'-CAT CTT CTA AAA ATC GAG TGA CAA-3', Reverse: 5'-TGG GAG TAG ACA AGG TAC AAC CC-3'), IFN-γ (Forward: 5'-TGC ATC TTG GCT TTG CAG CTC TTC-3', Reverse: 5'-GGG TTG TTG ACC TCA AAC TTG GCA-3'), interleukin-1 beta (Forward: 5'-CCA CCT TTT GAC AGT GAT GA-3', Reverse: 5'-GTT GTC TAA TGG GAA CGT CA-3'), IL-6 (Forward: 5'-CAG AGG ATA CCA CTC CCA ACA-3', Reverse: 5'-CAG AAT TGC CAT TGC ACA AC-3'), and IL-17 (Forward: 5'-TCT CAT CCA GCA AGA GAT CC-3', Reverse: 5'-AGT TTG GGA CCC CTT TAC AC-3').

Statistical analysis

Data are expressed as the mean ± standard deviation. The Non-parametric Mann–Whitney U test was performed to compare values among the groups using SPSS 25 statistical software (SPSS, Chicago, IL, USA). Statistical significance was set at $P < 0.05$.

Ethics approval

This study was approved by the Institutional Animal Care and Use Committee of SMG-SNU Boramae Medical Center (Institutional Review Board [IRB] No. 2016–0017). All procedures were performed in accordance with the ethical standards of the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health, and ARRIVE guidelines.

Data availability

The data supporting the findings of this study are included in this published article. Raw data generated and/or analysed during the current study are available from the corresponding author, upon reasonable request.

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References

1. Olén, O. et al. Colorectal cancer in ulcerative colitis: a scandinavian population-based cohort study. *Lancet.* **395**, 123–131 (2020).
2. Olén, O. et al. Colorectal cancer in Crohn's disease: a scandinavian population-based cohort study. *Lancet Gastroenterol. Hepatol.* **5**, 475–484 (2020).
3. Renz, B. W. et al. Clinical outcome of IBD-associated versus sporadic colorectal cancer: a matched-pair analysis. *J. Gastrointest. Surg.* **17**, 981–990 (2013).
4. Baker, A. M. et al. Evolutionary history of human colitis-associated colorectal cancer. *Gut.* **68**, 985–995 (2019).
5. Akira, S., Uematsu, S. & Takeuchi, O. Pathogen recognition and innate immunity. *Cell.* **124**, 783–801 (2006).
6. Cario, E. et al. Lipopolysaccharide activates distinct signaling pathways in intestinal epithelial cell lines expressing toll-like receptors. *J. Immunol.* **164**, 966–972 (2000).
7. Kumar, H., Kawai, T. & Akira, S. Pathogen recognition by the innate immune system. *Int. Rev. Immunol.* **30**, 16–34 (2011).
8. Vijay-Kumar, M. et al. Activation of toll-like receptor 3 protects against DSS-induced acute colitis. *Inflamm. Bowel Dis.* **13**, 856–864 (2007).

9. Zhao, H. W. et al. Effect of toll-like receptor 3 agonist poly I:C on intestinal mucosa and epithelial barrier function in mouse models of acute colitis. *World J. Gastroenterol.* **23**, 999–1009 (2017).
10. Fukata, M. & Abreu, M. T. TLR4 signalling in the intestine in health and disease. *Biochem. Soc. Trans.* **35**, 1473–1478 (2007).
11. Huang, B. et al. TLR signaling by tumor and immune cells: a double-edged sword. *Oncogene*. **27**, 218–224 (2008).
12. Khvalevsky, E. et al. TLR3 signaling in a hepatoma cell line is skewed towards apoptosis. *J. Cell. Biochem.* **100**, 1301–1312 (2007).
13. Salaun, B. et al. Toll-like receptor 3 expressed by melanoma cells as a target for therapy? *Clin. Cancer Res.* **13**, 4565–4574 (2007).
14. Sato, Y. et al. Cancer cells expressing toll-like receptors and the Tumor Microenvironment. *Cancer Microenviron.* **2** (Suppl 1), 205–214 (2009).
15. Yu, L. & Chen, S. Toll-like receptors expressed in tumor cells: targets for therapy. *Cancer Immunol. Immunother*. **57**, 1271–1278 (2008).
16. Dajon, M., Iribarren, K. & Cremer, I. Toll-like receptor stimulation in cancer: a pro- and anti-tumor double-edged sword. *Immunobiology*. **222**, 89–100 (2017).
17. Chew, V. et al. Toll-like receptor 3 expressing tumor parenchyma and infiltrating natural killer cells in hepatocellular carcinoma patients. *J. Natl. Cancer Inst.* **104**, 1796–1807 (2012).
18. Paone, A. et al. Toll-like receptor 3 triggers apoptosis of human prostate cancer cells through a PKC-alpha-dependent mechanism. *Carcinogenesis*. **29**, 1334–1342 (2008).
19. Salaun, B. et al. TLR3 can directly trigger apoptosis in human cancer cells. *J. Immunol.* **176**, 4894–4901 (2006).
20. Scheeren, F. A. et al. A cell-intrinsic role for TLR2-MYD88 in intestinal and breast epithelia and oncogenesis. *Nat. Cell. Biol.* **16**, 1238–1248 (2014).
21. Lowe, E. L. et al. Toll-like receptor 2 signaling protects mice from tumor development in a mouse model of colitis-induced cancer. *PLoS One*. **5**, e13027 (2010).
22. Huang, B. et al. Toll-like receptors on tumor cells facilitate evasion of immune surveillance. *Cancer Res.* **65**, 5009–5014 (2005).
23. Huang, Z. N. et al. Synergistic immunostimulation through the dual activation of toll-like receptor 3/9 with spherical nucleic acids. *ACS Nano*. **15**, 13329–13338 (2021).
24. Kawasaki, T. & Kawai, T. Toll-like receptor signaling pathways. *Front. Immunol.* **5**, 461 (2014).
25. Vaidya, S. A. & Cheng, G. Toll-like receptors and innate antiviral responses. *Curr. Opin. Immunol.* **15**, 402–407 (2003).
26. Alexopoulou, L. et al. Recognition of double-stranded RNA and activation of NF-kappaB by toll-like receptor 3. *Nature*. **413**, 732–738 (2001).
27. Hausmann, M. et al. Toll-like receptors 2 and 4 are up-regulated during intestinal inflammation. *Gastroenterology*. **122**, 1987–2000 (2002).
28. Yamamoto, M. et al. Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. *Science*. **301**, 640–643 (2003).
29. Everett, H. & McFadden, G. Apoptosis: an innate immune response to virus infection. *Trends Microbiol.* **7**, 160–165 (1999).
30. Kaiser, W. J. & Offermann, M. K. Apoptosis induced by the toll-like receptor adaptor TRIF is dependent on its receptor interacting protein homotypic interaction motif. *J. Immunol.* **174**, 4942–4952 (2005).
31. Javaid, N. & Choi, S. Toll-like receptors from the perspective of Cancer Treatment. *Cancers (Basel).* **12** (2),297 (2020).
32. Bourquin, C., Pommier, A. & Hotz, C. Harnessing the immune system to fight cancer with toll-like receptor and RIG-I-like receptor agonists. *Pharmacol. Res.* **154**, 104192 (2020).
33. Jiang, Q., Wei, H. & Tian, Z. Poly I:C enhances cycloheximide-induced apoptosis of tumor cells through TLR3 pathway. *BMC Cancer*. **8**, 12 (2008).
34. Nojiri, K. et al. The expression and function of toll-like receptors 3 and 9 in human colon carcinoma. *Oncol. Rep.* **29**, 1737–1743 (2013).
35. Yoshida, T. et al. Toll-like receptor 3 as a recurrence risk factor and a potential Molecular Therapeutic Target in Colorectal Cancer. *Clin. Exp. Gastroenterol.* **13**, 427–438 (2020).
36. Terzić, J. et al. Inflammation and colon cancer. *Gastroenterology*. **138**, 2101–2114e5 (2010).
37. Koh, S. J. et al. Matricellular protein periostin promotes colitis-associated colon tumorigenesis in mice. *Carcinogenesis*. **40**, 102–111 (2019).

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Author contributions

Chung KY, Kim SJ and Koh SJ designed the study; Yoon HT and Kwon SH collected the data; Chung KY and Kim SJ analyzed the data; Chung KY, Kim SJ and Koh SJ were involved in the interpretation of the result of analyzed data; Chung KY and Kim SJ wrote the original draft of the manuscript; All authors participated in the critical review of the results, reading and editing the manuscript, and approving the final manuscript. Chung KY and Kim SJ equally contributed to this study as co-first authors.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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