Received: 1 August 2021 | Revised: 3 November 2021 | Accepted: 8 November 2021

DOI: 10.1111/cas.15206

Cancer Science WILEY

ORIGINAL ARTICLE

Dvl2 facilitates the coordination of NF-κB and Wnt signaling to promote colitis-associated colorectal progression

Feiyu Tang[1,2] | Fuyang Cao[1,2] | Can Lu[3] | Xiang He[1,2] | Liang Weng[1,2,4,5,6] | Lunquan Sun[1,2,5,6,7]

[1]Department of Oncology, Xiangya Cancer Center, Xiangya Hospital, Central South University, Changsha, China

[2]Key Laboratory of Molecular Radiation Oncology Hunan Province, Changsha, China

[3]Department of Pathology, Xiangya Hospital, Central South University, Changsha, China

[4]Hunan International Science and Technology Collaboration Base of Precision Medicine for Cancer, Changsha, China

[5]Hunan Provincial Clinical Research Center for Respiratory Diseases, Changsha, China

[6]Center for Molecular Imaging of Central South University, Xiangya Hospital, Changsha, China

[7]Institute of Gerontological Cancer Research, National Clinical Research Center for Gerontology, Changsha, China

Correspondence
Liang Weng and Lunquan Sun, Key Laboratory of Molecular Radiation Oncology Hunan Province, Changsha 410008, China.
Emails: wengliang@csu.edu.cn (LW) and lunquansun@csu.edu.cn (LQS)

Funding information
Hunan Province Natural Science Funds for Excellent Young Scholars, Grant/Award Number: 2019JJ30043; National Natural Science Foundation of China, Grant/Award Number: 81530084, 81900199, 81974465 and 82030087; recruitment program for Huxiang Talents, Grant/Award Number: 2019RS1009

Abstract

Colitis-associated colorectal cancer (CAC) arises due to prolonged inflammation and has distinct molecular events compared with sporadic colorectal cancer (CRC). Although inflammatory NF-κB signaling was activated by pro-inflammatory cytokines (such as TNFα) in early stages of CAC, Wnt/β-catenin signaling later appears to function as a key regulator of CAC progression. However, the exact mechanism responsible for the cross-regulation between these 2 pathways remains unclear. Here, we found reciprocal inhibition between NF-κB and Wnt/β-catenin signaling in CAC samples, and the Dvl2, an adaptor protein of Wnt/β-catenin signaling, is responsible for NF-κB inhibition. Mechanistically, Dvl2 interacts with the C-terminus of tumor necrosis factor receptor 1 (TNFRI) and mediates TNFRI endocytosis, leading to NF-κB signal inhibition. In addition, increased infiltration of the pro-inflammatory cytokine interleukin-13 (IL-13) is responsible for upregulating Dvl2 expression through STAT6. Targeting STAT6 effectively decreases Dvl2 levels and restrains colony formation of cancer cells. These findings demonstrate a unique role for Dvl2 in TNFRI endocytosis, which facilitates the coordination of NF-κB and Wnt to promote CAC progression.

KEYWORDS
Dvl2, endocytosis, inflammation, NF-κB, TNFRI, Wnt

1 | INTRODUCTION

Colorectal cancer (CRC) is a common malignant cancer and has a high global mortality rate. Colitis-associated colorectal cancer (CAC) differs from the more common sporadic CRC and accounts for 2% of CRC.[1] CAC develops from long-standing colitis in inflammatory bowel disease, having a worse prognosis and an increased mortality rate. Early-stage CAC is markedly associated with gut microbiota-triggered inflammation, which promotes the infiltration of pro-inflammatory cytokines, the continuous activation of NF-κB, the generation of reactive oxygen species, and the formation of invasive carcinoma associated with a high frequency of over-activated Wnt/β-catenin signaling.[2] Nevertheless, it is unclear how inflammation interacts with tumorigenic signaling to contribute to the progression of CAC. Therefore, dynamic surveillance of CAC progression is required to better understand the molecular mechanisms underlying inflammation-induced tumorigenesis.

The Wnt/β-catenin signaling pathway plays a critical role in CAC initiation and progression.[3] Two components, membrane-associated signalosome and cytosolic Axin/glycogen synthase kinase 3 (GSK3)/adenomatous polyposis coli (APC) destruction complex, typically have opposite functions but together control the transcriptional cofactor β-catenin to integrate Wnt signaling.[4] Dishevelled (Dvl) is a key regulator that can be activated and recruited to the Wnt receptor upon Wnt stimulation, which further assembles the signalosome with Axin and GSK3β and breaks up the destruction complex.[5-9] Several pieces of evidence indicate that Dvl2 is involved in CRC progression,[10,11] however, the relevant regulatory mechanism has not yet been completely explored.

Inflammation is associated with the accelerated progression of CAC, partly due to the activation of the canonical NF-κB signaling pathway, which is involved in cellular processes such as cell growth, apoptosis, and inflammatory responses.[12] The canonical NF-κB signaling pathway is triggered by Toll-like microbial pattern recognition receptors (TLRs) and pro-inflammatory cytokines such as tumor necrosis factor alpha (TNFα) and interleukin-1 (IL-1). These molecules then activate NF-κB and facilitate the nuclear import of RelA/p65 (one of the subunits of NF-κB) to induce the expression of various inflammatory-related genes.[13] The clathrin-dependent endocytosis of TNFRI represents a switch in the downstream molecular composition, blocking inflammation and inducing pro-apoptotic signal.[14] However, little is currently known about the regulatory mechanism of the endocytosis of TNFRI.

Due to the critical role of NF-κB and Wnt signaling in CRC, several studies have investigated the relationship between these 2 pathways. Previous studies have reported that elevated NF-κB levels enhance Wnt signaling, with de-differentiation of intestinal epithelial cells and the promotion of tumor initiation.[15] Noteworthy, there was a reverse correlation between NF-κB and Wnt in CRC cell lines. β-Catenin interacted and inhibited RelA to enter the nucleus, while expression of RelA was positively modulated by β-catenin transcriptional activity.[16,17] Therefore, the mechanism behind how NF-κB and Wnt signaling regulate each other in CRC, especially during CAC progression, demands further clarification.

In this study, we comprehensively evaluated the molecular events during CAC progression. Our results demonstrated that there is a negative correlation between NF-κB signaling and Wnt signaling in CAC. During the early stages of CAC, infiltrated inflammatory cytokines within the microenvironment upregulate the expression of Dvl2. As the disease progresses, elevated Dvl2 levels inhibit TNFα–NF-κB by promoting TNFRI endocytosis and releasing the inhibitory function of NF-κB on Wnt3a-induced stems of cancer cells. We here reported for the first time that Dvl2 acts as a key regulator of NF-κB and Wnt signaling during CAC tumorigenesis.

2 | MATERIALS AND METHODS

2.1 | Animal procedures

This study was approved by the Institutional Animal Care and Use Committee (IACUC) of Central South University. C57BL/6 (6-8 wk old) mice were purchased from Hunan SJA Laboratory Animal Co., Ltd. and mated. The generation of azoxymethane (AOM)/dextran sodium sulfate (DSS)-induced colitis-associated colon cancer has been described previously.[18] Briefly, mice were given a single intraperitoneal injection of AOM (10 mg/mL) solution on day 0; on day 7, 2.5% DSS solution was administered for 1 wk followed by free water for 2 wk. This cycle was repeated 2 times. Disease progression was determined by body weight changes and the presence of rectal bleeding. Mice were sacrificed on day 0, day 40 and day 80 and divided into normal, inflammatory, and tumorous groups according to the presence or absence of nodules, levels of inflammatory cytokines (IL-1β, IL-6, TNFα), and histomorphology.

2.2 | TNFRI endocytosis assay and immunofluorescence staining

Cells were cultured under starvation conditions for 4 h, and then treated with TNFα (100 ng/mL) for 0 and 20 min. Cells were incubated with stripping buffer (0.5 mol/L NaCl, 3% acetic acid) to remove surface-bound TNFα. Cells were fixed with 4% paraformaldehyde for 10 min and permeabilized with 0.2% Triton X-100 at room temperature for 5 min. Cells were incubated with anti-TNFα antibody overnight at 4°C and then with secondary antibody (anti-rabbit Alexa Fluor 594 dye conjugate) for another 1 h at room temperature. The cells were imaged using Leica DM6500 confocal microscopy.

2.3 | Statistical analysis

The statistical analysis for comparisons between 2 groups was performed using Student *t* test, and the statistical analysis for multiple comparison was performed using two-way ANOVA. Quantitative data are presented as means ± SEM. Values of $P < .05$ were regarded as significant. See Supplementary material Doc. S1 for further details.

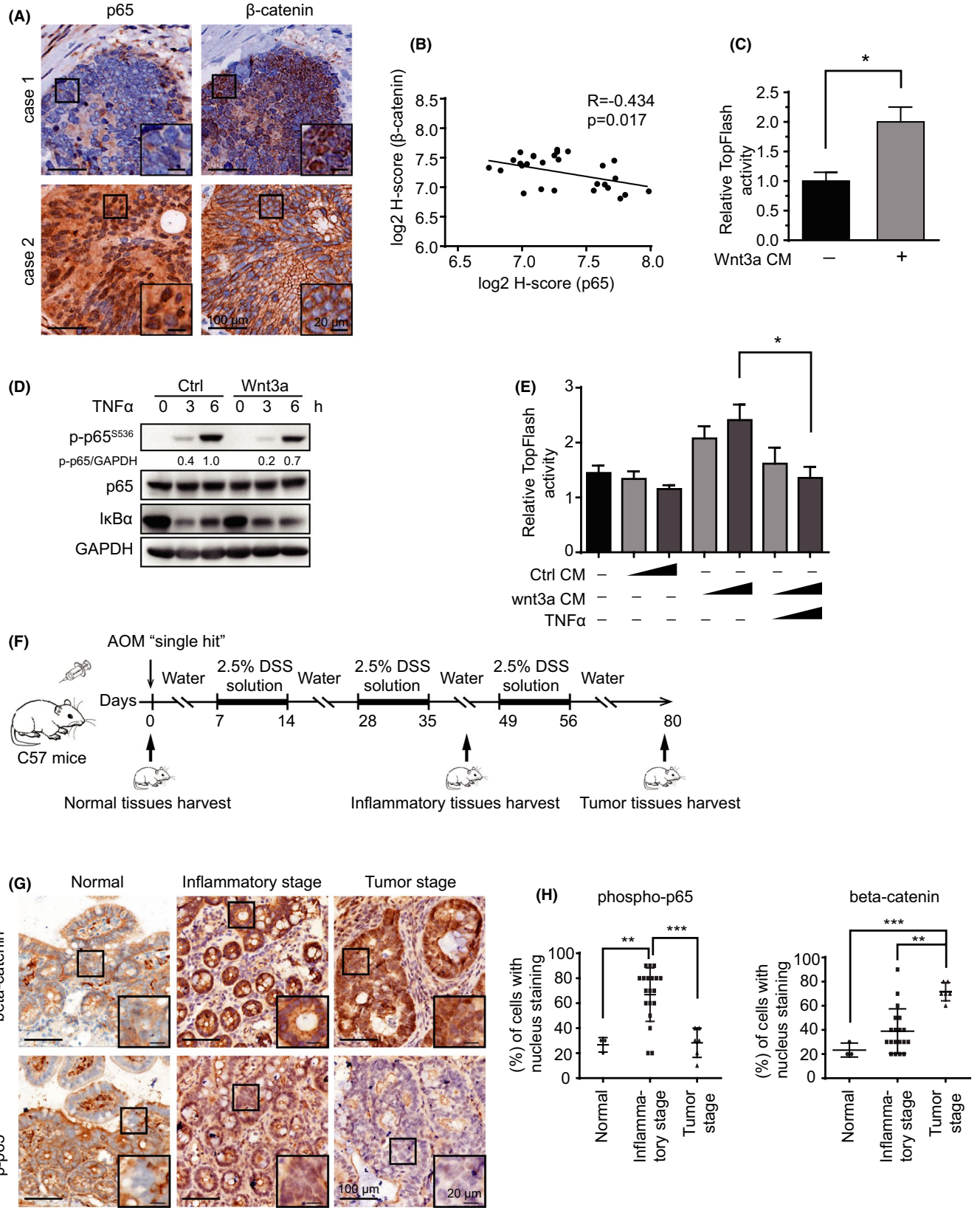

FIGURE 1 NF-κB is negatively correlated with Wnt signaling in inflammation-induced colon cancer. A and B, The immunochemistry staining of p65 and β-catenin in human colorectal cancer tissues. Representative images (A) and the quantification of nuclear accumulation (B) are shown. Scale bars, 100 μm. N = 30. C, The efficiency of Wnt3a conditioned medium verified using the TopFlash luciferase assay. D, The activity of TNFα-induced NF-κB in Wnt3a conditioned medium-treated RKO cells. The intensity of bands was quantified and normalized to GAPDH. E, The activity of Wnt3a-induced Wnt signaling upon TNFα treatment. F, Schematic of the inflammation driven colorectal tumorigenesis model. G, Immunochemistry staining of phospho-p65 and β-catenin in mice tissues, scale bars, 100 μm. N = 3, 19, 6, for normal, inflammatory stage, and tumor stage, respectively. H, Quantification of nuclear accumulation phospho-p65 and β-catenin. All statistical values are presented as means ± SEM; $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$

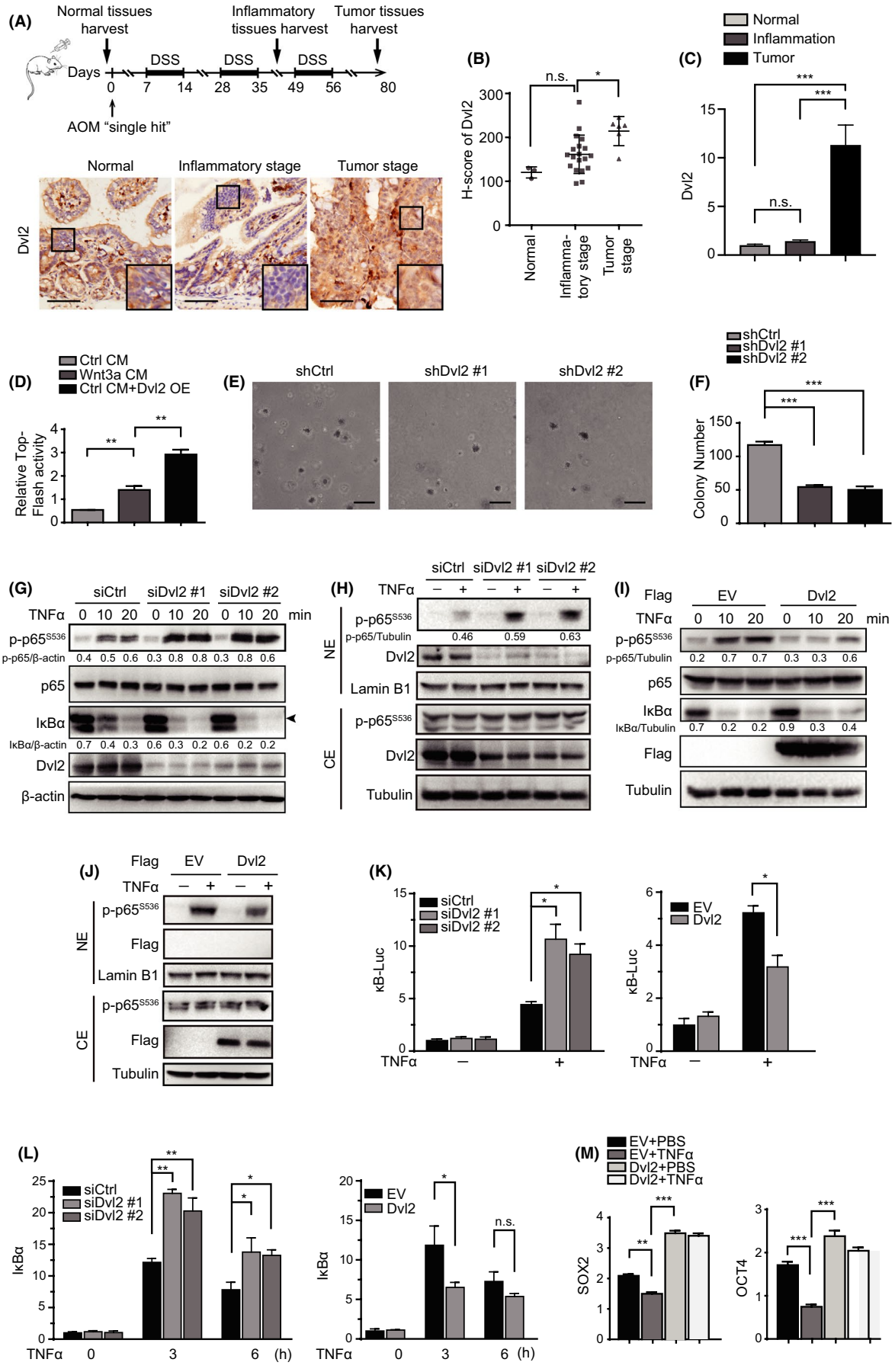

FIGURE 2 Legend on next page

FIGURE 2 Dvl2 inhibits TNFα-induced NF-κB signaling. A, Immunohistochemistry images of Dvl2 in normal, inflammatory, and colorectal cancer mice tissues. Scale bars, 100 μm. B, Quantification of Dvl2 level in mice tissues. N = 3, 19, 6, for normal, inflammatory stage, and tumor stage, respectively. C, qRT-PCR analysis of Dvl2 in normal, inflammatory, and colorectal cancer mice tissues. D, The activation of Wnt signaling in Dvl2-overexpressed RKO cells. E, RKO cells colony formation in soft agar with or without Dvl2 knockdown. The quantification data are shown in (F). G, The activation of NF-κB signaling in Dvl2-knockdown RKO cells. H, The expression of NF-κB in cytosol or nucleus in Dvl2-knockdown RKO cells. I, The activation of NF-κB signaling in Dvl2-overexpressed RKO cells. J, The expression of NF-κB in cytosol or nucleus in Dvl2-overexpressed RKO cells. K, TNFα-induced NF-κB reporter gene activity in Dvl2-knockdown or Dvl2-overexpressed RKO cells. L, qRT-PCR analysis of IκBα in Dvl2-knockdown or Dvl2-overexpressed RKO cells. M, qRT-PCR analysis of stem cell genes (*OCT4* and *SOX2*) in TNFα-treated or Dvl2 overexpressed RKO cells. The intensity of bands was quantified and normalized to β-actin or tubulin in (G), (H), and (I). All statistical values are presented as means ± SEM; $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$

3 | RESULTS

3.1 | Negative correlation between NF-κB and Wnt signaling in inflammation-induced colon cancer

To examine the role of NF-κB and Wnt signaling in CAC, we collected tumor tissues from 30 colon cancer patients. Immunohistochemical staining was performed on these cancer tissues with antibodies of p65 and β-catenin, with their nuclear staining representing activation of NF-κB and Wnt signal, respectively. We found the opposite nuclear expression pattern of p65 and β-catenin in human colon cancer tissues (Figure 1A,B), indicating the presence of a negative correlation between NF-κB and Wnt signaling. Among several colorectal cell lines, only RKO cells have no mutation in Wnt signaling-related genes and show low level activity in the absence of Wnt stimuli.[19,20] To further confirm the relationship between these 2 pathways, we used RKO cells to examine the effects of Wnt3a stimulation on NF-κB signaling. We prepared conditioned medium from L cells and Wnt3a-L cells and performed a Wnt reporter activity assay to confirm the activity of these conditioned media (Figure 1C). As shown in Figure 1D, upon Wnt3a-conditioned medium stimulation, the level of TNFα-induced phospho-p65^{S536}, which is the active form of p65, was significantly decreased, while the inhibitory member IκBα was upregulated, suggesting the inhibition of TNFα-induced NF-κB activity by Wnt3a signaling (Figure 1D). Additionally, Wnt/β-catenin activity was significantly decreased in the TNFα-treated RKO cells (Figure 1E).

To study the correlation between NF-κB and Wnt signaling during the progression of CAC in vivo, we used a well established mouse model mimicking CAC to study the role of inflammation in CRC carcinogenesis: the AOM/DSS-induced mouse CRC model. Carcinogen AOM was administered to the mice on day 0, followed by 3 rounds of DSS treatment (Figure 1F). Animals were then euthanized and the colorectal tissues were harvested from the inflammation-stage group (at approximately day 40) and from the tumor-stage group (at approximately day 80). Compared with inflammation tissues, less accumulated phospho-p65 and enhanced accumulation of β-catenin were shown in the nucleus of tumor tissues. This pattern was well correlated with the results from human CAC tissues (Figure 1G,H). Our findings therefore implied the presence of a negative relationship between NF-κB and Wnt signaling in CAC both in vivo and in vitro.

3.2 | Dvl2 inhibits TNFα-induced NF-κB signaling

To elucidate the negative regulatory mechanism between NF-κB and Wnt signaling in CAC, we assessed the effects of Dvl2 on NF-κB signaling due to its critical role in the Wnt signaling pathway and its high expression in CRC.[10,11] We first analyzed the expression level of Dvl2 in inflammatory and tumorous colon tissues. We observed that Dvl2 levels were elevated in tumors in mice colons compared with inflammatory tissues in both protein and mRNA level (Figure 2A-C). To confirm the effect of Dvl2 on Wnt signaling, we performed a TopFlash luciferase assay. Our result showed that overexpression of Dvl2 alone could mimic Wht3a-induced activation of Wnt signaling (Figure 2D). Furthermore, the knockdown of Dvl2 could significantly decrease the formation of colonies in the soft agar assay (Figure 2E,F), implying the crucial role of Dvl2 in the progression of colon cancer. The impact of Dvl2 upon NF-κB signaling was next assessed. Dvl2 knockdown significantly promoted TNFα-induced activation of NF-κB signaling in RKO cells, represented by increased levels of phospho-p65 and decreased expression of IκBα in cells, as well as accumulated phospho-p65 in the nucleus (Figure 2G,H). Furthermore, the overexpression of Dvl2 was capable of inhibiting NF-κB signaling activation (Figure 2I,J).

To further assess how Dvl2 regulated NF-κB signaling, we used a κB-Luc reporter gene to evaluate the effects of Dvl2 on NF-κB transcriptional activity. The knockdown of Dvl2 significantly promoted TNFα-induced NF-κB transcriptional activity, while the overexpression of Dvl2 led to a dramatic repression of NF-κB transcriptional activity (Figure 2K). We also investigated the alteration of NF-κB-dependent target gene expression by Dvl2. As shown in Figure 2L, the induction of IκBα was negatively modulated by Dvl2 (Figure 2L). Additionally, we found that TNFα treatment, which activates NF-κB signaling, reduced the stemness of colon cancer cells. Intriguingly, the overexpression of Dvl2 in TNFα-treated cells impaired the inhibitory effect of TNFα on the stemness (Figure 2M). In brief, our results demonstrated that Wnt signaling negatively regulates TNFα-induced NF-κB signaling activation via Dvl2 in CRC cells, which could facilitate tumor progression by increasing cancer cell stemness.

3.3 | Dvl2 interacts with TNFRI

Dvl2 was previously reported to bind to the Wnt receptor as an adaptor.[21] To understand how Dvl2 modulates NF-κB signaling, we

first assessed whether Dvl2 interacts with TNFRI, one of the main upstream receptors that activate NF-κB. The endogenous interaction of Dvl2 with TNFRI was detected via co-immunoprecipitation in RKO cells, supporting the hypothesis that Dvl2 interacts with TNFRI in vivo (Figure 3A). Mapping the interacting domains indicated that the Dvl2 carboxyl-terminus (CT) domain was responsible for complexing with TNFRI (Figure 3B).

We next examined whether the interaction between Dvl2 and TNFRI was regulated by Wnt signaling, which demonstrated elevated activity during the progression of CAC in our mouse model (Figure 3C). We found that Wnt3a stimulation promoted Dvl2/TNFRI interaction (Figure 3D). Previous studies have reported that Wnt3a can induce the phosphorylation of Dvl2 at 4 sites (S594/T595/S597/T604) within the Dvl2 CT domain and subsequently activate Dvl2.[22] We found that the interaction of TNFRI with the Dvl2-CT alanine mutant (S594A/T595A/S597A/T604A; Figure) was attenuated and did not respond to Wnt3a treatment (Figure 3D), implying that the interaction between Dvl2 and TNFRI was dependent on Wnt3a-induced Dvl2 phosphorylation. All together, these results demonstrated that the Dvl2/TNFRI interaction is regulated by Wnt signaling in CRC cells.

3.4 | Dvl2 promotes the endocytosis of TNFRI

We then assessed how the Dvl2/TNFRI engagement modulates NF-κB signaling. NF-κB signaling is inhibited by the processing and internalization of the TNFα/TNFRI complex via clathrin-coated vehicles.[14,23] A significant increase in NF-κB signaling activation after TNFα treatment was consistently observed in clathrin knockdown RKO cells (Figure 4A). The YHEL motif within the Dvl2 C-terminus is responsible for the activation of clathrin-dependent Wnt receptor internalization,[24] suggesting that Dvl2 could contribute to TNFα-induced TNFRI endocytosis. To examine this hypothesis, we labeled membrane proteins with biotin and isolated surface biotinylated proteins using NeutrAvidin agarose (Figure 4B). Surface TNFRI increased after inhibition of Dvl2, which was similar to the clathrin knockdown, postulating an attenuated internalization of TNFRI (Figure 4C). In addition, the effects of Dvl2 on TNFRI endocytosis were also investigated by endocytosis of TNFα, which was reported to reflect the internalization of TNFRI.[14] No endogenous TNFα staining was detected in cells without TNFα stimulation, however clear TNFα staining could be observed in cells incubated with TNFα for 20 min, which was indicative of the endocytosis of TNFα.

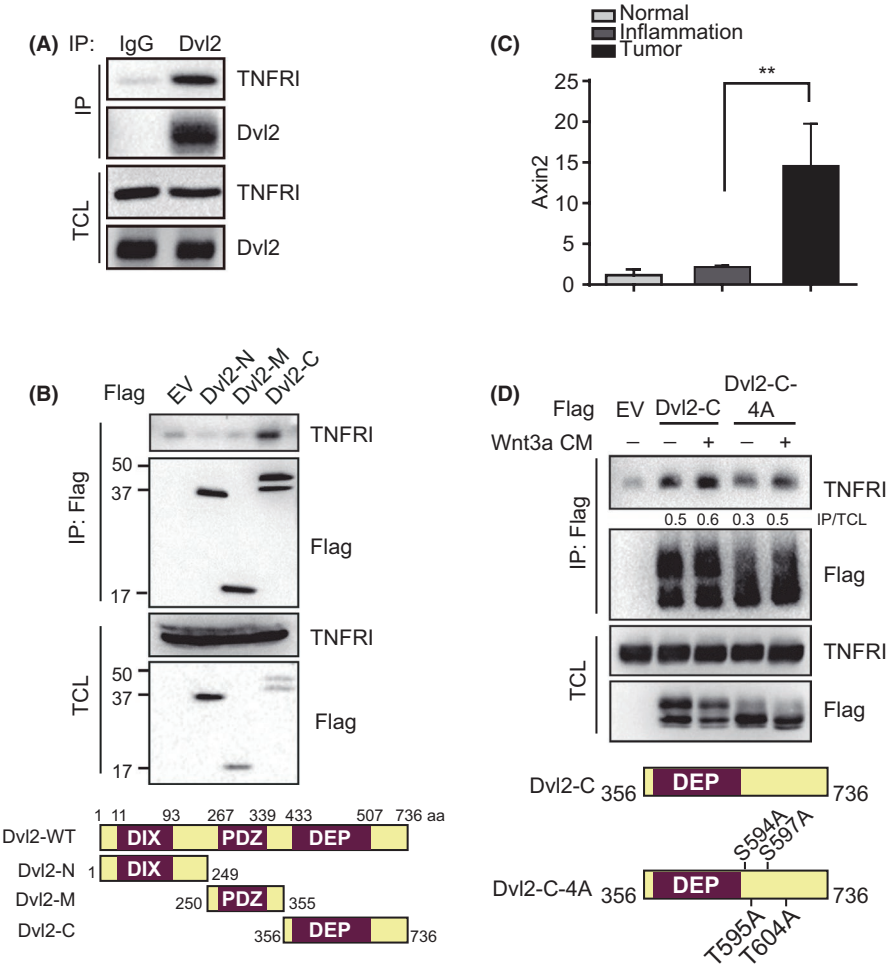

FIGURE 3 Dvl2 interacts with TNFRI. A, Endogenous interaction between Dvl2 and TNFRI was detected in RKO cell lysates. B, The interaction between Dvl2 truncation and TNFRI. C, qRT-PCR analysis of axin2 expression in mice inflammatory and tumorous tissues. D, The interaction between Dvl2 and TNFRI upon Wnt3a stimulation. The intensities of immunoprecipitated TNFRI protein bands were quantified and normalized to TNFRI in TCL

Consistently, endocytosis of TNFα was impeded by downregulating Dvl2 and clathrin (Figure 4D,E). Taken together, our results indicated that Dvl2 inhibited NF-κB signaling activation by promoting TNFα/TNFRI endocytosis.

3.5 | Dvl2 expression is upregulated by inflammatory cytokines during CAC progression

This study demonstrated that highly expressed Dvl2 in CRC cells inhibited NF-κB signaling activation, leading us to ponder how Dvl2 expression level is upregulated. Analysis of hematoxylin and eosin (H&E)-stained colorectal tissues from the AOM-DSS mouse model revealed the presence of inflammation at both the inflammatory stage and the tumor stage (Figure 5A), suggesting continuous inflammation in tumorigenesis that is incompatible with NF-κB inhibition at the tumor stage (Figure 1G). The regulation of inflammation and Dvl2 in CRC is largely unknown to date. Given that many pro-inflammatory cytokines play pivotal roles in tumor initiation and progression, we hypothesized that some inflammatory cytokines within the tumor microenvironment may be responsible for Dvl2 expression in cancer cells. Therefore, we tested the expression of several classical cytokines in AOM-DSS mice model tissues. qRT-PCR analysis uncovered a marked increase in TNFα and IL-13, whereas levels of IL-1β, IL-4, and IL-6 did not show significant changes (Figure 5B). We further treated the RKO cells with these pro-inflammatory cytokines, accompanied with another cytokine TGFβ, which is also related to inflammation, to assess how they affected Dvl2 expression. Our results demonstrated that levels of Dvl2 expression upon IL-13 and IL-4 stimulation was markedly elevated over a 48-h time period. TNFα also promoted Dvl2 expression in a short term, but had no effect after long-term treatment. In contrast, TGFβ significantly decreased Dvl2 expression (Figure 5C). As the upregulation of Dvl2 in tumor stage was consistent with the upregulation of IL-13 instead of IL-4 (Figure 5B), we assessed the underlying mechanisms of IL-13-induced Dvl2 upregulation. Most biological effects of IL-13 are linked to a single transcription factor, the signal transducer and activator of transcription 6 (STAT6).[25,26] Our ChIP assay demonstrated that STAT6 can recognize the Dvl2 promotor region (Figure 5D). Additionally, treatment of the STAT6 inhibitor AS1517499 significantly blocked the STAT6-Dvl2 promoter interaction and reversed the effects of IL-13 on Dvl2 expression (Figure 5E,F). In RKO cells, IL-13 treatment consistently resulted in Wnt activation (Figure 6A). In the presence of IL-13, we observed an increased number of colonies in the soft agar medium, while STAT6 interference inhibited colony formation, indicating the effect of IL-13–STAT6 axis on tumor progression (Figure 6B,C). To sum up, our results propose that the effect of inflammation is constantly present as CAC progresses, and infiltrated pro-inflammatory cytokine IL-13 plays an important role in upregulating Dvl2 expression by acting as a molecular switch between the NF-κB signaling and the Wnt signaling.

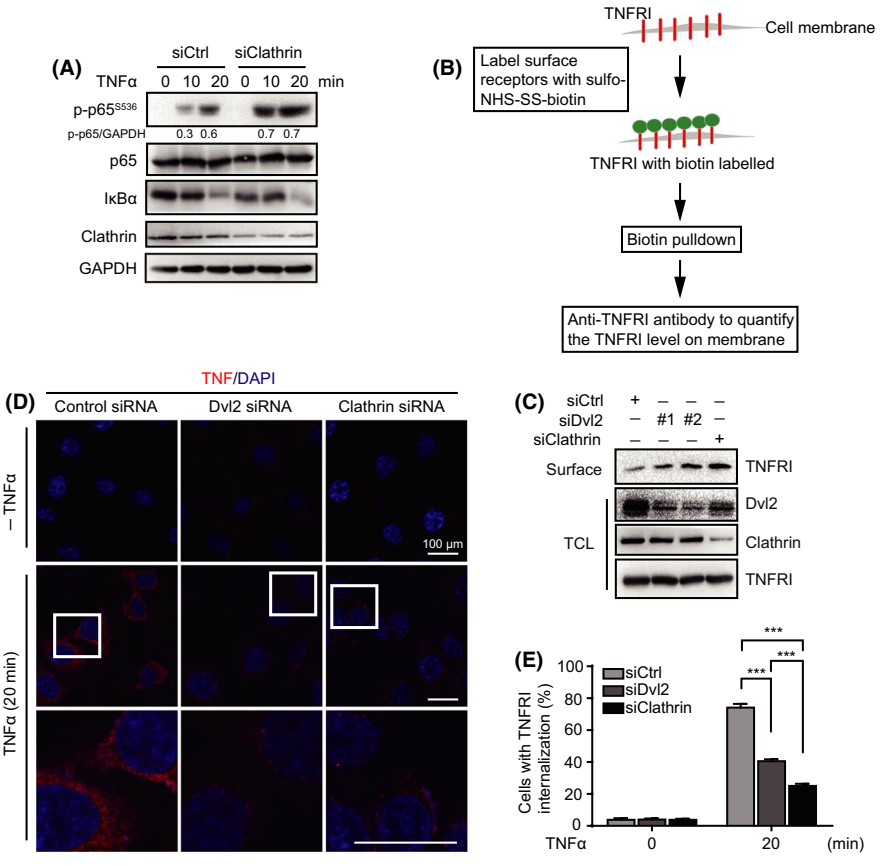

FIGURE 4 Dvl2 promotes the endocytosis of TNFRI. A, NF-κB activity in clathrin-depleted RKO cells. The intensity of bands was quantified and normalized to GAPDH. B, Schematic model for experimental design to check cell surface protein expression. C, Surface level of TNFRI in Dvl2-knockdown RKO cells. D and E, Endocytosis level of TNFα (red) in Dvl2 or clathrin knockdown RKO cells. 4′,6-Diamidino-2-phenylindole was used to stain nucleus (blue). Representative images (D) and the quantification of internalization (E) are both shown. Scale bars, 100 μm. All statistical values were presented as means ± SEM; $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$

4 | DISCUSSION

In this study, we identified a novel function of Dvl2, which acts as a switch that can elaborately regulate activation of the NF-κB signaling and the Wnt signaling to promote CAC progression. We demonstrated that pro-inflammatory cytokine IL-13 was upregulated during CAC progression, which subsequently elevated the expression of Dvl2 via STAT6. Upregulated Dvl2 then promoted the internalization of TNFRI, suppressing the NF-κB signaling and relieving the inhibitory function of TNFα on the Wnt-dependent stemness of CRC cells (Figure 6D).

Somatic mutations in Wnt-related genes such as APC[4] often occur in sporadic CRC, while APC mutation is present less during CAC development. In contrast, inflammatory cytokines are

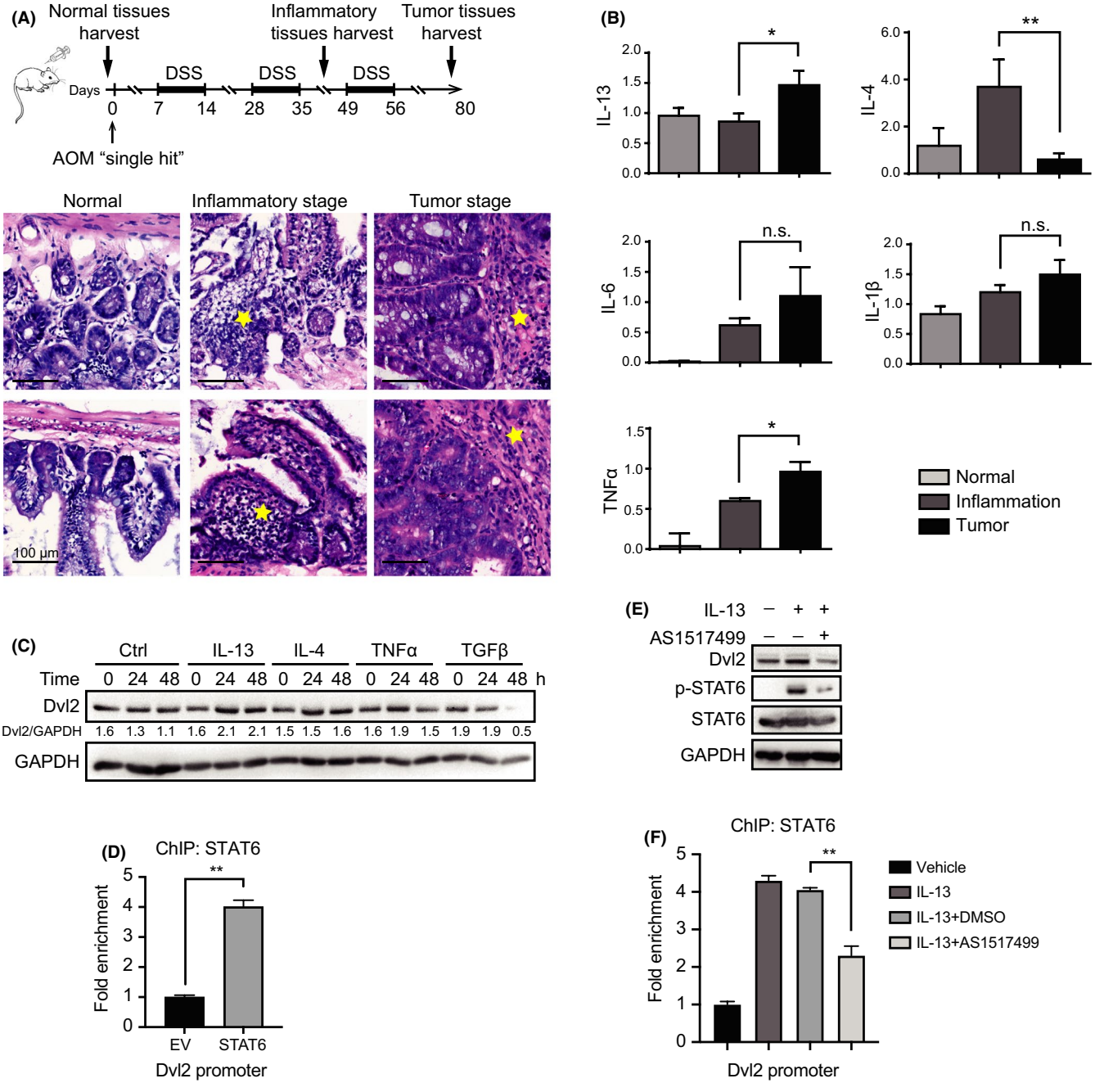

FIGURE 5 Expression of cytokines in tumor progression. A, H&E staining of normal, inflammatory, and tumorous mice tissues. The yellow asterisks indicate the inflammatory area. B, qRT-PCR analysis of pro-inflammatory cytokine expression in mouse tissues. C, Dvl2 expression with treatment of pro-inflammatory cytokines in CCD 841 CoN cells. The intensity of bands was quantified and normalized to GAPDH. D, The binding of STAT6 to the Dvl2 promotor region was detected by ChIP assay. E, Dvl2 expression with treatment of IL-13 and STAT6 inhibitor AS1517499 in CCD 841 CoN cells. F, The binding of STAT6 to the Dvl2 promotor region in the presence or absence of IL-13 and STAT6 inhibitor AS1517499

constantly present in the intestinal tissue in CAC.[27] However, the detailed mechanism behind how inflammation contributes to the CAC is not fully characterized. Dvl2 is an adaptor protein essential for the activation of Wnt signaling, and is overexpressed during CAC progression. We found that the inflammatory cytokine IL-13 is upregulated in CAC, which subsequently increases Dvl2 expression through STAT6. IL-13 was previously reported to play a critical role in colon cancer invasion and liver metastasis via activation of PI3K-AKT signaling.[3,28] This study identified a new role of IL-13 in Wnt signaling and uncovered a novel mechanism for inflammatory-induced Wnt signaling activation, however the mechanism by which pro-inflammatory signaling promotes IL-13 secretion in CAC demands further study.

NF-κB signaling is one of the primary pathways involved in colorectal tumor development. IL-6, which is induced by NF-κB, activates STAT3 in intestinal epithelial cells (IECs) and promotes tumorigenesis by inducing cell proliferation and the inhibition of apoptosis in CAC.[29] Wnt signaling is believed to affect the activation of NF-κB signaling.[30] However, the effects of Wnt activation on NF-κB signaling during the progression of CAC remain unclear. One study reported that constitutive activation of Wnt induces TNF, which leads to enhanced activation of NF-κB, the

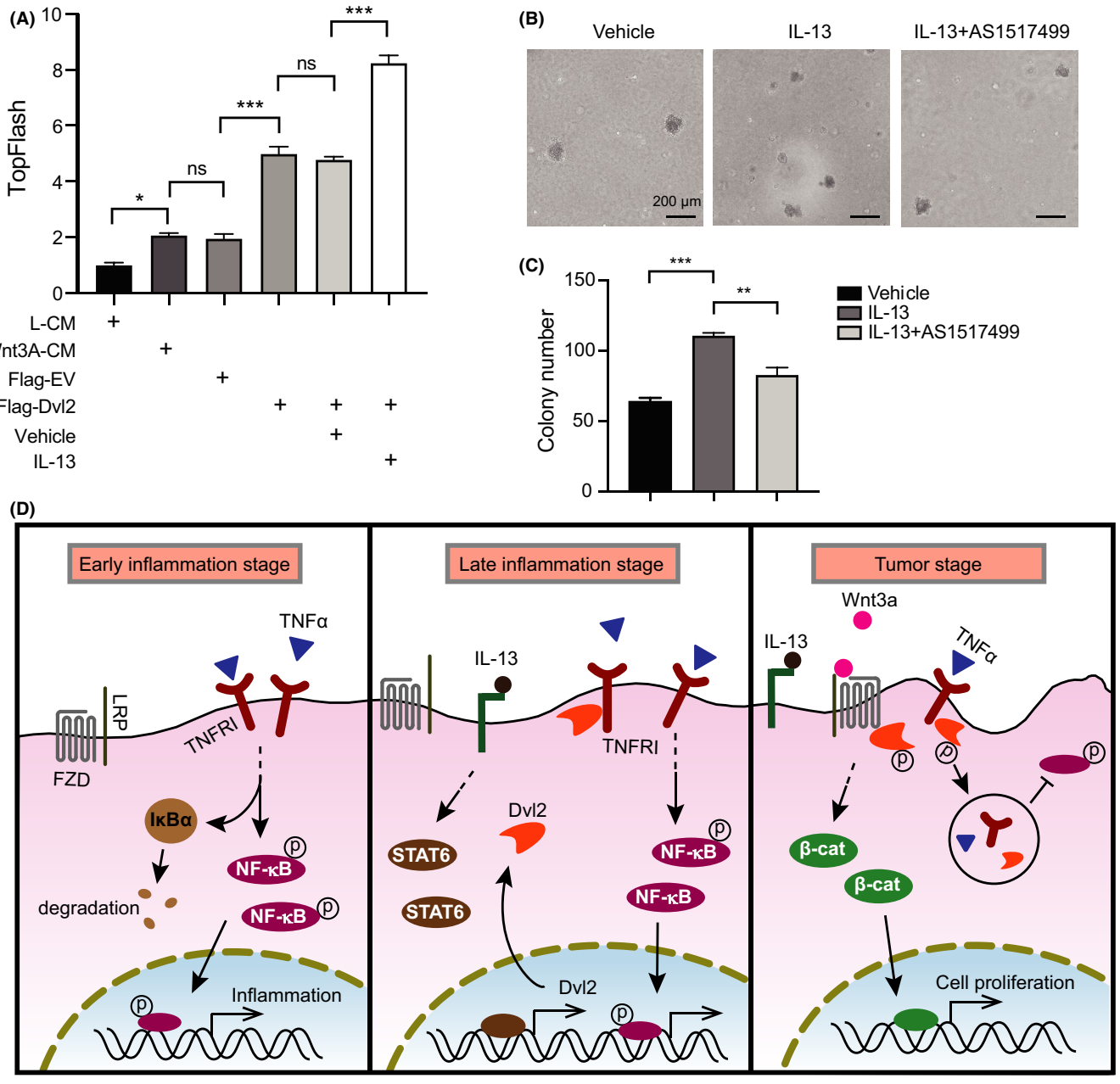

FIGURE 6 IL-13-mediated increase of Dvl2 is responsible for colony formation in RKO. A, TopFlash luciferase assay to determine the effect of IL-13 stimulation on Wnt signaling activation. B, RKO cell colony formation in soft agar in the presence of IL-13 with or without AS1517499. The quantification data are shown in (C). All statistical values are presented as means ± SEM $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$. D, Proposed model for the relationship between NF-κB and Wnt signaling in CAC progression

de-differentiation of IECs, and tumor initiation.[15] Another study found that activated β-catenin represses the TNFα-induced nuclear translocation of NF-κB p50 in CRC cells.[16] We used an AOM-DSS mouse model to monitor the crosstalk between these 2 pathways and clarify the role of Wnt signaling in NF-κB activation. Although the AOM-DSS mouse model cannot fully simulate the development process of CAC, such as increased mutation of gene encoding β-catenin[31] which happens more frequently in CRC, it is still a useful method and is extensively used in studying CAC for its function on inflammation induction and different patterns of somatic mutations. For instance, *APC*, *TP53*, and *KRAS*, which are highly mutated genes in CRC, are rarely observed in this model. Our results strongly indicate that Wnt activation inhibits NF-κB signaling through Dvl2 in both mouse and human CRC tissue.

The expression of Dvl2 increases in human CRC tissues, however, its expression during CAC progression has not yet been elucidated. In this study, we observed a significant upregulation of Dvl2 during CAC progression. Furthermore, we identified a critical role of Dvl2 as a negative regulator of canonical NF-κB signaling, independent of its traditional role in the Wnt/β-catenin pathway. Dvl2 has been reported to interact with p65 in the nucleus, where it attenuates the binding of p65 to the promoter of its target genes and inhibits NF-κB signaling.[32] Dvl2 is primarily expressed in the cytosol, suggesting that Dvl2 may also negatively regulate NF-κB signaling via a novel mechanism. Endocytosis is a critical mechanism involved in the regulation of TNF signaling. The clathrin-mediated internalization of TNFRI in the presence of TNFα switches the downstream signal from the pro-inflammatory NF-κB to the pro-apoptotic pathway.[14] Given that Dvl2 is typically an endocytic adaptor protein that facilitates Wnt receptor internalization, we demonstrated that Dvl2 interacts with TNFRI in response to Wnt3a treatment, inducing TNFRI endocytosis and suppressing NF-κB signaling. We consistently observed an opposite expression pattern in the Dvl2 and nuclear p65 in CAC tissue. These findings imply that Wnt activation inhibits NF-κB signaling via Dvl2-mediated TNFRI endocytosis in CAC. It is worth noting that the TNFα-mediated death signal in tumor cells is much weaker compared with the inflammatory NF-κB signal (Figure S1A,B).[33] Acting as a multifaceted protein, Dvl2 not only inhibited TNFα-NF-κB signaling, but also activated Wnt signaling to promote tumor growth. Here, the high expression of Dvl2 in CRC cells promotes tumor progression instead of leading to apoptosis.

Taken together, our data proposed a cross-regulation between Wnt and NF-κB signaling in the progression of CAC. Further study on how the Wnt-induced inhibition upon NF-κB signaling contributes to CAC progression is needed to develop novel therapeutic approaches for treating CAC.

ACKNOWLEDGMENTS

National Natural Science Foundations of China (82030087 and 81530084 to LQS, 81974465 and 81900199 to LW), Hunan province natural science funds for Excellent Yong scholars (2019JJ30043) and the recruitment program for Huxiang Talents (2019RS1009).

DISCLOSURE

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

ORCID

Feiyu Tang https://orcid.org/0000-0003-0380-9537
Fuyang Cao https://orcid.org/0000-0002-1096-268X
Can Lu https://orcid.org/0000-0003-0898-1854
Xiang He https://orcid.org/0000-0003-3784-105X
Liang Weng https://orcid.org/0000-0001-7058-8274
Lunquan Sun https://orcid.org/0000-0002-0749-1995

REFERENCES

1. Popivanova BK, Kitamura K, Wu Y, et al. Blocking TNF-alpha in mice reduces colorectal carcinogenesis associated with chronic colitis. *J Clin Investig*. 2008;118:560-570.
2. Lasry A, Zinger A, Ben-Neriah Y. Inflammatory networks underlying colorectal cancer. *Nat Immunol*. 2016;17:230-240.
3. Lee PJ, Zhang X, Shan P, et al. ERK1/2 mitogen-activated protein kinase selectively mediates IL-13-induced lung inflammation and remodeling in vivo. *J Clin Investig*. 2006;116:163-173.
4. Bugter JM, Fenderico N, Maurice MM. Mutations and mechanisms of WNT pathway tumour suppressors in cancer. *Nat Rev Cancer*. 2021;21(1):5-21.
5. Rubinfeld B, Souza B, Albert I, et al. Association of the APC gene product with beta-catenin. *Science*. 1993;262:1731-1734.
6. Ikeda S, Kishida S, Yamamoto H, Murai H, Koyama S, Kikuchi A. Axin, a negative regulator of the Wnt signaling pathway, forms a complex with GSK-3beta and beta-catenin and promotes GSK-3beta-dependent phosphorylation of beta-catenin. *EMBO J*. 1998;17:1371-1384.
7. Rubinfeld B, Albert I, Porfiri E, Fiol C, Munemitsu S, Polakis P. Binding of GSK3beta to the APC-beta-catenin complex and regulation of complex assembly. *Science*. 1996;272:1023-1026.
8. Hart M, Concordet JP, Lassot I, et al. The F-box protein beta-TrCP associates with phosphorylated beta-catenin and regulates its activity in the cell. *Curr Biol*. 1999;9:207-210.
9. Amit S, Hatzubai A, Birman Y, et al. Axin-mediated CKI phosphorylation of beta-catenin at Ser 45: a molecular switch for the Wnt pathway. *Genes Dev*. 2002;16:1066-1076.
10. Chang B, Tessneer KL, McManus J, et al. Epsin is required for Dishevelled stability and Wnt signalling activation in colon cancer development. *Nat Commun*. 2015;6:6380.
11. Gao C, Cao W, Bao L, et al. Autophagy negatively regulates Wnt signalling by promoting Dishevelled degradation. *Nat Cell Biol*. 2010;12:781-790.
12. Ben-Neriah Y, Karin M. Inflammation meets cancer, with NF-κB as the matchmaker. *Nat Immunol*. 2011;12:715-723.
13. Perkins ND. The diverse and complex roles of NF-κB subunits in cancer. *Nat Rev Cancer*. 2012;12:121-132.
14. Schneider-Brachert W, Tchikov V, Neumeyer J, et al. Compartmentalization of TNF receptor 1 signaling: internalized TNF receptosomes as death signaling vesicles. *Immunity*. 2004;21:415-428.
15. Schwitalla S, Fingerle A, Cammareri P, et al. Intestinal tumorigenesis initiated by dedifferentiation and acquisition of stem-cell-like properties. *Cell*. 2013;152:25-38.
16. Deng J, Miller SA, Wang H-Y, et al. beta-catenin interacts with and inhibits NF-kappa B in human colon and breast cancer. *Cancer Cell*. 2002;2:323-334.

17. Du Q, Zhang X, Cardinal J, et al. Wnt/beta-catenin signaling regulates cytokine-induced human inducible nitric oxide synthase expression by inhibiting nuclear factor-kappaB activation in cancer cells. *Cancer Res*. 2009;69:3764-3771.
18. Allen I, Wilson J, Schneider M, et al. NLRP12 suppresses colon inflammation and tumorigenesis through the negative regulation of noncanonical NF-κB signaling. *Immunity*. 2012;36:742-754.
19. Scholer-Dahirel A, Schlabach MR, Loo A, et al. Maintenance of adenomatous polyposis coli (APC)-mutant colorectal cancer is dependent on Wnt/beta-catenin signaling. *Proc Natl Acad Sci USA*. 2011;108:17135-17140.
20. Tanaka N, Mashima T, Mizutani A, et al. Mutations as a potential biomarker for sensitivity to Tankyrase inhibitors in colorectal cancer. *Mol Cancer Ther*. 2017;16:752-762.
21. Chen W, ten Berge D, Brown J, et al. Dishevelled 2 recruits beta-arrestin 2 to mediate Wnt5A-stimulated endocytosis of Frizzled 4. *Science*. 2003;301:1391-1394.
22. González-Sancho JM, Greer YE, Abrahams CL, et al. Functional consequences of Wnt-induced dishevelled 2 phosphorylation in canonical and noncanonical Wnt signaling. *J Biol Chem*. 2013;288:9428-9437.
23. McMahon HT, Boucrot E. Molecular mechanism and physiological functions of clathrin-mediated endocytosis. *Nat Rev Mol Cell Biol*. 2011;12:517-533.
24. Yu A, Rual J-F, Tamai K, et al. Association of Dishevelled with the clathrin AP-2 adaptor is required for Frizzled endocytosis and planar cell polarity signaling. *Dev Cell*. 2007;12:129-141.
25. Obiri NI, Murata T, Debinski W, Puri RK. Modulation of interleukin (IL)-13 binding and signaling by the gammac chain of the IL-2 receptor. *J Biol Chem*. 1997;272:20251-20258.
26. Murata T, Noguchi PD, Puri RK. IL-13 induces phosphorylation and activation of JAK2 Janus kinase in human colon carcinoma cell lines: similarities between IL-4 and IL-13 signaling. *J Immunol*. 1996;156:2972-2978.
27. Comprehensive molecular characterization of human colon and rectal cancer. *Nature*. 2012;487:330-337.
28. Bartolomé RA, García-Palmero I, Torres S, López-Lucendo M, Balyasnikova IV, Casal JI. IL13 receptor α2 signaling requires a scaffold protein, FAM120A, to activate the FAK and PI3K pathways in colon cancer metastasis. *Cancer Res*. 2015;75:2434-2444.
29. Greten FR, Eckmann L, Greten TF, et al. IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. *Cell*. 2004;118:285-296.
30. Ma B, Hottiger MO. Crosstalk between Wnt/β-Catenin and NF-κB signaling pathway during inflammation. *Front Immunol*. 2016;7:378.
31. Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. *Nature*. 2011;474:307-317.
32. Deng N, Ye Y, Wang W, Li L. Dishevelled interacts with p65 and acts as a repressor of NF-κB-mediated transcription. *Cell Res*. 2010;20:1117-1127.
33. Wajant H, Pfizenmaier K, Scheurich P. Tumor necrosis factor signaling. *Cell Death Differ*. 2003;10:45-65.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Tang F, Cao F, Lu C, He X, Weng L, Sun L. Dvl2 facilitates the coordination of NF-κB and Wnt signaling to promote colitis-associated colorectal progression. *Cancer Sci*. 2022;113:565–575. doi:10.1111/cas.15206