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ORIGINAL RESEARCH

YAP Activates STAT3 Signalling to Promote Colonic Epithelial Cell Proliferation in DSS-Induced Colitis and Colitis Associated Cancer

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Background and Aims: Yes-associated protein (YAP) is a key transcriptional coactivator of cell proliferation and differentiation. In this study, we sought to identify the roles of YAP in colonic epithelial regeneration and tumourigenesis.

Methods: Murine DSS-induced colitis and YAP overexpression models were constructed via lentiviral intraperitoneal injection. Stable YAP-overexpressing cells, protein immunoprecipitation, and ChIP were used to deeply explore the molecular mechanism.

Results: We found that the expression of YAP was dramatically diminished in the colonic crypts during the acute colitis phase, while YAP was strikingly enhanced to initiate tissue repair after DSS withdrawal. Overexpressing YAP in mice drastically accelerated epithelial regeneration, presenting with more intact structural integrity and reduced inflammatory cell infiltration in the mucosa. Further mechanistic studies showed that the expression of YAP in the nucleus was significantly increased by 2 h post-DSS removal, accompanied by upregulated protein levels of activated STAT3. Overexpression of YAP (YAP^{WT}) elevated the expression of activated STAT3 and its transcriptional targets and strengthened the proliferation and "wound healing" ability of colonic cells. However, these effects were reversed when STAT3 was silenced in YAP^{WT} cells. Moreover, YAP could directly interact with STAT3 in the nucleus, and c-Myc and CyclinD1 were the transcriptional targets. Finally, during colitis-associated cancer (CAC), YAP^{WT} promoted the progression of CAC, while the phosphomimetic YAP downregulated the expression of STAT3 and inhibited the development and progression of CAC.

Conclusion: YAP activates STAT3 signalling to facilitate mucosal regeneration after DSS-induced colitis. However, excessive YAP activation in the colonic epithelium promotes CAC development.

Keywords: dextran sodium sulfate, DSS induced colitis, mucosal repair, YAP, STAT3, colitis-associated cancer

Introduction

Ulcerative colitis (UC) is a chronic, relapsing inflammation of the colon. The aetiology of UC is unclear and is most often reported to be associated with genetic susceptibility, environmental changes, immune dysregulation and microbial infections.[1,2] UC is prevalent in Western countries, but in recent years, there has been a significant increase in previously low-incidence regions, such as Asia.[2] Common complications of UC include colonic perforation, bleeding and colorectal cancers.[3] UC-associated colorectal cancer generally presents as flat, focal or multifocal lesions and is difficult to detect during colonoscopy screening, accounting for nearly 10%-15% of deaths in UC patients.[4,5]

UC has two important biological features: dysregulation of the innate immune system and impaired epithelial repair.[6] The current treatment for UC is mainly based on the suppression of inflammation and modulation of the immune system, but the efficiency of drugs is limited, and individual efficacy varies greatly. Accelerating intestinal epithelial repair to achieve endoscopic mucosal healing has become a key goal in UC.[7,8]

YAP is a key transcriptional coactivator that regulates organ size, tissue growth and tumourigenesis.[9–11] At home-ostasis, YAP is localized at the base of the crypt in the intestinal epithelium, and its activity mainly depends on phosphorylation by Lats or Mst kinases of the Hippo pathway.[12] When Lats or Mst kinases are inactivated, YAP can be translocated into the nucleus where it then interacts with transcription factors, such as TEADs, P73 and SMAD, to regulate the transcription of target genes.[12–14] Mice deficient in both Mst1 and Mst2 exhibited significantly increased proliferation of intestinal epithelial cells and higher expression of intestinal stem cell markers in the intestinal mucosa than control wild-type mice.[15] In addition, conditional knockout of YAP in the intestinal epithelium of mice clearly delayed the restoration of epithelial structure and mucosal barrier function after DSS-induced colitis.[16] In intestinal crypt cells, Group 3 innate lymphoid cells (ILC3s) maintained the adequate initiation of tissue repair, and crypt proliferation was mediated by YAP.[17]

As the Hippo/YAP pathway plays an important role in the regulation of intestinal epithelial cell proliferation and regeneration, we suppose that YAP is also an essential regulator in the development of UC and colitis-associated cancer (CAC). In this study, we used a DSS-induced mouse model to simulate the onset of UC and colitis-associated cancer in humans and found that overexpressing YAP could distinctly promote colonic epithelial repair after acute colitis. During the AOM/DSS procedure, nuclear overexpression of YAP caused excessive proliferation of epithelial cells and promoted the development of CAC; in contrast, cytoplasmic YAP overexpression in mice inhibited CAC tumour growth.

Methods and Materials

Human Samples

20 UC patients were recruited, the inflamed colonic specimens and relatively non-inflamed (endoscopically normal) tissues were obtained from each UC patient undergoing colonoscopy screening with the approval of the Medical Ethics Committee of the Second Xiangya Hospital of Central South University, and were conducted in accordance with in accordance with the Declaration of Helsinki. All samples were immediately frozen in liquid nitrogen and stored at −80 °C. All patients were enrolled with informed consent.

Cell Culture

The human normal colonic epithelial cell line FHC was purchased from ATCC, and cells were cultured in RPMI-1640 medium containing 10% foetal bovine serum in a 37 °C incubator with 5% CO_2. For the dextran sodium sulfate (DSS)-induced cellular inflammation model, FHC cells were precultured overnight in serum-free medium for starvation, followed by treatment with reduced serum medium (2% FBS) containing 1% DSS for 0 and 4 h and then replaced with reduced serum medium without DSS for 1 h (4+1 h), 2 h (4+2 h) and 4 h (4+4 h).

Reagents and Antibodies

AOM (azoxymethane) was purchased from Sigma, and DSS (MW 40000–50000 kDa) was purchased from MP Biomedicals. Antibodies against YAP, phosphorylated YAP-S127, STAT3, phosphorylated STAT3-Tyr705, Ki67, c-Myc, cyclin D1, and PCNA were purchased from CST, and GAPDH was purchased from Santa Cruz Biotechnology. The STAT3 inhibitor S31-201 was purchased from Selleckchem (USA).

Plasmids, siRNA and Transfection

YAP wild-type overexpressed (YAP^WT) and phosphorylated mutant (YAP^S112D) lentiviruses were purchased from Genechem (Shanghai, China). The packaging vector was GV358. The sequences of lentiviral plasmids for human and mouse YAP are detailed in Supplementary Table 1. The YAP-S112D mutant is a recombinant lentiviral plasmid constructed by mutating serine 112 of YAP^WT to aspartic acid and then inserting it into the GV358 vector. The lentiviral and control empty plasmids transfected into cells were screened by 2 μg/mL puromycin. The siRNA sequence was purchased from Genepharma (Suzhou, China). The specific sequences of the siRNAs are detailed in Supplementary Table 2. Cells were transfected with lentiviral plasmids or siRNA using Lipofectamine 3000, and the transfection efficiency was verified by fluorescence microscopy and Western blotting.

Nuclear and Cytosolic Fractionation

A Nuclear and Cytoplasmic Protein Extraction Kit was purchased from Beyotime (China). FHC cells ($1*10^7$) were washed 3 times with PBS and collected in 200 µL cytoplasm protein extraction reagent A/protease inhibitor mixture on ice for 10–15 minutes. Then, 10 µL cytoplasm protein extraction reagent B was added to the mixture, vortexed for 5 seconds and incubated on ice for 1 minute, followed by centrifugation at 120,000 g at 4 °C for 10 minutes. The supernatant was the cytoplasmic fraction. Fifty microlitres of nuclear protein extraction buffer was added to the cell precipitate, vortexed for 30 seconds at 5-minute intervals for 30 minutes and then centrifuged at 4 °C at $120,000 \times g$ for 10 minutes. The final supernatant was the cytosolic fraction.

RNA Extraction and Real-Time PCR

Total RNA was extracted using TRIzol reagent (Takara) according to the manufacturer's instructions. Reverse transcription to generate cDNA was conducted using a reverse transcriptase kit (Takara), and the real-time PCR procedure was performed using a SYBR Green PCR Mix with the ABI system following the manufacturer's instructions. The specific primer sequences used for qPCR are detailed in Supplementary Table 3.

Western Blot and Protein Immunoprecipitation

Equal amounts of protein samples were loaded onto 10–12% SDS-polyacrylamide gels and electrophoresed for 45 min to 1 h. After transferring onto polyvinylidene difluoride membranes, the protein bands were incubated with 1:1000 primary antibody overnight at 4 °C and then incubated with 1:3000 secondary antibody for 1 hour at room temperature, followed by detection with the enhanced chemiluminescence detection system.

For immunoprecipitation, cells were collected in precooled RIPA lysis buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 1%TritonX-100, 1% sodium deoxycholate, 0.1% SDS, 2 mM sodium pyrophosphate, 25 mM β-glycerophosphate, 1 mM EDTA and cocktail protease inhibitors), followed by shaking at 4°C for 15 minutes and centrifugation at $120,000 \times g$ for 15 minutes at 4 °C. Fifty microlitres of Protein A+G-agarose working solution was added to the protein sample, shaken at 4 °C for 10 minutes and centrifuged at $120,000 \times g$ for 15 minutes at 4 °C to obtain the supernatant. Then, the Protein A/G-agarose microspheres were removed. The total protein concentration was tested, the primary antibody was added to the lysates and shaken at 4 °C overnight to obtain immune complexes, and the precipitate was collected after centrifugation for subsequent Western blot analysis.

Chromatin Immunoprecipitation

Chromatin immunoprecipitation (ChIP) was performed by using a ChIP kit (Invitrogen). First, different treated FHC cells were cross-linked by 1% formaldehyde for 10 min at 37 °C. After sonication, ChIP was conducted using Protein G-agarose beads containing anti-STAT3 or IgG control antibody overnight at 4 °C. The DNA–protein complexes were reversed at 65 °C for 4 h, and the DNA fragments were then purified and amplified by qPCR using promoter-specific primers.

Histochemistry and Immunohistochemistry (IHC)

Mouse colonic specimens were fixed in 4% paraformaldehyde for 24 hours, embedded in paraffin and cut into 5-µm-thick sections for haematoxylin and eosin staining. The histological score of mucosal inflammation was assessed as previously reported.[18] Histological scores were analyzed from 3–5 independent fields of colons by calculating the total score of epithelial damage (ranking 0–6; normal=0, hyperproliferation and irregular crypts=1, mild to moderate crypt loss (10–50%) =2, severe crypt loss (50–90%)=3, complete crypt loss=4, small to medium sized ulcer=5, large ulcer=6], with inflammatory cells infiltrating in mucosa (normal=0; mild=1; modest=2; severe=3), submucosa (normal=0; mild to modest=1; severe=2), and muscle/serosa (normal=0; moderate to severe=1). Scores for epithelial damage and inflammatory cell infiltration were added.

For IHC, tissue sections were dehydrated, endogenous peroxidase was inhibited and blocked with 1% FBS, the indicated primary antibody was incubated overnight, and the secondary antibody was then incubated for 2 hours at room

temperature. Finally, the sections were washed and incubated with DAB for visualization. Immunoreactive scores (IRSs) were applied for IHC assessment as previously described.[19] Immunoreactivity was evaluated by immunoreactive scores [IRSs] according to positive area of the cell population in the biopsy specimen staining [<25%=1; 25–50%=2; 50–75% =3; >75%=4] and positive degree [negative=0; mild =1; moderate=2; strong=3]. Scores for positive area and degree were multiplied, resulting in a total scoring range of 0–12. Assessment of colonic inflammation and tumour grading was performed independently by two senior pathologists.

Cell Proliferation and Scratch Assay
FHC cells were grown in 96-well plates at a density of 2000 cells/well. Ten microlitres of CCK-8 reagent was added to each well and incubated at 37 °C for 1 hour at different time points.

The purpose of the scratch assay was to detect the wound healing ability of cells after transfection with different plasmids or siRNAs. A scratch was made by rapidly scratching the cell monolayer with a 10 µL pipette tip, and the "wound healing" area of the cells at different time points was observed by an inverted microscope.

Mouse Model Construction
Six- to eight-week-old BALB/c mice were purchased from Xiangya School of Medicine of Central South University. All of the experiments were carried out following the Institutional Animal Care and Use Committee guidelines and permitted by the Second Xiangya Hospital of Central South University under a Project License (2022661) authorized by Central South University. For the YAP lentivirus transfection model, male BALB/c mice at 6–8 weeks were injected intraperitoneally with 1×10^7 loading of virus per mouse, and gene expression was measured by Western blot 3 days after injection to ensure the successful construction of the model. For DSS-induced colitis, the degree of diarrhea, and occult blood or bleeding in the stool of the mice were monitored to evaluate the disease activity index.[20] Mice were examined for occult blood by using a hemoccult test kit (Fisher HealthCare). Occult blood was scored as follows: 0: negative; 2: guiac; 4: gross blood.[21] For AOM/DSS-induced colorectal carcinoma, mice were first intraperitoneally injected with 10 mg/kg AOM and then rested for 1 week, followed by treatment with 3% DSS for 7 successive days and then normal drinking water for 14 days. The cycle was repeated 3 times.

Intestinal Permeability Assessment
Intestinal permeability was assessed through the oral administration of FITC-labelled dextran as previously described.[22]

Statistical Analysis
All results are presented as the means ± standard error of the mean (SEM). Statistical analysis between groups was performed using Student's t test, and more than two groups were analysed by one-way ANOVA. P values <0.05 were considered statistically significant.

Results
YAP Expression is Decreased in the Colonic Mucosa of UC and DSS-Treated Mice and Elevated During Epithelial Regeneration
Based on the key role of YAP in intestinal epithelial proliferation and regeneration, we first tested YAP expression in inflamed and non-inflamed colonic epitheliums of UC patients. We found that the inflamed colons of UC patients manifested with severe epithelial damage and extensive inflammatory cell infiltration, concomitant with significantly lower expression of YAP in the mucosa than in non-inflamed tissues (Figure 1A-C). Using the classical inflammation and repair mouse model (administration of 3% DSS for 5 successive days followed by 5 days of normal drinking water), we then explored the role of YAP in epithelial regeneration after DSS-induced injury. We found that, at homeostasis, YAP was generally localized at the base of colonic epithelial crypts (the region where intestinal stem cells are located) and mainly accumulated in the nucleus (Figure 1C). Continuous DSS induction caused massive destruction of glands and widespread infiltration of inflammatory cells in the colonic mucosa of mice; meanwhile, the distribution of YAP in the colonic mucosa of inflamed mice was visibly dispersed, and both the crypts and the mesenchyme could be weakly
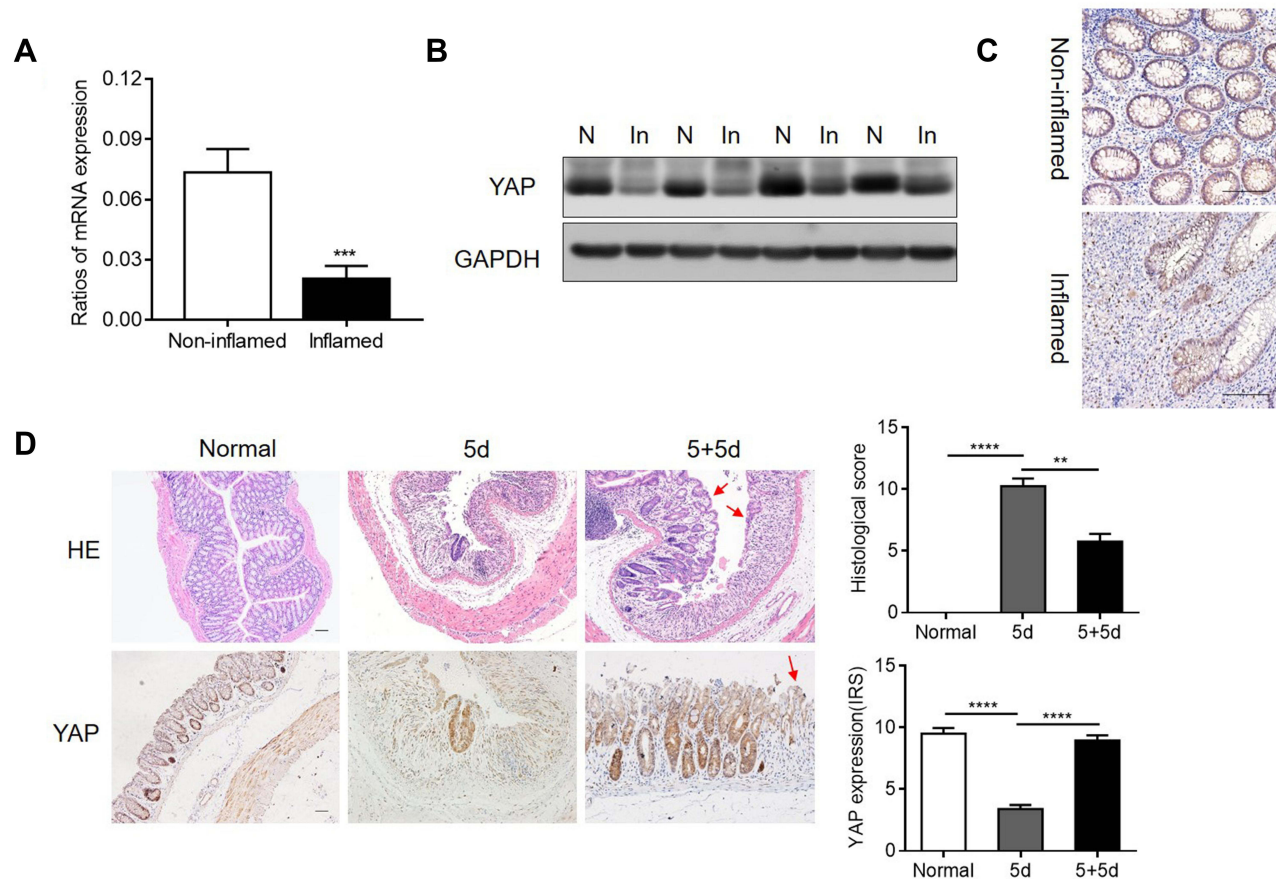
Figure 1 YAP expression was downregulated in human UC lesions and DSS induced colitis of mice. (**A**) mRNA levels; (**B**) protein levels and (**C**) IHC analysis of YAP expression in inflamed and non-inflamed colonic tissues of human UC. (**D**). HE staining and YAP expressions in colonic mucosa of DSS induced mice with successive 5 days DSS induction and 5 days after DSS removal (n=4 each), the experiments were carried out in three independent replications. The bar graph represented histological scores and IHC statistical analysis of YAP among different groups. N represented with non-inflamed; In represented with inflamed. Scale bars =50μm. Data were presented as mean ± SEM, **p <0.01, ***P< 0.001, ****P< 0.0001.

detected. Moreover, YAP expression in the mucosa of mice at 5 d was significantly lower than that in normal mice by nearly four-fold (p<0.0001) (Figure 1D). Importantly, after mucosal wounding, channels of epithelial cells move to exposed surfaces to begin to recreate normal crypt architecture.[23,24] We found that, at 5 days after DSS removal, new colonic crypts adjacent to the wound site could be detected, accompanied by improved epithelial inflammation and enhanced expression of YAP in these new colonic crypts (Figure 1D), suggesting that YAP may act as a regenerative factor to affect epithelial repair after mucosal injury. Therefore, these results indicated that YAP expression was clearly reduced in the inflamed mucosa of UC patients and DSS-treated mice and that YAP may play an important role in the process of epithelial regeneration after mucosal damage.

Overexpression of YAP in a DSS-Induced Mouse Model Promotes Colonic Mucosal Repair

To further explore the role of YAP in epithelial repair after DSS-induced injury, we constructed a YAP overexpression mouse model by intraperitoneal lentivirus injection. We then analysed the mucosal pathology of different mice on Day 5 after withdrawal of DSS. We found that YAP-overexpressing mice (YAP^WT) presented elevated protein expression of Flag and YAP in the colonic mucosa (Figure 2A), accompanied by a specific EGFP signal (Figure 2B left), indicating that the YAP overexpression mouse model was successfully constructed. At Day 5 after DSS withdrawal, the colon lengths of YAP^WT mice were approximately 9 mm longer than those of negative control lentivirus-transfected mice (NC) (p<0.05) (Figure 2C). Additionally, the NC group showed extensive crypt loss and epithelial structure destruction, with a large
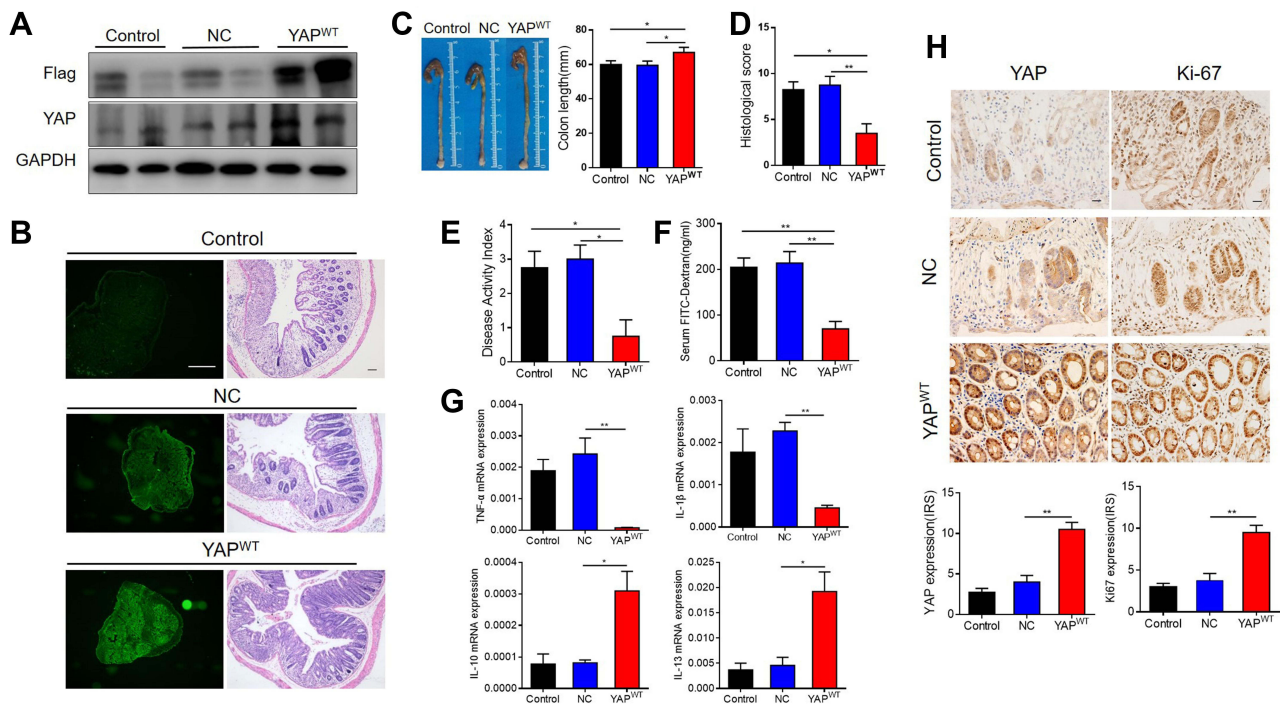
Figure 2 Overexpressed YAP promoted mucosal repair after DSS induced acute colitis. All mice were received 5 days 3%DSS induction followed by 5 days normal drinking water, and were sacrificed at 5d+5 for subsequent analysis (n=8 each), the experiments were carried out in three independent replications. (**A**) Western Blot analysis of indicated proteins in colons of control (PBS administrated mice), NC (negative control lentivirus-transfected mice) and YAP^{WT} mice. (**B**) Histological analyzed the mucosal lesions among different treated groups, figures at the left column presented as the EGFR imaging under fluorescent microscope. (**C**) Murine colon lengths of different treated mice at 5d+5. The Bar graph represented the statistical analysis of colon lengths among different groups. (**D**) Histological score of colonic mucosa of control, NC and YAP^{WT} mice at 5 days DSS withdraw. (**E**) Disease Activity Index of control, NC and YAP^{WT} mice at 5d+5. (**F**) Serum FITC-labelled dextran assessment of control, NC and YAP^{WT} mice at 5d+5. (**G**)Real-time PCR analyzed the mRNA expression levels of cytokines of murine colons of control, NC and YAP^{WT} mice at 5d+5. (**H**) IHC analysis of YAP expression in colonic epithelium of different treated mice at 5 days DSS withdraw. Bar graphs represented IHC statistical analysis from different groups. Scale bars =50μm. Data were presented as mean ± SEM, *P< 0.05, **P< 0.01.

number of inflammatory cells infiltrating the mucosa. However, the epithelial structure of YAP^{WT} mice nearly recovered to normal after the removal of DSS, presenting with more intact epithelium and less inflammatory cell infiltration (Figure 2B right), suggesting accelerated recovery from colitis in YAP-overexpressing mice than in the control group. The results of the inflamed pathology score and intestinal permeability assessment of colons in different groups also supported this finding (Figure 2D and F). Moreover, YAP^{WT} mice also showed lower disease activity index than control and NC mice (Figure 2E). When comparing with the NC group, expressions of inflammatory cytokines in murine colons including TNF-α and IL-1β were downregulated and the anti-inflammatory cytokines IL-10 and IL-13 were upregulated in YAP^{WT} mice (Figure 2G). Next, by immunohistochemistry, we found that the expression levels of YAP and Ki67 in the colonic mucosa of YAP^{WT} mice were significantly increased compared to those in the NC group (Figure 2H), indicating that YAP^{WT} mice had a stronger proliferative capacity of epithelial cells than NC mice. In conclusion, YAP overexpression increased the proliferative capacity of colonic epithelial cells and promoted epithelial repair after DSS-induced colitis.

Nuclear YAP Overexpression Activates STAT3 Signalling and Accelerates Epithelial Cell Proliferation and Wound Healing

Based on the critical role of YAP in epithelial repair after DSS-induced injury, we next investigated the molecular mechanisms by which YAP promotes epithelial cell proliferation and regeneration. By constructing a cellular inflammation model, we found that DSS induction led to a marked decrease in YAP expression, while the protein expression of YAP gradually increased after DSS withdrawal, with peaked expression at 2 h. Nuclear YAP expression also peaked at this time (Figure 3A). STAT3 is an important transcription factor involved in cellular inflammation, tissue repair and
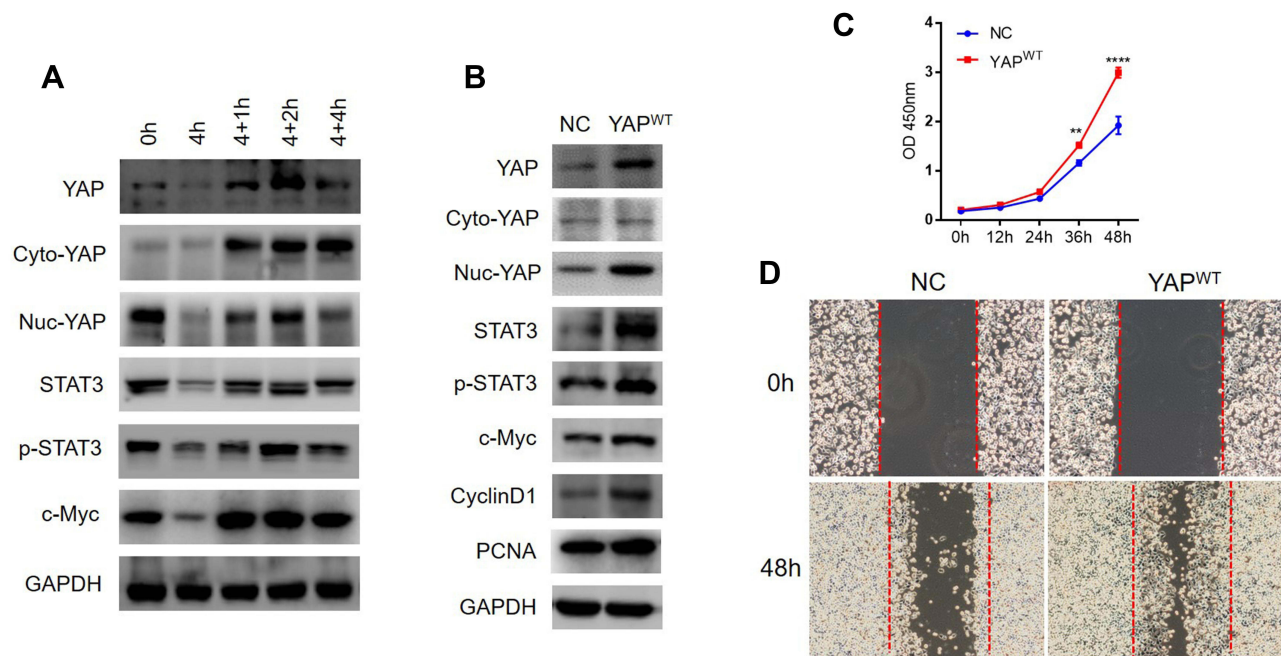
Figure 3 Overexpressed YAP activated STAT3 signalling and accelerated epithelial cell proliferation and "wound healing". (**A**) Western Blot analysis of YAP and STAT3 expressions after 4 hour DSS stimulation followed by different time incubation in reduced serum medium without DSS. (**B**) Western Blot analysis of protein levels of YAP and STAT3 signalling related key factors between NC and YAP^{WT} FHC cells. (**C**) CCK-8 analysis of epithelial cell proliferation between NC and YAP^{WT} FHC cells at different time points. (**D**) Scratch assay analysis of epithelial cell "wound healing" areas between NC and YAP^{WT} FHC cells at different time points. Data were presented as mean ± SEM, **P< 0.01, ****P< 0.0001.

tumourigenesis,[25–27] and its phosphorylation at tyrosine 705 is thought to represent the activated state.[28] In this process, we also examined the expression of STAT3 and its related transcriptional targets, and the results showed that STAT3 expression progressively increased after DSS withdrawal, peaking at 2 h post-DSS cessation. Additionally, the expression of phosphorylated STAT3 and its transcriptional target gene c-Myc was also elevated during epithelial repair after DSS treatment (Figure 3A). The consistent expression of nuclear YAP and activated STAT3 during the repair process suggested that STAT3 may cooperate with YAP to participate in epithelial repair after DSS induction.

Next, we constructed a stable YAP-overexpressing cell line by lentiviral transfection into FHC cells. We found that transfection with YAP^{WT} lentivirus resulted in YAP being mainly overexpressed in the nucleus. YAP^{WT} FHC cells showed clearly increased expression of STAT3 and p-STAT3, accompanied by elevated expression of the transcriptional targets c-Myc and CyclinD1 (Figure 3B). Further CCK-8 and scratch assays demonstrated that colonic epithelial cells in the YAP^{WT} group had a stronger proliferation and "wound healing" capacity than those in the NC group (Figure 3C and D). Therefore, in the process of epithelial cell repair, overexpressing YAP could improve the ability of epithelial proliferation and wound healing, and nuclear YAP overexpression may activate STAT3 signalling to regulate the proliferation and repair of colonic epithelial cells.

YAP Interacts with STAT3 to Form a Transcriptional Complex to Regulate Epithelial Cell Proliferation

Based on the above relationship between YAP and STAT3, we further explored the molecular mechanisms by which YAP regulates STAT3 activity. By silencing STAT3 in YAP-overexpressing FHC cells, we compared the differences in the expression of STAT3 transcriptional targets in YAP^{WT} and YAP^{WT}siSTAT3 cells, and the results showed that the expression levels of c-Myc and CyclinD1 were significantly decreased in the YAP^{WT}siSTAT3 group (Figure 4A). The scratch assay also showed that silencing STAT3 significantly reduced the "wound healing" ability of YAP^{WT} cells (Figure 4B). Further protein immunoprecipitation assays revealed that YAP could directly interact with STAT3 in the nucleus (Figure 4C). In addition, by using the STAT3 inhibitor S31-201 to destroy STAT3 transcriptional activities,[29] we
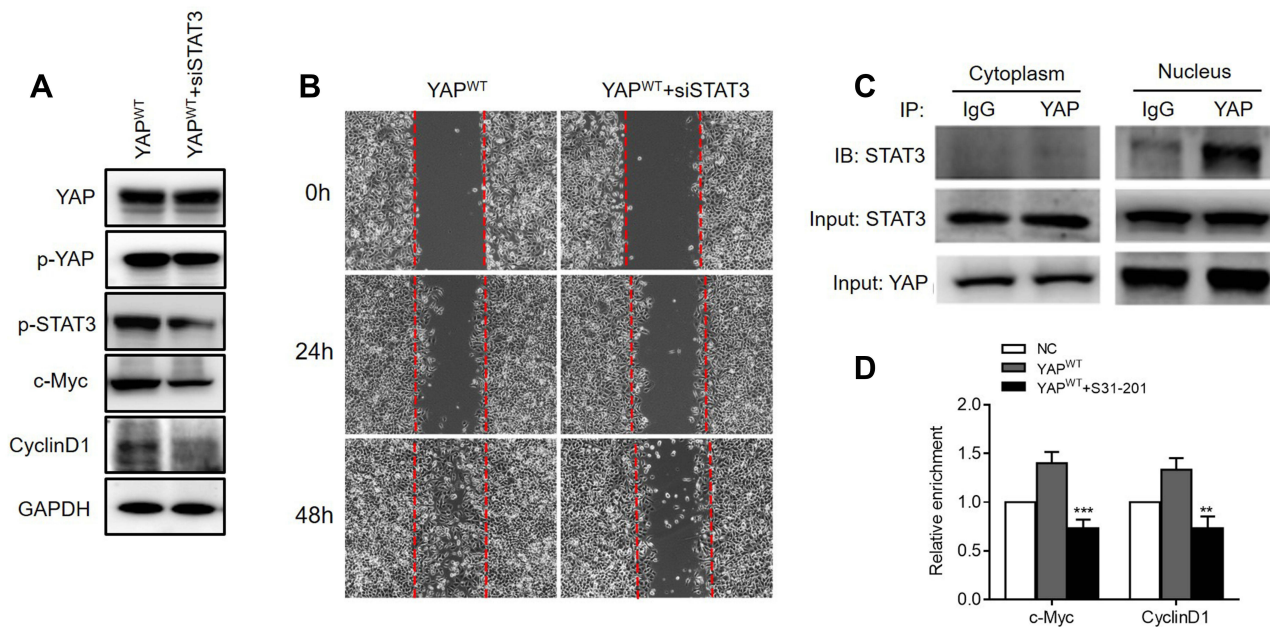
Figure 4 YAP interacted with STAT3 in nucleus forming a complex to transcriptionally regulate c-Myc and CyclinD1 to facilitate epithelial cell wound healing. (**A**) Western Blot analysis of YAP and STAT3 expressions in YAP^WT and YAP^WT+siSTAT3 FHC cells. (**B**) Scratch assay analysis of epithelial cell "wound healing" areas between YAP^WT and YAP^WT+siSTAT3 FHC cells at different time points. (**C**) Protein immunoprecipitation analysis of YAP and STAT3 interaction in cytoplasm and nucleus of FHC cells. (**D**) qPCR analysis of c-MYC and CyclinD1 ChIP with anti-STAT3 antibody in NC, YAP^WT and YAP^WT +S31-201 FHC cells. Data were presented as mean ± SEM, **P< 0.01, ***P< 0.001.

found that the transcriptional targets c-Myc and CyclinD1 were enhanced in YAP^WT cells, while their expression was significantly downregulated in YAP^WT +S31-201 cells (Figure 4D). This result indicates that YAP promoted the proliferation of colonic epithelial cells via direct interaction with STAT3 in the nucleus and that c-Myc and cyclin D1 are targets of the YAP/STAT3 transcriptional complex in epithelial cells.

Nuclear Overexpression of YAP Activates STAT3 to Promote the Development of CAC

Finally, using the AOM/DSS-induced colitis-associated cancer mouse model, we explored the role of YAP in epithelial repair during chronic colitis. We constructed YAP nuclear overexpression (YAP^WT) and cytoplasmic overexpression (YAP^S112D) mouse models through intraperitoneal lentivirus injection. YAP^S112D, with a mutation of serine 112 to aspartate, is in a persistent inactivation state of murine YAP.[30] After 3 cycles, we found that tumours in the YAP^WT group were mostly identified as high-grade intraepithelial neoplasia, whereas tumours in the colons of YAP^S112D-treated mice were largely low-grade intraepithelial neoplasia (Figure 5A). Moreover, the tumour numbers and areas in the colons of YAP^S112D mice were smaller than those in YAP^WT mice (Figure 5B). Additionally, the colonic neoplasms of YAP^S112D mice manifested with weaker STAT3 staining than YAP^WT mice (Figure 5C and D), indicating that cytoplasmic YAP overexpression inhibits the expression of STAT3 in epithelial cells. Therefore, in the background of genetic mutation during chronic and repeated inflammation, nuclear YAP overexpression activated STAT3 signalling and caused excessive epithelial cell proliferation, which accelerated the development of CAC. However, overexpression of cytoplasmic YAP is unable to translocate to the nucleus to trigger the transcriptional process of STAT3, resulting in repressed epithelial cell proliferation and progression of CAC.

Discussion

YAP is a transcriptional coactivator in the Hippo pathway that plays a critical role in organ growth, tissue regeneration and tumourigenesis.[10,31–33] In recent years, an increasing number of studies have shown that YAP can be involved in the regenerative process of many organs, such as the cornea,[34] lung[35,36] and heart.[37] Impaired mucosal regeneration is an important biological feature of UC. In this study, we found that YAP expression was significantly decreased in the
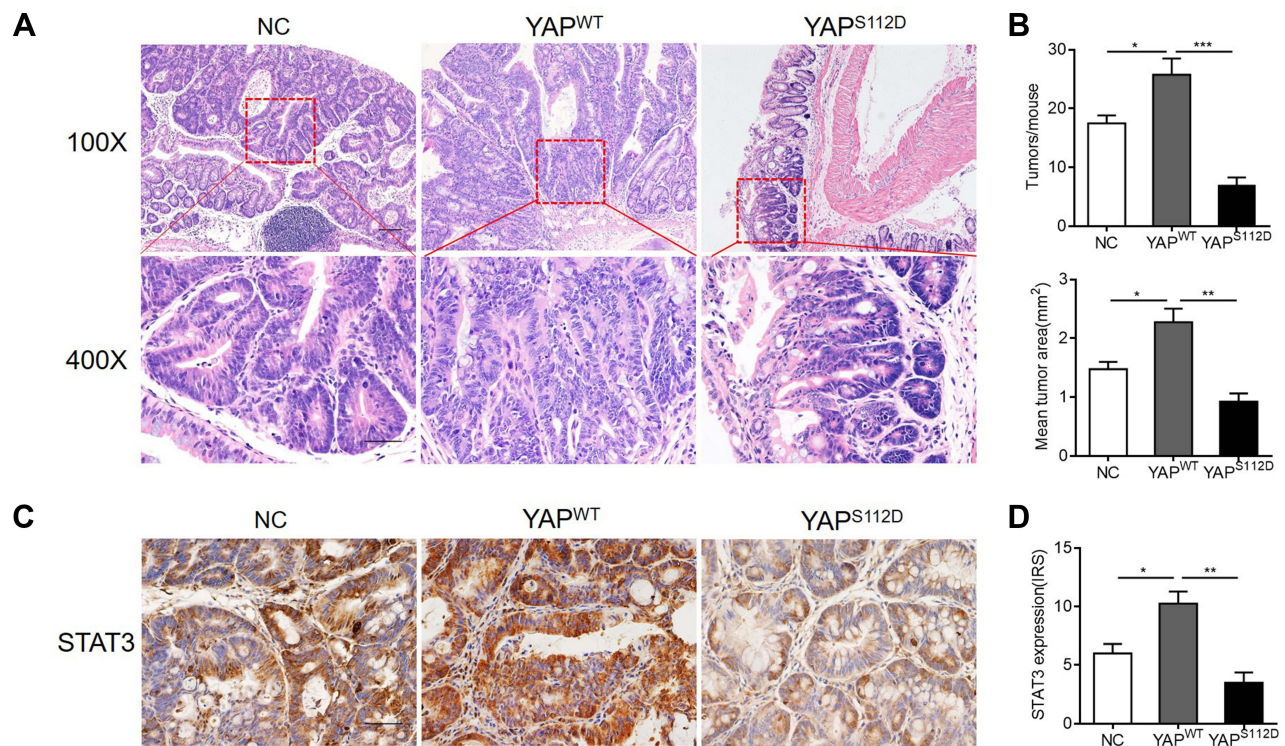
Figure 5 Nuclear YAP overexpression (YAP^WT) promoted the development of CAC, cytoplasmic YAP overexpression(YAP^S112D) downregulated STAT3 expression and inhibited CAC progression. All mice were sacrificed after the 3 cycles of AOM/DSS procedure ended (n=6 each), the experiments were carried out in three independent replications. (**A**) Histological analysis of colorectal tumors from NC, YAP^WT and YAP^S112D mice. (**B**) Statistical analysis of tumor numbers and areas in colons of NC, YAP^WT and YAP^S112D mice. (**C**) IHC staining of STAT3 in tumors from NC, YAP^WT and YAP^S112D mice. (**D**) The bar graph was presented the statistical results of STAT3 IHC staining in different groups. Scale bars =20μm. Data were presented as mean ± SEM, *P< 0.05, **P< 0.01, ***P< 0.001.

colonic mucosa of mice after DSS-induced acute colitis and was clearly enhanced during the phase of epithelial repair. YAP overexpression in mice significantly promoted colonic mucosal repair after acute colitis. However, upon sustained chronic inflammation, overexpressed YAP caused excessive epithelial cell proliferation and induced the development of CAC, suggesting that YAP is an important regulator in the control of colonic epithelial repair and tumourigenesis.

YAP is a key factor in the Hippo pathway and generally regulates cell proliferation and tumourigenesis through interactions with various transcription factors.[13] Mothers against decapentaplegic homologue (SMAD)7 can activate the YAP/NOTCH signalling pathway and increase cholangiocellular gene expression to promote liver carcinogenesis.[38] In addition, YAP can interact with TEAD to form a transcriptional complex to upregulate the expression of the apoptosis-inhibitory protein Bcl-2, negatively regulating autophagy in colorectal cancer cells (CRC) and promoting CRC tumour progression.[39] The molecular mechanisms by which YAP regulates intestinal epithelial repair after injury are less well studied. In this research, we found that YAP expression was significantly increased in the colonic mucosa of DSS-treated mice during the process of epithelial repair. YAP overexpression in mice clearly promoted mucosal repair after acute DSS injury. More importantly, we found that the nuclear expression of YAP was consistent with the expression of activated STAT3 and STAT3 transcriptional targets during the repair phase, suggesting that YAP may be involved in the repair process of colonic epithelial cells through activation of the STAT3 signalling pathway.

STAT3 is an important transcription factor that regulates cell proliferation and inflammation, tissue regeneration and tumourigenesis.[25,40,41] STAT3 is crucial for both organoid formation and intestinal stem cell-mediated epithelial regeneration in an ILC/IL-22-dependent manner.[42] The STAT3-BDNF-TrkB axis plays an important role in alveolar epithelial regeneration.[43] Based on the similar roles of YAP and STAT3 in tissue regeneration, we probed the effect of STAT3 in colonic epithelial repair and further investigated the mutual regulation between YAP and STAT3 in epithelial cells. Using the cellular inflammation model, we found that YAP nuclear expression was significantly elevated 2 h after DSS withdrawal, as well as STAT3 and p-STAT3 expression. Nuclear YAP overexpression

markedly upregulated activated STAT3 expression and promoted proliferation and "wound healing" of colonic epithelial cells, while silencing STAT3 in these cells reversed the effect. Previous studies have reported that STAT3 is a target gene of the YAP/TEAD4 transcriptional complex, and YAP can promote pancreatic cancer development through activation of the JAK/STAT3 pathway.[44] Moreover, YAP can directly interact with the transcription factor STAT3 in the nucleus, and the expression of its transcriptional target genes is closely associated with poor prognosis in patients with triple-negative breast cancer.[28] Meanwhile, YAP could directly interact with STAT3 in the nucleus and then transcriptionally regulate VEGF expression to upregulate PD-L1 expression in M2-like tumour-associated macrophages, which ultimately promoted laryngeal cancer cell proliferation in vivo.[45] In our study, YAP could directly interact with STAT3 in the nucleus of epithelial cells, and c-Myc and CyclinD1 were the targets of the YAP/STAT3 transcriptional complex. This suggested that, in the nucleus, YAP interacts with STAT3 to transcriptionally regulate the expression of epithelial cell proliferation-related genes, thereby promoting the proliferation and wound healing of epithelial cells to accelerate mucosal repair after colitis. However, upon chronic inflammation, nuclear overexpression of YAP caused hyperproliferation of epithelial cells and increased susceptibility to CAC, whereas a sustained YAP cytoplasmic phosphorylation state suppressed STAT3 expression and thereby inhibited the development of CAC.

In conclusion, impaired epithelial repair can lead to the development of inflammatory bowel disease (IBD), while sustained, excessive epithelial proliferation may result in the occurrence of colitis-associated colorectal cancer. Achieving endoscopic mucosal healing as early as possible is essential for the treatment of IBD and the prevention of associated complications. In this study, YAP directly interacted with the transcription factor STAT3 to activate the STAT3 transcriptional process, causing accelerated proliferation of colonic epithelial cells and wound healing after mucosal damage. We conclude that YAP may serve as an important therapeutic target for mucosal healing in UC and that systemic administration of YAP phosphorylation mutants may provide a new therapeutic strategy for CAC.

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Disclosure

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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