Periostin Promotes Colorectal Tumorigenesis through Integrin-FAK-Src Pathway-Mediated YAP/TAZ Activation

Graphical Abstract

Highlights

- Periostin deficiency inhibits colorectal tumor formation in mice
- Periostin is mainly secreted by fibroblasts to promote tumor cell proliferation
- Periostin promotes YAP/TAZ nuclear localization and IL-6 expression in tumor cells
- IL-6 promotes fibroblast activation and periostin expression during tumorigenesis

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In Brief

Ma et al. demonstrate that fibroblast-derived periostin promotes colorectal tumorigenesis by enhancing YAP/TAZ nuclear localization and IL-6 expression in tumor cells. Conversely, IL-6 promotes periostin expression in fibroblasts via STAT3 signaling. This work identifies crosstalk between stromal cells and tumor cells via periostin and IL-6 during colorectal tumorigenesis.
Periostin Promotes Colorectal Tumorigenesis through Integrin-FAK-Src Pathway-Mediated YAP/TAZ Activation

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SUMMARY

Periostin is a multifunctional extracellular matrix protein involved in various inflammatory diseases and tumor metastasis; however, evidence regarding whether and how periostin actively contributes to inflammation-associated tumorigenesis remains elusive. Here, we demonstrate that periostin deficiency significantly inhibits the occurrence of colorectal cancer in azoxymethane/dextran sulfate sodium-treated mice and in ApcMin/+ mice. Moreover, periostin deficiency attenuates the severity of colitis and reduces the proliferation of tumor cells. Mechanistically, stromal fibroblast-derived periostin activates FAK-Src kinases through integrin-mediated outside-in signaling, which results in the activation of YAP/TAZ and, subsequently, IL-6 expression in tumor cells. Conversely, IL-6 induces periostin expression in fibroblasts by activating STAT3, which ultimately facilitates colorectal tumor development. These findings provide the evidence that periostin promotes colorectal tumorigenesis, and identify periostin- and IL-6-mediated tumor-stroma interaction as a promising target for treating colitis-associated colorectal cancer.

INTRODUCTION

Colorectal cancer (CRC) is one of the most common malignant tumors, with a high incidence and mortality. Previous studies have demonstrated that genetic mutation of adenomatous polyposis coli (APC) is closely associated with colorectal tumorigenesis (Moser et al., 1990; Rakoff-Nahoum and Medzhitov, 2007; Karki et al., 2016). Chronic inflammation is another recognized driver of CRC (Grivennikov et al., 2010; Huber et al., 2012). Current evidence has revealed that the interactions between tumor cells and their adjacent stromal cells play a critical role in colorectal tumorigenesis (Koliaraki et al., 2012). However, it is still unclear how the resident stromal cells are activated by their adjacent tumor cells under the inflammatory condition, and how stromal cell-derived cytokines and extracellular matrix (ECM) proteins educate tumor cells in the pathogenesis of CRC.

Periostin (encoded by Postn) was originally identified as an adhesion protein in mouse osteoblastic cells (Takeshita et al., 1993; Horuchi et al., 1999). Periostin is rarely detected in most normal adult tissues, but it is highly induced in lesions, inflammation, and tumors (Conway et al., 2014; Liu et al., 2014; Izuhara et al., 2017; Kudo, 2017; Ma et al., 2019). As a multifunctional ECM protein, periostin contributes to tumor microenvironment remodeling during tumor progression. During breast tumor metastasis, cancer-associated fibroblast (CAF)-derived periostin is involved in the formation of the cancer stem cell niche, the perivascular niche, and the premetastatic niche in the lung (Malanchi et al., 2011; Wang and Ouyang, 2012; Ghajar et al., 2013; Wang et al., 2016b). Activated hepatic stellate cell-derived periostin promotes the formation of a fibrotic microenvironment to facilitate the liver metastatic outgrowth of pancreatic cancer cells (Nielsen et al., 2016). Our previous study demonstrated that periostin promotes the metastatic outgrowth of colon tumors in the liver by enhancing cell survival and angiogenesis (Bao et al., 2004). However, it remains largely unclear whether and how periostin is involved in colorectal tumorigenesis, especially in the development of colitis-associated CRC (CAC).

Here, we demonstrate that periostin deficiency suppresses intestinal tumorigenesis in azoxymethane (AOM)/dextran sulfate sodium (DSS)-treated mice and ApcMin/+ mice. We further observe that the deletion of periostin significantly reduces the proliferative cells and decreases nuclear YAP/TAZ in colorectal tumors in mice. Our results unveil that CAF-derived periostin promotes colorectal tumorigenesis through integrin-focal adhesion kinase (FAK)-Src signaling-mediated YAP/TAZ activation.
RESULTS

Periostin Deficiency Inhibits Intestinal Tumorigenesis in AOM/DSS-Treated Mice and ApcMin/+ Mice

To determine the impact of periostin on colorectal tumorigenesis, we used AOM/DSS to induce CAC in mice. To this end, mice received a single injection of AOM followed by three cycles of DSS administration (Figure S1A). During the course of AOM/DSS treatment, periostin knock-out (Postn−/−) mice exhibited a significant reduction in body weight loss in comparison to wild-type (WT) mice (Figure 1A). During the first cycle of DSS treatment, the disease activity index was significantly decreased in Postn−/− mice compared to WT mice (Figure S1B). Histologically, AOM/DSS-administered Postn−/− mice displayed clearly attenuated inflammation severity (Figure 1B). Moreover, the histopathological score was greatly reduced in the distal colon of Postn−/− mice compared to that of WT mice (Figure 1C). The enhanced colitis is invariably accompanied by more infiltrated inflammatory cells in the lamina propria. To further investigate whether periostin has an effect on immune cell infiltration under inflammatory conditions, we performed immunohistochemical assays to analyze the dense infiltration of inflammatory cells in the distal colon of WT and Postn−/− mice during the early and late stages of AOM/DSS administration. We found fewer infiltrated inflammatory cells in the colon tissues of Postn−/− mice than in those of WT controls (Figures 1D and 1E), suggesting that the deletion of periostin retards inflammatory cell infiltration in the distal colon of AOM/DSS-treated mice. At the end point of the experimental protocol, the colons of both WT and Postn−/− mice were collected and tumors were analyzed. Tumor incidence in WT mice was 100%, whereas <80% of Postn−/− mice formed tumors or polyps in the distal colon. In particular, a significant decrease in tumor numbers and a remarkable reduction in tumor size were observed in the colons of Postn−/− mice compared to WT mice (Figures 1F–1I, S1C, and S1D). The average tumor number per colon in Postn−/− mice (2.5 ± 0.4) was less than in WT mice (5.0 ± 0.4) (Figure 1F). Moreover, Postn−/− mice showed a significant reduction in the average tumor size compared with WT controls (Figure 1H). Although there was no significant difference in the number of small tumors (≤1 mm diameter) between WT and Postn−/− mice, 53.1% of tumors in WT mice developed into large tumors (>2 mm diameter), while only 37.5% of tumors in Postn−/− mice developed into large tumors (Figure 1I). The number of proliferative cells was dramatically decreased in the tumors of Postn−/− mice compared to those in WT tumors (Figures 1J–1L). Furthermore, the mRNA levels of many inflammation-associated cytokines such as interleukin-6 (IL-6), tumor necrosis factor α (TNF-α), and IL-1β were significantly reduced in the distal colonic tissues of Postn−/− mice compared with those of WT mice after AOM/DSS treatment (Figures 1M, S1E, and S1F). We also found that the protein levels of IL-6, TNF-α, and IL-1β were markedly decreased in the distal colonic tissues of Postn−/− mice (Figure 1N). Consistent with these results, we observed greatly decreased levels of inducible nitric oxide synthase (iNOS), extracellular signal-regulated kinase 1/2 (ERK1/2), and signal transducer and activator of transcription 3 (STAT3) phosphorylation in the colon tissue of Postn−/− mice compared with WT mice at day 80 of AOM/DSS treatment (Figure S1G), indicating that the absence of periostin prevents the activation of inflammatory signaling pathways in mice. These findings suggest that periostin deficiency inhibits colitis-associated colorectal carcinogenesis.

To further investigate whether periostin promotes colorectal tumorigenesis in a spontaneous mouse intestinal tumor model, we used ApcMin/+ mice and Postn−/− mice to generate Postn−/− ApcMin/+ mice and Postn−/− ApcMin/+ mice. After 6 months, tumors in the whole intestinal tract were detected. We found that Postn−/− ApcMin/+ mice had a lower tumor burden than Postn−/− ApcMin/+ mice both in the small and large intestines (Figures S2A–S2E). In addition, fewer Ki67+ cells were observed in the small and large intestines of Postn−/− ApcMin/+ mice compared with Postn−/− ApcMin/+ mice (Figures S2F and S2G). These findings were similar to the observation obtained from the AOM/DSS-induced CAC model, suggesting that the absence of periostin significantly suppresses intestinal tumorigenesis in different mouse models of colorectal tumors.

Periostin Deficiency Inhibits the Precancerous Lesion Formation in the Inflamed Colon Tissues of Mice

As periostin has a critical role during the early phases of the inflammation (Figures 1A–1E, 1M, 1N, and S1B), we reasoned that pathological change differences between WT and Postn−/− mice may exist during early stages of the CAC progression. To this end, we examined the expression of periostin in the inflamed colonic tissue sections of WT mice treated with AOM/DSS for 15 days. Immunohistochemical staining showed that a higher level of periostin was deposited in the inflamed colon tissues compared to the normal colon tissues (Figure S3A). As shown in Figures S3B and S3C, a significant decrease in the incidence of dysplasia in Postn−/− mice was observed compared with that in WT mice. In contrast, the number of goblet cells in the colon of Postn−/− mice was greater than that in WT mice (Figures S3D and S3E), indicating that genetic ablation of periostin may protect secretory epithelial cells from damage under the inflammatory disorder. Furthermore, Postn−/− mice had fewer proliferative cells in the distal colon sections than WT mice after the first cycle of AOM/DSS administration (Figures S3F–S3I). Epithelial barrier dysfunction is prominent in patients with inflammatory bowel disease and is always related to the severity of inflammation disorder in animals (Sharma et al., 2018). After DSS administration, the epithelial barrier is permeable and tight junction is lost within 2 days (Huber et al., 2012). Thus, we further analyzed the effect of periostin deficiency on tight junctions during the early stage (on day 8) of AOM/DSS administration. We found that AOM/DSS-treated Postn−/− mice exhibited a significant increase in the expression of tight junction markers compared with AOM/DSS-treated WT mice (Figure S3J). In addition, even at this early time point, we observed significantly decreased production of inflammatory mediator markers and reduced levels of tumor surrogate markers in the distal colon lysates of Postn−/− mice compared with WT mice (Figures S3K and S3L). These results demonstrate that the absence of periostin prevents precancerous lesion formation in colon tissues during the early phase of CAC progression.
Figure 1. Periostin Deficiency Attenuates Colitis-Associated Colorectal Tumorigenesis in Mice

(A) Body weight changes in WT (n = 5) and Postn−/− (n = 4) mice during the course of AOM/DSS treatment.

(B and C) H&E staining (B) and histopathological score (C) of the distal colon sections of AOM/DSS-treated WT (n = 6) and Postn−/− (n = 6) mice. Scale bars, 100 μm.

(D and E) Immunohistochemical staining of CD45, F4/80, and Gr-1 (D) and quantification of CD45⁺, F4/80⁺, and Gr-1⁺ cells (E) in the inflamed colon sections of WT (n = 6) and Postn−/− (n = 6) mice. Scale bars, 50 μm.

(F and G) Colon tumors (F) and tumor load (G) of WT (n = 19) and Postn−/− (n = 16) mice after AOM/DSS colitis.

(H) The average tumor size in WT (n = 16) and Postn−/− (n = 14) mice.

(I) The number of small, medium, and large tumors of WT (n = 19) and Postn−/− (n = 16) mice.

(J–L) Immunohistochemical staining of Ki67 and BrdU (J) and quantification of Ki67⁺ (K) and BrdU⁺ (L) cells in tumor sections of WT (n = 6) and Postn−/− (n = 6) mice. Scale bars, 50 μm.

(M and N) Relative mRNA (M) and protein (N) levels of the indicated inflammatory mediators (n = 5-6 mice each group).

Each symbol represents one mouse (C, E, F–I, and K–N). In all of the panels, data are the means ± SEs; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001; n.s., not significant. Two-way ANOVA (A) or Student’s t test (C, E, F–I, and K–N). See also Figures S1, S2, and S3.
Figure 2. Periostin Is Mainly Derived from Stromal Fibroblasts in Mouse Colorectal Tumors

(A) Periostin expression and deposition in normal and colorectal tumor tissues. Scale bars, 50 μm.

(B–D) Co-staining of periostin with α-SMA (B), vimentin (C), or epithelial cell adhesion molecule (EpCAM) (D) in AOM/DSS-induced tumors. Scale bars, 25 μm.

(E) Body weight changes in BM chimera mice during AOM/DSS treatment (n = 5–10 mice per group). For statistical comparison, the asterisk indicates Postn/c0/c0 BM > Postn/c0/c0 versus WT BM > WT; # indicates WT BM > Postn/c0/c0 versus WT BM > WT.

(F and G) H&E staining (F) and histological score analysis (G) of colon tissue sections of BM chimera mice after AOM/DSS colitis (n = 5–9 mice per group). Scale bars, 100 μm.

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Periostin Is Mainly Derived from Stromal Fibroblasts in Mouse Colorectal Tumors

Next, we determined the cellular source of periostin in colorectal tumors. We found that the level of periostin was low in normal colon tissues and its deposition closely surrounded the pericryptal fibroblasts (Figure 2A). By contrast, a highly elevated level of periostin was observed both in the AOM/DSS-induced mouse colorectal tumors and in the Apc mutation-related mouse tumors (Figures 2A and S4A). Immunohistochemical staining assays also revealed that periostin was deposited in tumor stromal cells but not in tumor cells (Figure 2A). Immunofluorescence co-staining assays further determined that α-smooth muscle actin-positive (α-SMA+) or vimentin+ stromal cells, but not epithelial-derived tumor cells, contributed to the production of periostin (Figures 2B–2D), indicating that periostin is mainly derived from tumor stromal fibroblasts. We also observed an extremely low amount of periostin deposition in surrounding macrophages in colitis-associated colorectal tumors (Figure S4B). To further determine what kinds of stromal cells, stromal fibroblasts, or bone marrow (BM)-derived macrophages were the dominant source of periostin in colorectal tumorigenesis, we performed BM transplantation assays to generate four groups of BM chimera mice: Postn−/− mice transplanted with BM from WT mice (WT BM > Postn−/−), WT mice transplanted with BM from Postn−/− mice (Postn−/− BM > WT), and the two control groups consisting of Postn−/− mice transplanted with BM from Postn−/− mice (Postn−/− BM > Postn−/−), and WT mice transplanted with BM from WT mice (WT BM > WT). Next, we administered these four groups of BM chimera mice with AOM/DSS to induce colitis-associated tumor formation. Postn−/− mice receiving BM from WT or Postn−/− mice showed body weight loss at a significant reduction level, compared to WT mice receiving either BM from Postn−/− or WT mice (Figure 2E). During the early phases of tumor progression, the disease activity index was significantly lower in Postn−/− mice transplanted with BM from WT or Postn−/− mice than in WT mice transplanted with BM from WT or Postn−/− mice (Figure S4G). Moreover, compared with irradiated WT mice, Postn−/− mice receiving lethal irradiation exhibited significantly decreased histological scores in the distal colonic tissue sections after AOM/DSS treatment for 80 days (Figures 2F and 2G), as well as fewer infiltrated inflammatory cells in the irradiation-insensitive compartment of Postn−/− mice (Figures 2H and 2I). These data suggest that periostin-deficient mice transplanted with BM cells have weakened colitis disease severity after AOM/DSS colitis. Postn−/− mice receiving either WT or Postn−/− mice-derived BM cells developed significantly decreased tumor numbers and reduced tumor loads than WT mice receiving Postn−/− or WT mice-derived BM cells (Figures 2J, 2K, and S4D–S4F). However, we observed no significant difference in tumor numbers or tumor load between WT mice transplanted with either WT mice-derived BM cells or Postn−/− mice-derived BM cells, as well as between Postn−/− mice transplanted with either Postn−/− mice-derived BM cells or WT mice-derived BM cells (Figures 2J, 2K, and S4D–S4F). Thus, these results demonstrate that periostin is mainly derived from stromal fibroblasts during colorectal tumorigenesis.

Periostin Promotes Colorectal Tumor Cell Proliferation In Vitro

Given that our results (Figures 1J–1L, S2F, and S2G) demonstrated that periostin deficiency decreases proliferative cells in the tumors of AOM/DSS-treated mice and ApcMin/+ mice, we further detected the proliferation-promoting capacity of periostin in vitro. We treated the CRC cell line CMT93 with the conditioned medium (CM) of the primary CAFs isolated from colorectal tumors in WT and Postn−/− mice. We observed that there were fewer Ki67+ or BrdU+ cells in CMT93 cells treated with Postn−/− CAF CM than in WT CAF CM-treated cells (Figures 3A–3D). Moreover, recombinant mouse periostin protein (rmPeriostin)-treated CMT93 cells showed many more proliferative cells than control groups (Figures 3E–3H). MTT assays also showed that rmPeriostin-treated CMT93 cells had a higher proliferative capability than PBS-treated cells (Figure 3I).

Periostin Induces YAP/TAZ Activation in Colorectal Tumor Cells

Next, we sought to explore the mechanisms by which periostin promotes cell proliferation and colorectal tumorigenesis. Previous studies demonstrate that the transcriptional cofactor YAP plays a critical role in regulating tissue regeneration and tumorigenesis in the intestine (Zhou et al., 2011; Cai et al., 2015; Yui et al., 2019). Periostin is a well-known extracellular player in tissue regeneration, inflammation, and tumor progression (Conway et al., 2014; Liu et al., 2014). Thus, we determined whether periostin acts as an extracellular regulator of YAP signaling in colorectal tumor development. To this end, we determined the levels of YAP and TAZ in the colon tumor tissues of WT and Postn−/− mice. We found that both the protein and mRNA levels of YAP and TAZ were greatly decreased in the tumors of Postn−/− mice compared with WT mice (Figures 4A–4C). The depletion of periostin resulted in attenuated YAP/TAZ nuclear localization in the colorectal tumor cells (Figure 4B), indicating that periostin deficiency decreases YAP/TAZ activation during colorectal tumorigenesis. Activated YAP serves as a transcriptional co-factor of TEADs to induce their target gene expression. qRT-PCR analysis further revealed that the production of their target genes, such as Ankrd1, Ctgf, and Cyr61, was markedly reduced in the tumor lysates of Postn−/− mice relative to the production in WT tumor lysates (Figure 4C). To further determine whether periostin induces YAP/TAZ activation in colorectal tumor cells, we detected nuclear translocation of YAP/TAZ with immunohistochemical staining using antibodies against YAP and TAZ (Figures 4D and 4E). The results showed that the nuclear localization of YAP/TAZ was significantly increased in the tumors of AOM/DSS-treated mice and ApcMin/+ mice

(H and I) Immunohistochemical staining of CD45, F4/80, and Gr-1 (H) and quantification of CD45+, F4/80+, and Gr-1+ cells (I) in inflamed colon sections of BM chimera mice (n = 5–9 mice per group). Scale bars, 50 µm.

(J and K) Tumor number (J) and tumor load (K) in AOM/DSS-treated BM chimera mice (n = 5–9 mice per group).

Each symbol represents one mouse (G and I–K). In all of the panels, data are presented as means ± SEMs; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001; n.s., not significant. Two-way ANOVA (E) or Student’s t test (G and I–K). See also Figure S4.
YAP/TAZ in CMT93 cells treated with WT and Postn−/− CAF CM, respectively. Notably, Postn−/− CAF-CM, but not WT CAF-CM, failed to enhance YAP/TAZ nuclear translocation in CMT93 cells (Figures 4D and 4E). These results were further confirmed by rmPeriostin-induced YAP/TAZ nuclear localization in CMT93 cells (Figures 4F and 4G). Moreover, rmPeriostin treatment strongly elevated the levels of YAP and TAZ in a time- and dose-dependent manner (Figures 4H and 4I). Administration of CMT93 cells with rmPeriostin showed a significant increase in the expression of YAP/TAZ target genes, but this effect was prevented by knocking down YAP/TAZ with short interfering RNAs (Figures 4J and S5A). These findings suggest that periostin enhances YAP/TAZ activation in colorectal tumor cells in vivo and in vitro.

As a downstream effector of the Hippo signaling pathway, YAP can be directly phosphorylated by large tumor suppressor kinases 1 and 2 (LATS1/2) on several residues (Moroishi et al., 2015; Gregorieff et al., 2015; Hong et al., 2016). Phosphorylation of YAP at Ser127 is known to enhance YAP cytoplasmic localization and degradation by the proteasome (Guo et al., 2018). Phosphorylation-dependent nucleocytoplasmic shuttling of YAP is tightly associated with its activity (Zhao et al., 2010). We found that periostin significantly increased the abundance of YAP but reduced the phosphorylated level of YAP (Figures 4A, 4H, and 4I). Moreover, the treatment of rmPeriostin rescued the nuclear localization of YAP/TAZ in CMT93 cells grown even at high confluence (Figure S5B). To determine whether LATS1/2 were involved in regulating YAP phosphorylation and nuclear localization induced by periostin, we adopted short interfering RNAs to knock down LATS1/2 in CMT93 cells (Figure S5C) and treated them with rmPeriostin alone or in combination with RGD, an Arg-Gly-Asp motif peptide for binding and inhibiting integrin receptors. Notably, RGD treatment significantly prevented periostin-induced YAP/TAZ nuclear translocation, while LATS1/2 knockdown entirely reversed this effect, as evidenced by a highly increased percentage of YAP/TAZ nuclear localization (Figures 4K and S5D). In line with this, rmPeriostin treatment caused a significant reduction in LATS1 phosphorylation (Figure S5E).
Figure 4. Periostin Enhances the Activation of YAP/TAZ in Colorectal Tumor Cells

(A) Western blot analysis of YAP, TAZ, CTGF, and p-YAP in the lysates of AOM/DSS-induced colorectal tumors (n = 3 mice each group). The experiments were repeated three times.

(B) Immunohistochemical staining of YAP/TAZ in the colorectal tumor sections from AOM/DSS-treated WT and Postn+/− mice or from Postn+/−ApcMin/+ and Postn−/−ApcMin/+ mice. Scale bars, 50 μm.

(C) Relative mRNA levels of YAP, TAZ, and their target genes in colorectal tumors of WT (n = 5) and Postn−/− (n = 5) mice.

(D–G) Immunofluorescence staining of YAP/TAZ (D and F) and quantification of nuclear YAP/TAZ (E and G) in CMT93 cells treated with WT or Postn−/− CAF-CM (D and E), or treated with rmPeriostin (F and G) for 24 h (n = 3 biological replicates). Scale bars, 25 μm. Each point represents one microscopic field.

(H and I) Western blot analysis of YAP, TAZ, CTGF, and p-YAP in CMT93 or DLD1 cells treated with recombinant periostin for the indicated time (H) or treated with the indicated concentrations of recombinant periostin for 24 h (I). The experiments were repeated at least twice.

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Furthermore, the forced decrease in YAP/TAZ expression in tumor cells markedly reduced tumor cell proliferation to a very low level, even in the presence of periostin stimulation (Figures 4L, SSF, and S5G). These results suggest that periostin promotes tumor cell proliferation and colorectal tumorigenesis through the activation of YAP/TAZ in a Hippo pathway-dependent manner.

**Integrin-FAK-Src Axis Mediates Periostin-Induced YAP/TAZ Activation in Colorectal Tumor Cells**

Next, we investigated the molecular mechanisms by which periostin induces YAP and TAZ activation in tumor cells. YAP can be directly phosphorylated and activated by active Src in intestine epithelial cells and breast cancer cells (Taniguchi et al., 2015; Sorrentino et al., 2017). The current evidence demonstrates that periostin is one of the ligands and regulators of integrin/FAK signaling in tumor progression. In tumor microenvironments, the enhanced interaction of the ECM proteins, such as collagens, periostin, tenascin-C, and fibronectin, leads to integrin-mediated outside-in signaling, including FAK and Src activation. As an ECM component, periostin is highly expressed in the colorectal tumors and deposited in the matrix (Figures 2A–2D). Thus, we proposed that integrin-FAK-Src signaling may be involved in periostin-induced YAP activation in colorectal tumorigenesis. To test this hypothesis, we examined the protein levels of these signaling cascades in two different tumor cell lines by treatment with mouse or human recombinant periostin protein. We found that recombinant periostin treatment significantly elevated the levels of FAK and Src phosphorylation, as well as YAP, TAZ, and connective tissue growth factor (CTGF) in CMT93 and DLD1 tumor cells, which were significantly blocked by adding integrin inhibitory RGD peptide (Figures 5A–5C). We also found that periostin strongly enhanced AKT phosphorylation in the two different tumor cell lines (Figure 5A), which was in line with our previous work (Bao et al., 2004). As an ECM component, periostin usually binds to integrins such as \( \alpha v \beta 1, \alpha v \beta 3, \alpha v \beta 5 \), or \( \alpha v \beta 4 \) to perform its biological functions. Among these integrins, \( \alpha v \beta 3 \) and \( \alpha v \beta 5 \) are primarily expressed on tumor cells. Similar to RGD peptide, \( \alpha v \beta 3 \) or \( \alpha v \beta 5 \) neutralizing antibody treatment increased the level of YAP phosphorylation but markedly reversed the elevated levels of FAK and Src phosphorylation, as well as YAP, TAZ, and their target genes induced by periostin in tumor cells through disrupting integrin-mediated outside-in signaling (Figure 5D). We further confirmed that inhibition of FAK or Src kinase, but not inhibition of Akt, led to the increased level of YAP phosphorylation and significantly decreased the levels of YAP/TAZ and their target gene expression, indicating the important contributions of FAK and Src to the periostin-induced YAP and TAZ increase (Figure 5E). Our further results showed that the inhibition of FAK or Src kinase, rather than the inhibition of Akt, greatly decreased periostin-induced YAP/TAZ nuclear accumulation (Figures 5F and S6A). To further assess whether the FAK-Src axis was involved in periostin-enhanced colorectal tumorigenesis in vivo, we performed western blotting and immunohistochemical assays and found that the levels of phosphorylated FAK and Src in the tumors of Postn−/− mice were significantly reduced compared with the tumors from WT mice (Figures 5G and S5H), suggesting that the FAK-Src axis is responsible for periostin-enhanced colorectal tumorigenesis. We also found that the inhibition of Src kinase activation strongly prevented periostin-induced YAP/TAZ nuclear localization, but this was significantly reversed by knocking down LATS1/2 in tumor cells (Figures 5I and S6B). Furthermore, we found that treating YAP-WT transiently transfected cells with dasatinib significantly prevented periostin-induced cell proliferation, while YAP-SSA (a mutant form of YAP) transfected cells were almost completely insensitive to the inhibitory effect of dasatinib on periostin-induced cell proliferation, as evidenced by the higher increased proliferative cells (Figures 5J, 5K, and S6C–S6E). These data indicate that the integrin-FAK-Src axis mediates periostin-induced YAP/TAZ activation and tumor cell proliferation.

**Periostin-Dependent YAP Activation Induces IL-6 Secretion in Tumor Cells to Stimulate Periostin Expression in Colon Myofibroblasts**

After having demonstrated that fibroblast-derived periostin enhances YAP/TAZ activation in colorectal tumor cells, we further determined which cytokine(s) from tumor cells in turn induced fibroblast activation and, subsequently, periostin expression. It has been reported that YAP/TAZ activation leads to the expression of pro-inflammation genes such as \( Il6, Il8 \), and \( Il1b \) (Wang et al., 2016a). Another recent report indicates that IL-6 is a target of YAP and plays a key role in maintaining the stemness of breast cancer cells (Kim et al., 2015). Consistent with these findings, we found that YAP knockdown was sufficient to downregulate \( Il6 \), but not \( Il1b \) or \( Tnfa \) expression, in colorectal tumor cells (Figure S7A). Decreased YAP expression significantly reversed periostin-induced \( Il6 \) production (Figure 6A), indicating that periostin induces \( Il6 \) expression in tumor cells via YAP activation. Previous reports have revealed that IL-6 treatment increases collagen expression in fibroblasts (Duncan and Berman, 1991); thus, we proposed that tumor cell-derived IL-6 may contribute to fibroblast activation and periostin expression. To test this hypothesis, we analyzed the changes in periostin production in the isolated primary colon myofibroblasts treated with recombinant IL-6 protein.

(J) Relative mRNA levels of the indicated genes in CMT93 cells transfected with indicated small interfering RNAs (siRNAs) for 48 h and treated with or without rmPeriostin for 24 h (n = 3 biological replicates). siCtrl is control siRNA; YAP/TAZ siRNA A is composed of oligos YAP 1# and TAZ 1#; YAP/TAZ siRNA B is composed of oligos YAP 2# and TAZ 2#.

(K) Quantification of YAP/TAZ nuclear localization in CMT93 cells transfected with indicated siRNAs for 48 h and treated with or without RGD for 6 h in serum-free medium containing rmPeriostin (n = 3 biological replicates). LATS1/2 siRNA A is composed of oligos LATS1 1# and LATS2 1#; LATS1/2 siRNA B is composed of oligos LATS1 2# and LATS2 2#.

(L) Quantification of Ki67+ and BrdU+ cells in CMT93 cells treated as in (J); n = 3 biological replicates.

Each value represents mean ± SEM; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. Student’s t test. See also Figure S5.
Figure 5. Integrin-FAK-Src Signaling Mediates Periostin-Induced YAP/TAZ Activation in Tumor Cells

(A) Western blot analysis of the indicated proteins in CMT93 and DLD1 cells treated with recombinant periostin alone or in combination with RGD for 6 h in serum-free medium.

(B and C) Immunofluorescence staining of YAP/TAZ (B) and quantification of nuclear YAP/TAZ (C) in CMT93 cells treated as in (A); n = 4 biological replicates. Scale bars, 25 μm. Each point represents one macroscopic field.

(D) Western blot analysis of the indicated proteins in CMT93 cells treated with rmPeriostin in the presence or absence of αvβ3 or αvβ5 integrin neutralizing antibody.

(E) Western blot analysis of the indicated proteins in CMT93 cells treated with rmPeriostin alone or together with the indicated inhibitors for 24 h.

(F) Quantification of YAP/TAZ nuclear localization in CMT93 cells treated as in (E) by immunofluorescence (n = 3 biological replicates).

(G) Western blot analysis of p-FAK, FAK, p-Src, and Src in colorectal tumor tissue lysates of AOM/DSS-treated WT (n = 3) and Postn−/− (n = 3) mice.

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Accompanying the activation of colon myofibroblasts, the abundance of Postn mRNA was significantly increased in a time- and dose-dependent manner by IL-6 stimulation (Figures 6B and 6C). Moreover, STAT3-signaling inhibitor Stattic treatment significantly reduced the increase in the mRNA and protein levels of periostin induced by IL-6 (Figures 6D and S7B), suggesting that IL-6-STAT3 signaling is responsible for periostin expression in activated fibroblasts. To further determine whether IL-6-STAT3 signaling was associated with fibroblast activation and periostin increase during CAC progression, we analyzed the expression of Il6, Acta2, and Postn during the course of AOM/DSS colitis. We found that the levels of Il6, Acta2, and Postn were significantly increased during the development of AOM/DSS-induced CAC (Figures 6E and S7C), which were further confirmed by western blot analysis (Figure 6F). Furthermore, immunohistochemical staining assays showed that p-STAT3, α-SMA, and periostin were highly expressed in stromal cells of the inflamed colonic and tumorous tissues (Figure 6G). These results demonstrate that periostin-induced YAP activation results in IL-6 expression in tumor cells, which in turn stimulates colon myofibroblast activation and periostin expression. Therefore, we conclude that under the pathological conditions of inflammation and/or tumor, elevated IL-6 can stimulate colon myofibroblast activation and periostin expression to facilitate colorectal tumorigenesis.

**Periostin Is Highly Expressed in Patients with Intestinal Diseases and Correlates with YAP/TAZ Expression in Human Colorectal Tumors**

Next, we analyzed periostin expression in human intestinal tissues and tissues from patients with inflammatory bowel diseases (ulcerative colitis and Crohn disease) or colorectal tumors. We found that the expression of periostin in ulcerative colitis and Crohn disease samples and tumors was higher than that in normal tissues (Figures 7A and 7B) and that periostin was deposited in the stromal component (Figure 7A). Notably, YAP/TAZ was significantly increased in human colorectal tumors (Figure 7B) and was mainly localized in tumor cells (Figure 7A). We also detected a weak increase in YAP/TAZ in inflammatory disorder samples (Figures 7A and 7B). In contrast, the level of YAP phosphorylation was strongly reduced in human tumors (Figure 7B). The analysis of the tissue microarray further revealed significant increases in periostin and YAP/TAZ expression in colorectal tumors compared with adjacent non-tumorous tissues (Figures 7C and 7D). Moreover, we found a stronger positive correlation between periostin and YAP/TAZ expression in colorectal tumors (Figure 7E). These data suggest that periostin positively correlates with inflammatory intestinal disease development and especially with YAP/TAZ expression in human colorectal tumors.

**DISCUSSION**

Accumulating evidence shows that periostin plays important roles in mediating inflammatory responses and tumor progression. Our previous study demonstrates that periostin can promote liver metastasis of CRC (Bao et al., 2004). However, the roles of periostin in CAC development remain controversial (Shimoyama et al., 2018; Koh et al., 2019), and the underlying molecular mechanisms are still largely unknown. Here, we applied an AOM/DSS-induced inflammation-associated CRC model and an Apc<sup>Cmin</sup>/– spontaneous intestinal tumor model to investigate the role of periostin in colorectal tumor development. We found that knockout of periostin exhibits a tumor-inhibiting role, not only in the CAC mouse model but also in the Apc<sup>Cmin</sup>/– mouse intestine tumor model. Therefore, our data definitely demonstrate that periostin deficiency attenuates colorectal tumorigenesis in mice. Moreover, we found that periostin-knockout mice exhibit fewer proliferative cells in colorectal tumor tissues and that periostin treatment promotes colorectal tumor cell proliferation in vitro. These data suggest that periostin has the capacity for enhancing epithelial cell proliferation during colorectal tumorigenesis.

The Hippo pathway has been reported to be involved in the development of intestinal diseases, including inflammatory bowel diseases and CRC. In DSS-induced mouse colitis tissues, increased YAP nuclear localization was found in gp130-active intestinal epithelial cells (Taniguchi et al., 2015). In Mst1/Mst2-deficient intestinal epithelium, YAP is preferential nuclear localization (Zhou et al., 2011). YAP is overexpressed in human colorectal tumors, and depletion of YAP can inhibit cell proliferation and survival (Hong et al., 2016). Moreover, higher YAP or TAZ expression is positively correlated with poor survival outcomes in patients with colon cancer (Hong et al., 2016; Wang et al., 2013; Yuen et al., 2013). Here, we found that periostin deficiency significantly reduces YAP/TAZ total protein levels and their nuclear localization in mouse tumors. Moreover, recombinant periostin treatment can significantly increase total YAP/TAZ levels and enhance the nuclear translocation of YAP/TAZ in colorectal tumor cells. Ctgf, Cyr61, and Ankrd1 are strikingly upregulated after periostin treatment, and knockdown of YAP/TAZ can reverse the effect induced by periostin. Knocking down YAP/TAZ expression in tumor cells significantly suppresses cell proliferation, even in the presence of periostin treatment. Therefore, our findings indicate that periostin enhances tumor cell proliferation via regulating YAP/TAZ activation during colorectal carcinogenesis.

Tumor cells can release many types of soluble factors to educate stromal cells and remodel tissue microenvironments to sustain their own survival and growth (Grivennikov et al., 2010). A recent study demonstrated that IL-6 plays a major role in mediating dynamic cross-talk between tumor cells...
and activated fibroblasts in head and neck squamous cell carcinoma, esophageal cancer, and gastric adenocarcinoma by supporting the growth of tumor cells and promoting the activation of fibroblasts (Karakasheva et al., 2018). Another report reveals a key role for the IL-6-STAT3 axis in fibroblast growth factor 19 (FGF19)-driven hepatocarcinogenesis, while blocking this axis in hepatocytes abolishes FGF19-induced tumorigenesis (Zhou et al., 2017). STAT3 can induce periostin expression by directly binding to its promoter (Amara et al., 2015).

Here, we found that the deletion of periostin significantly decreases the level of IL-6 in AOM/DSS-induced mouse tumor tissues. We also observed that knocking down YAP is sufficient to downregulate Il6 expression and that periostin-induced YAP leads to the increase of Il6 in tumor cells.

**Figure 6. IL-6-STAT3 Signaling Is Involved in Fibroblast Activation and Periostin Expression during CAC Development**

(A) Relative mRNA level of Il6 in CMT93 cells transfected with the indicated siRNAs for 48 h and treated with or without rmPeriostin for 24 h (n = 3 biological replicates).

(B) Relative mRNA levels of Acta2 and Postn in isolated CMFs treated with rmIL-6 for the indicated times (n = 3 biological replicates).

(C) Relative mRNA expression of Acta2 and Postn in CMFs treated with the indicated concentrations of rmIL-6 for 48 h (n = 3 biological replicates).

(D) Western blot analysis of p-STAT3, α-SMA, and periostin in isolated primary CMFs treated with rmIL-6 alone or together with STAT3 inhibitor Stattic for 48 h.

(E) Relative mRNA levels of Il6, Acta2, and Postn in the distal colonic tissue lysates of WT mice (n = 3) during the course of colitis-associated tumor formation.

(F) Western blot analysis of p-STAT3, α-SMA, and periostin in the colonic tissue lysates of WT mice treated as in (E).

(G) H&E staining and immunohistochemical staining of p-STAT3, α-SMA, and periostin in the non-tumorous (days 0–57) and tumors (day 80) tissues of AOM/DSS-treated WT mice. Scale bars, 50 μm.

Each value represents mean ± SEM; *p < 0.05, **p < 0.01, ***p < 0.001; Student’s t test. See also Figure S7.
Moreover, recombinant IL-6 protein can increase periostin expression in isolated colon myofibroblasts via STAT3 activation. Therefore, IL-6-STAT3 signaling is responsible for periostin expression in activated fibroblasts during colorectal tumorigenesis.

Immune and/or inflammatory cells can produce tumor-promoting cytokines to remodel the initial hostile tissue microenvironment, which may occur in the early or late stages of tumor development (Grivennikov et al., 2010). In this study, we found that Postn 

\[ \text{Postn}^{-/-} \]

mice have a significant reduction in inflammation such as less infiltrated inflammatory cells and reduced chemokines during the first cycle of AOM/DSS treatment. We further demonstrated that the significantly increased level of periostin is deposited in the stromal component and that the absence of periostin prevents precancerous lesion formation in the inflamed colon tissue. Our findings suggest that the released inflammatory mediators by infiltrated immune cells may also be involved in inducing the secretion of periostin and thereby facilitate the precancerous lesion formation during the early phase of CAC progression. Therefore, it is worthwhile to further characterize how stromal periostin facilitates tumor onset and outgrowth by recruiting inflammatory immune cells in tissues.

In conclusion, we demonstrate that CAF-derived periostin enhances the activation of YAP via the integrin-FAK-Src pathway, thereby increasing the expression of IL-6 in tumor cells, which in turn stimulates fibroblasts to produce periostin via STAT3 activation. Our study provides strong evidence to support the hypothesis that the periostin-IL-6 loop contributes to regulating the interaction between tumor cells and fibroblasts during colorectal tumorigenesis. Targeting periostin- and IL-6-mediated tumor-stroma interaction may be an attractive therapeutic strategy for human colorectal tumors.

**STAR+METHODS**

Detailed methods are provided in the online version of this paper and include the following:
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- **LEAD CONTACT AND MATERIALS AVAILABILITY**
- **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
  - Animal Studies
  - Human Tissue Samples
  - Cell Culture
  - Primary Colon Myofibroblast and CAF Isolation

Figure 7. Periostin Is Highly Expressed in Human Intestinal Diseases and Positively Correlates with YAP/TAZ Expression in Human Colorectal Tumors

(A) Representative images of H&E staining and the immunohistochemical staining of periostin and YAP/TAZ in human normal intestine, inflammatory bowel diseases (ulcerative colitis [UC] and Crohn disease [CD]), and colorectal tumors. Scale bars, 50 μm.

(B) Western blot analysis of periostin, p-YAP, YAP, and TAZ in the tissue lysates of human normal mucosa, inflammatory bowel diseases, and tumors.

(C and D) Analysis of the immunoreactive score (IRS) of periostin (C) and YAP/TAZ (D) expression by immunohistochemistry in colorectal tumorous tissues and the adjacent non-tumorous tissues. IRS scores of 0–1 were analyzed as negative, 2–3 as mild, 4–8 as moderate, and 9–12 as strongly positive. Each point represents the IRS score of periostin (C) or YAP/TAZ (D) in one individual tissue section. Data are means ± SEMs (n = 49); **p < 0.01, ****p < 0.0001; Student’s t test.

(E) Correlation analysis of periostin and YAP/TAZ expression in human colorectal tumors.
METHOD DETAILS
- Determination of Disease Activity Index
- Histological Score and Tumors Analysis
- Bone Marrow Chimeric Mice Generation
- Western Blot Analysis
- Immunohistochemical Staining
- Immunofluorescent Staining
- Real-Time Polymerase Chain Reaction
- Enzyme-Linked Immunosorbent Assay for Cytokines
- Transfection and Reagents

QUANTIFICATION AND STATISTICAL ANALYSIS

DATA AND CODE AVAILABILITY

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.celrep.2019.12.075.

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AUTHOR CONTRIBUTIONS

Conception & Design, H.M., Y.L., and G.O.; Methodology, H.M., J.W., X.Z., and D.C.; Manuscript Writing, H.M., Y.L., and G.O.; Study Supervision, Y.L. and G.O.; Funding Acquisition, G.O.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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## STAR METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| Mouse monoclonal anti-Periostin | Adipogen | Cat# AG-20B-0033; RRID: AB_2490231 |
| Rabbit monoclonal anti-Phospho-STAT3 | Cell Signaling Technology | Cat# 9145; RRID: AB_2491009 |
| Mouse monoclonal anti-STAT3 | Cell Signaling Technology | Cat# 9139; RRID: AB_331757 |
| Rabbit monoclonal anti-Phospho-AKT | Cell Signaling Technology | Cat# 4060; RRID: AB_2315049 |
| Rabbit monoclonal anti-AKT | Cell Signaling Technology | Cat# 4691; RRID: AB_915783 |
| Rabbit polyclonal anti-Phospho-FAK | Cell Signaling Technology | Cat# 3283; RRID: AB_2173659 |
| Rabbit monoclonal anti-FAK | Abcam | Cat# ab40794; RRID: AB_732300 |
| Rabbit monoclonal anti-Phospho-ERK1/2 | Cell Signaling Technology | Cat# 9102; RRID: AB_331646 |
| Rabbit monoclonal anti-ERK1/2 | Cell Signaling Technology | Cat# 8457; RRID: AB_10950489 |
| Rabbit monoclonal anti-β-actin | Cell Signaling Technology | Cat# MAB1876; RRID: AB_569486 |
| Rabbit monoclonal anti-In-1 antibody | Millipore/Merck | Cat# MAB1961Z; RRID: AB_94466 |
| Rabbit monoclonal anti-β3 integrin | Cell Signaling Technology | Cat# 12202; RRID: AB_2620142 |
| Rabbit monoclonal anti-β5 integrin | Cell Signaling Technology | Cat# 8480; RRID: AB_11127855 |
| Rabbit monoclonal anti-INOS | Cell Signaling Technology | Cat# 13120; RRID: AB_2687529 |
| Rabbit monoclonal anti-Cyclin D1 | Cell Signaling Technology | Cat# 2978; RRID: AB_2259616 |
| Rabbit monoclonal anti-c-Myc | Abcam | Cat# ab32072; RRID: AB_731658 |
| Rabbit monoclonal anti-Phospho-Src | Cell Signaling Technology | Cat# 2101; RRID: AB_331697 |
| Rabbit monoclonal anti-Src | Cell Signaling Technology | Cat# 2109; RRID: AB_2106059 |
| Rabbit monoclonal anti-YAP | Cell Signaling Technology | Cat# 14074; RRID: AB_2650491 |
| Rabbit monoclonal anti-Phospho-YAP | Cell Signaling Technology | Cat# 13008; RRID: AB_2650553 |
| Rabbit polyclonal anti-CTGF | Abcam | Cat# ab6992; RRID: AB_305688 |
| Rabbit monoclonal anti-Phospho-LATS1 | Cell Signaling Technology | Cat# 8654; RRID: AB_10971635 |
| Rabbit monoclonal anti-LATS1 | Abcam | Cat# ab79561; RRID: AB_1209494 |
| Rabbit polyclonal anti-αSMA | Abcam | Cat# ab84927; RRID: AB_1925489 |
| Rabbit monoclonal anti-YAP/TAZ | Cell Signaling Technology | Cat# 8418; RRID: AB_10950494 |
| Rabbit monoclonal anti-GAPDH | Cell Signaling Technology | Cat# 5174; RRID: AB_10622025 |
| Rabbit polyclonal anti-αSMA | Abcam | Cat# ab52218; RRID: AB_870573 |
| Rabbit polyclonal anti-LAT2 | Abcam | Cat# ab714499; RRID: N/A |
| Rabbit polyclonal anti-ANKRD1 | BBI Life Science | Cat# D121628; RRID: N/A |
| Rabbit polyclonal anti-CYR61 | BBI Life Science | Cat# D222190; RRID: N/A |
| Rat monoclonal anti-F4/80 | Abcam | Cat# ab16911; RRID: AB_443548 |
| Rat monoclonal anti-Ly6g | Abcam | Cat# ab25377; RRID: AB_470492 |
| Rabbit polyclonal anti-CD45 | Abcam | Cat# 208002; RRID: N/A |
| Mouse monoclonal anti-BrdU | Cell Signaling Technology | Cat# 5292; RRID: AB_10548898 |
| Rabbit monoclonal anti-Vimentin | Cell Signaling Technology | Cat# 5741; RRID: AB_10695459 |
| Rabbit polyclonal anti-Epcam | Abcam | Cat# ab71916; RRID: AB_1603782 |
| **Biological Samples** | | |
| Human inflammatory bowel disease and tumor samples | Shanghai General Hospital of Shanghai Jiao Tong University | N/A |
| Tissue arrays (Col015-118e) | Alenabio | http://alenabio.bioon.com.cn/ |

(Continued on next page)
| REAGENT or RESOURCE NAME | SOURCE | IDENTIFIER |
|--------------------------|--------|------------|
| Chemicals, Peptides, and Recombinant Proteins | | |
| Lipofectamine RNAi-MAX Reagent | Invitrogen | Cat# 13778030 |
| STAT3 inhibitor Stattic | Selleck Chemicals | Cat# S7024 |
| Src inhibitor PP2 | Selleck Chemicals | Cat# S7008 |
| Src inhibitor dasatinib | Selleck Chemicals | Cat# S1021 |
| AKT inhibitor LY294002 | Selleck Chemicals | Cat# S1105 |
| RGD peptide | PEPTIDES | Cat# PCI-3661-PI |
| Recombinant Human Periostin/OSF-2 Protein | R&D Systems | Cat# 3548-F2-050 |
| Recombinant Mouse Periostin/OSF-2 Protein | R&D Systems | Cat# 2955-F2-050 |
| Recombinant Mouse IL-6 Protein | R&D Systems | Cat# 406-ML-025 |
| Azoxy methane (AOM) | Sigma-Aldrich | Cat# A5486 |
| Dextran sulfate sodium salt (DSS) | MP Biomedicals | Cat# 160110 |
| Lipofectamine 3000 Transfection Reagent | Invitrogen | Cat# L3000001 |
| FAK inhibitor PF573228 | Selleck Chemicals | Cat# S2013 |

Critical Commercial Assays

| REAGENT or RESOURCE NAME | SOURCE | IDENTIFIER |
|--------------------------|--------|------------|
| Mouse IL-6 ELISA Kit | R&D Systems | Cat# DY406 |
| Mouse TNFα ELISA Kit | R&D Systems | Cat# DY410 |
| Mouse IL-1β ELISA Kit | R&D Systems | Cat# DY401 |

Experimental Models: Cell Lines

| REAGENT or RESOURCE NAME | SOURCE | IDENTIFIER |
|--------------------------|--------|------------|
| Mouse: CMT93 | ATCC | CCL-223 |
| Human: DLD1 | ATCC | CCL-221 |
| Mouse: CMF (colon myofibroblast) | This paper | N/A |
| Mouse: CAF (cancer-associated fibroblast) | This paper | N/A |

Experimental Models: Organisms/Strains

| REAGENT or RESOURCE NAME | SOURCE | IDENTIFIER |
|--------------------------|--------|------------|
| Mouse: B6.129-Postrn1tsm1j/J | Jackson Laboratory | JAX: 009067 |
| Mouse: C57BL/6 | Nanjing Biomedical Research Institute of Nanjing University | N000013 |
| Mouse: C57BL/6J-ApcMin/J | Jackson Laboratory | JAX: 002020 |

Oligonucleotides

| REAGENT or RESOURCE NAME | SOURCE | IDENTIFIER |
|--------------------------|--------|------------|
| siRNA targeting sequence: siYAP 1# | This paper | N/A |
| | GCAUGAGGAGCGCUAACGAUUTT |
| siRNA targeting sequence: siYAP 2# | This paper | N/A |
| | GCCUGAAUACUCGGCUUGATT |
| siRNA targeting sequence: siYAP 3# | This paper | N/A |
| | GAUCCUGAUGAUGUACATT |
| siRNA targeting sequence: siTAZ 1# | Calvo et al., 2013 | N/A |
| | GAUUGAUGCGUAGACAGATT |
| siRNA targeting sequence: siLATS1 1# | This paper | N/A |
| | GCAUGCACUCUCUGCUAAUUTT |
| siRNA targeting sequence: siLATS1 2# | This paper | N/A |
| | GGUGAAGUCUCUGCUAGCATT |
| siRNA targeting sequence: siLATS2 1# | This paper | N/A |
| | GCCCAACUACUCUACUGAATT |
| siRNA targeting sequence: siLATS2 2# | This paper | N/A |
| | GCCCUAGUCUGACUGUUGATT |
| siRNA targeting sequence: siTAZ 2# | Calvo et al., 2013 | N/A |
| | CGGAUGGAUGACAGGUAGAATT |

See Table S1 for qRT-PCR primer sequences | This paper | N/A |
LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Gaoliang Ouyang (oygldz@xmu.edu.cn).

All unique and stable reagents generated in this study are available from the Lead Contact with a completed Materials Transfer Agreement.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animal Studies
We maintained all animals under specific pathogen-free conditions at the Laboratory Animal Center of Xiamen University. All animal experiments were performed in accordance with the animal study protocols approved by the Animal Care and Use Committee of Xiamen University. C57BL/6 mice were originally obtained from Nanjing Biomedical Research Institute of Nanjing University and were maintained in the Laboratory Animal Center of Xiamen University. Postn/+− and ApcMin/+ mice were originally purchased from the Jackson Laboratory. Wild-type and periostin-deficient mice were generated by crossing Postn/+− mice with Postn/+− mice as described previously (Ma et al., 2019). For the generation of Postn+/− ApcMin/+ mice, ApcMin/+ mice in a C57BL/6 background were crossed with Postn+/− mice in a C57BL/6 background. Age- and sex-matched Postn+/− ApcMin/+ mice and Postn+/+ ApcMin/+ mice were analyzed for intestine tumor formation at 6-month-old. Minimum of 3 mice per group were used in this research. Mouse with dermatitis, urethritis or fight wounds was excluded from the experimental analysis.

Colitis-associated colorectal tumor formation was induced in mice as previously described (Karki et al., 2016; Huber et al., 2012). In brief, 6- to 8-week-old male wild-type and Postn+/− mice were injected intraperitoneally with mutagen AOM (Sigma-Aldrich) at a dose of 10 mg/kg body weight. Five days after AOM injection, mice were subjected to 3 cycles of 3% DSS (MP Biomedicals, molecular mass 36,000-50,000) in drinking water for 5 days followed by regular drinking water for 16 days. Mice were sacrificed on day 80 of the experimental protocol. The colons were collected from the mice and placed on the ice-cold PBS wetted gauze. After the colons were cut open longitudinally and cleaned with ice-cold PBS, the distal colon tissues were kept in different conditions depending on the subsequent experimental requirements. For enzyme-linked immunosorbent assay (ELISA) measurements of inflammatory cytokines, fresh colon tissues were homogenized, snap frozen and thawed on ice, then cleared by centrifugation at 4°C and the supernatants were used for test. Samples for western blotting or RNA isolation, distal colons were snap-frozen in liquid nitrogen for 10 min and then stored at −80°C. For tumor sample collection, AOM/DSS-induced colon tumors or tumors in the small and large intestines of 6-month-old ApcMin/+ mice were counted and the size of each tumor was measured in each mouse. Some intestine tumors either from AOM/DSS-induced mice or from ApcMin/+ mice were placed on 4% PFA wetted gauze for 30 min to be fixed, then dehydrated and Paraffin-embedded for further immunohistochemical assay.

Human Tissue Samples
The collection and study of human tissue samples was performed in accordance with the approved guidelines of the Ethics and Scientific Committees of Shanghai Jiao Tong University. Clinical samples were collected from colorectal carcinoma patients (n = 7), ulcerative colitis patients (n = 5) and Crohn’s disease patients (n = 5) who underwent surgical resection or colonoscopy in Shanghai General Hospital. All of these obtained specimens were from previously untreated patients and both the male and female patients were included. After excision, specimens were fixed in 4% formalin or stored at −80°C for further studies. 5 paired tumor and relative normal mucosa samples, 3 ulcerative colitis samples and 3 Crohn’s disease specimens were available for performing western blotting assay. 2 paired tumor and normal mucosa samples, 2 ulcerative colitis samples and 2 Crohn’s disease samples were available for performing immunohistochemical study. Tissue arrays (Col015-118e) were purchased from Alenabio.

Cell Culture
CMT93 and DLD1 cell lines were purchased from American Type Culture Collection (ATCC). CMT93 cells were cultured in DMEM (GIBCO) supplemented with 10% FBS (HyClone) and 1% penicillin and streptomycin (GIBCO); DLD1 cells were cultured in RPMI 1640 medium (GIBCO) with 10% FBS and 1% penicillin and streptomycin. All cell lines were maintained at 37°C with 5% CO2 in
a humidified atmosphere. All cell lines were regularly tested for mycoplasma contamination by PCR-based method and were found to be negative.

**Primary Colon Myofibroblast and CAF Isolation**

Primary colon myofibroblasts (CMFs) were isolated as reported previously (Khalil et al., 2013). In brief, 4- to 5-week-old wild-type mouse colon was removed and linearized, cleared of feces by flushing with ice-cold PBS. The colon was incubated in HBSS supplemented with 5 mM EDTA for 15 minutes at room temperature with shacking to remove the normal epithelial cells. After twice washing, colon tissue was digested for 1.5 hours at 37°C in the following solution: 20 mL of RPMI-5 containing 10.5 mg of Dispase (Roche) and 7.2 mg of collagenase D (Sigma). Centrifugation and resuspending the pellet with 10 mL RPMI-5 medium and passing through 70 μm mesh strainer into a 100 mm dish. After 3 hours, the nonadherent cells were washed off with PBS and cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin and streptomycin. All primary colon myofibroblasts were used before reaching the third passage.

For CAF isolation (Kesselring et al., 2016), tumors were obtained from tumor burden colons of AOM/DSS-treated wild-type and Postn−/− mice. Tumor tissues were incubated in the EDTA chelation buffer for 1 hour on ice to remove normal epithelia cells. Tumor fragments were digested in the following buffer for 2 hours at 37°C: DMEM medium containing 2.5% FBS, 1% penicillin and streptomycin, 125 μg/ml dispase II, 200 U/ml collagenase IV and amphotericin B (Invitrogen). The supernatant was collected and followed by passing through 70 μm mesh strainer into a 6 cm dish. After 2-3 hours, the nonadherent cells were washed off and new medium was changed. When reaching 75%–90% confluence, the cells were washed twice with PBS and continued to be cultured in serum-free medium for 1 hour. After twice washing again, cells were cultured in the serum-free medium for 48 hours and conditioned media were collected.

**METHOD DETAILS**

**Determination of Disease Activity Index**

Disease activity index was assessed as described previously in detail (Hartmann et al., 2000; Siegmund et al., 2001). Body weights, stool consistency and occult blood were monitored daily or weekly according to the protocol. For body weight loss analysis, no body weight loss was analyzed as 0 score, increased body weight loss between 1 and 5% as 1 score, 5 to 10% as 2 scores, 10 to 20% as 3 scores, and a score of 4 was analyzed as more than 20% body weight loss. For the stool consistency, well-formed pellets were analyzed as 0 score, pasty and semi-formed stools that did not adhere to the anus were analyzed as 2 scores, and a score of 4 was analyzed for liquid stools that remained adhered to the anus. For occult blood measurement, no blood in hemoccult was analyzed as 0 score, positive hemoccult was analyzed as 2 scores, and a score of 4 was analyzed as gross bleeding from the rectum. All these scores were added and divided by 3 to obtain a total disease activity index ranging from 0 to 4 in one individual mouse.

**Histological Score and Tumors Analysis**

After cervical dislocation, the entire colon was removed from the cecum to the anus and placed on gauze wetted with ice-cold PBS, the colon was opened longitudinally and cleared of feces by flushing with ice-cold PBS, followed by division into thirds from the proximal, middle and distal colon, then placed on gauze wetted with 4% PFA for 30 minutes at room temperature. After performing the “petit four” arrangement composed of tissues and agar and fixed overnight at 4°C in 4% PFA (Zhang et al., 2015), the colon tissues were embedded in paraffin for histological analysis. Colonic tissue sections (5 μm) were performed by H&E staining, and histologic scoring was analyzed according to the protocol described previously (Kesselring et al., 2016). Tumor load was analyzed according to the formula: tumor load = (number of small tumors) × 1 + (number of medium tumors) × 2 + (number of large tumors) × 3.

**Bone Marrow Chimeric Mice Generation**

BM chimeric mice were generated to determine the cellular basis contribution to the development of colorectal tumorigenesis. BM from donor wild-type and Postn−/− mice was prepared from femurs and tibias. Two month-old recipient wild-type and Postn−/− mice were irradiated with a single lethal dose (9 Gry), and 2 × 10⁷ BM cells from donor mice were intravenously injected 4 hours later. We performed 4 groups of BM chimeric mice as following: wild-type BM to Postn−/−, Postn−/− BM to wild-type, Postn−/− BM to Postn−/−, and wild-type BM to wild-type mice. Four weeks after BM transplantation, efficiency of BM transplantation was determined as described previously (Popivanova et al., 2008). After 2 months, BM chimeric mice were subjected to AOM/DSS to induce colorectal tumor formation and sacrificed on day 80.

**Western Blot Analysis**

For immunoblotting, total proteins were extracted from colon tissue or cells using RIPA lysis buffer supplemented with protease and phosphatase inhibitors (Roche) as previously reported (Karki et al., 2016). Samples were separated on 8%–10% SDS-PAGE gels and transferred onto PVDF membranes (Millipore). Membranes were blocked in 5% BSA for at least 1 hour at room temperature and then incubated in primary antibodies overnight at 4°C. After washing with TBST for 30 minutes, the membranes were incubated with HRP-conjugated secondary antibody for 1 hour at room temperature. After washing again, proteins were visualized on X-ray films. The primary antibodies to β-actin, p-AKT, AKT, β-Catenin, Cyclin D1, p-ERK1/2, ERK1/2, p-FAK, GAPDH, iNOS, p-LATS1, p-Src, p-AKT, AKT, β-Catenin, Cyclin D1, p-ERK1/2, ERK1/2, p-FAK, GAPDH, iNOS, p-LATS1, p-Src, p-AKT, AKT, β-Catenin, Cyclin D1, p-ERK1/2, ERK1/2, p-FAK, GAPDH, iNOS, p-LATS1, p-Src, p-AKT, AKT, β-Catenin, Cyclin D1, p-ERK1/2, ERK1/2, p-FAK, GAPDH, iNOS, p-LATS1, p-Src, p-AKT, AKT, β-Catenin, Cyclin D1, p-ERK1/2, ERK1/2, p-FAK, GAPDH, iNOS, p-LATS1, p-Src, p-AKT, AKT, β-Catenin, Cyclin D1, p-ERK1/2, ERK1/2, p-FAK, GAPDH, iNOS, p-LATS1, p-Src, p-AKT, AKT, β-Catenin, Cyclin D1, p-ERK1/2, ERK1/2, p-FAK, GAPDH, iNOS, p-LATS1, p-Src, p-AKT, AKT, β-Catenin, Cyclin D1, p-ERK1/2, ERK1/2, p-FAK, GAPDH, iNOS, p-LATS1, p-Src, p-AKT, AKT, β-Catenin, Cyclin D1, p-ERK1/2, ERK1/2, p-FAK, GAPDH, iNOS, p-LATS1, p-Src, p-AKT, AKT, β-Catenin, Cyclin D1, p-ERK1/2, ERK1/2, p-FAK, GAPDH, iNOS, p-LATS1, p-Src, p-AKT, AKT, β-Catenin, Cyclin D1, p-ERK1/2, ERK1/2, p-FAK, GAPDH, iNOS, p-LATS1, p-Src, p-AKT, AKT, β-Catenin, Cyclin D1, p-ERK1/2, ERK1/2, p-FAK, GAPDH, iNOS, p-LATS1, p-Src, p-AKT, AKT, β-Catenin, Cyclin D1, p-ERK1/2, ERK1/2, p-FAK, GAPDH, iNOS, p-LATS1, p-Src, p-AKT, AKT, β-Catenin, Cyclin D1, p-ERK1/2, ERK1/2, p-FAK, GAPDH, iNOS, p-LATS1, p-Src, p-AKT, AKT, β-Catenin, Cyclin D1, p-ERK1/2, ERK1/2, p-FAK, GAPDH, iNOS, p-LATS1, p-Src, p-AKT, AKT, β-Catenin, Cyclin D1, p-ERK1/2, ERK1/2, p-FAK, GAPDH, iNOS, p-LATS1, p-Src, p-AKT, AKT, β-Catenin, Cyclin D1, p-ERK1/2, ERK1/2, p-FAK, GAPDH, iNOS, p-LATS1, p-Src, p-AKT, AKT, β-Catenin, Cyclin D1, p-ERK1/2, ERK1/2, p-FAK, GAPDH, iNOS, p-LATS1, p-Src, p-AKT, AKT, β-Catenin, Cyclin D1, p-ERK1/2, ERK1/2, p-FAK, GAPDH, iNOS, p-LATS1, p-Src, p-AKT, AKT, β-Catenin, Cyclin D1, p-ERK1/2, ERK1/2, p-FAK, GAPDH, iNOS, p-LATS1, p-Src, p-AKT, AKT, β-Catenin, Cyclin D1, p-ERK1/2, ERK1/2, p-FAK, GAPDH, iNOS, p-LATS1, p-Src, p-AKT, AKT, β-Catenin, Cyclin D1, p-ERK1/2, ERK1/2, p-FAK, GAPDH, iNOS, p-LATS1, p-Src, p-AKT, AKT, β-Catenin, Cyclin D1, p-ERK1/2, ERK1/2, p-FAK, GAPDH, iNOS, p-LATS1, p-Src, p-AKT, AKT, β-Catenin, Cyclin D1, p-ERK1/2, ERK1/2, p-FAK, GAPDH, iNOS, p-LATS1, p-Src, p-AKT, AKT, β-Catenin, Cyclin D1, p-ERK1/2, ERK1/2, p-FAK, GAPDH, iNOS, p-LATS1, p-Src.
Src, p-STAT3, STAT3, YAP, p-YAP and YAP/TAZ were purchased from Cell Signaling Technology. Anti-periostin antibody was obtained from Adipogen. Anti-c-Myc, anti-CTGF, anti-LATS1, anti-TAZ, anti-FAK, anti-α-SMA and anti-LATS2 antibodies were obtained from Abcam. Anti-ANKRD1 and anti-CYR61 antibodies were purchased from BBI Life Science.

**Immunohistochemical Staining**

For immunohistochemical analysis, colonic tissue sections were placed at 65°C for 1 hour and then deparaffinized in xylene and alcohol. Antigen retrieval was performed in 0.01 M sodium citrate buffer at 98°C for 10 minutes. Slides were blocked with normal goat serum, followed by incubating overnight at 4°C with the primary antibodies as the following: anti-Ki67 (Cell Signaling, #12202, 1:200), anti-F4/80 (Abcam, #ab16911, 1:200), anti-Gr-1 (Abcam, #ab25377, 1:200), anti-CD45 (Abcam, #208002, 1:200), anti-YAP/TAZ (Cell Signaling, #8418, 1:200), anti-p-Src (Abcam, #ab40660, 1:200), anti-periostin (Adipogen, #AG-20B-0033, 1:200), anti-BrdU (Cell Signaling, #5292, 1:500), anti-p-STAT3 (Cell Signaling, #9145, 1:200) and anti-α-SMA (Abcam, #ab52218, 1:400). After washing for 30 minutes, slides were incubated with anti-mouse or rabbit secondary antibody for 45 minutes at room temperature. After washing for 30 minutes, slides were stained with streptavidin-horseradish peroxidase conjugates and incubated for 20 minutes. The colonic tissue sections were washed 4 times, followed by staining with DAB, and then counterstained with hematoxylin. The number of Ki67- or BrdU-positive cells per crypt in one individual mouse was counted for at least 9 crypts per mouse, and at least 3 mice per experimental group were analyzed.

**Immunofluorescent Staining**

For immunofluorescent staining of colon tumor sections, tissues were dehydrated in increasing concentrations of sucrose, and embedded in OCT followed by placing at −80°C for sectioning. 8 μm thickness tissue sections were baked at 55°C for 30 minutes and then fixed with ice-cold acetone for 10 minutes at −20°C. After washing with PBS for three times, slides were incubated with 3% BSA solution and then incubated overnight at 4°C with the primary antibodies: anti-periostin (Adipogen, #AG-20B-0033, 1:200), anti-F4/80 (Abcam, #ab16911, 1:200), anti-α-SMA (Abcam, #ab52218, 1:300), anti-Vimentin (Cell Signaling, #5741, 1:200), anti-EpCAM (Abcam, #ab71916, 1:200). For 30 minutes washing, slides were incubated with secondary antibodies for 1 hour, followed by counterstaining with Hoechst. Images were taken with a Zeiss LSM 780 confocal microscope.

For cell immunofluorescent staining, CMT93 cells were seeded on 20- or 25-mm diameter glass slides placed in 12- or 6-well plate. After serum starvation overnight or transfection, cells were treated with mouse recombinant periostin protein or conditioned medium for 24 hours. 2 μM BrdU was added into cell culture medium 3 hours before cells fixed. Cells were fixed with 4% PFA for 30 minutes, then slides were washed and permeabilized, followed by blocking with 10% FBS for 1 hour and then incubated with the following antibodies overnight at 4°C: anti-Ki67 (Cell Signaling, #12202, 1:200), anti-BrdU (Cell Signaling, #5292, 1:500), anti-YAP/TAZ (Cell Signaling, #8418, 1:200). Slides were washed with PBS and then secondary antibodies were incubated for 1 hour at room temperature. Hoechst was dyed for visualizing cell nuclei. Images were taken with a Zeiss LSM 780 confocal microscope.

**Real-Time Polymerase Chain Reaction**

RNAs were extracted from colon tissue or cells using Trizol (Invitrogen) and complementary DNA were generated using ReverTra Ace RT kit (FSQ-101, TOYOBO) according to the manufacturer’s instructions. Real-time PCR was performed using the 2× Trans-Start Top Green qPCR SuperMix (Transgene). Primer sequences were listed in the Table S1. The PCR results were normalized to Gapdh expression.

**Enzyme-Linked Immunosorbtent Assay for Cytokines**

Cytokines in the colon tissue were analyzed by mouse IL-6 ELISA kit (R&D Systems), TNFα ELISA kit (R&D Systems) and IL-1β ELISA kit (R&D Systems) according to the manufacturer’s instructions, respectively.

**Transfection and Reagents**

Cells were cultured in standard conditions and siRNA transfection was performed with Lipofectamine RNAi-MAX (Invitrogen) in serum- and antibiotics-free medium according to the manufacturer instructions. When reaching 60% confluence, cells were subjected to 100 nM final concentration of siRNAs. siRNAs were purchased from Sangon Biotech (Shanghai, China), and the sequences of the siRNAs were listed in Key Resources Table. pCMV5-Flag-YAP wild-type and pCMV5-Flag-YAP 5SA plasmids were used as described previously (Liu et al., 2018).

The following reagents and inhibitors were applied: recombinant mouse peristin protein (#2955-F2-050, R&D Systems); recombinant human periostin protein (#3548-F2-050, R&D Systems); recombinant mouse IL-6 protein (#406-ML-025/CF, R&D Systems); Stattic, 2.5 μM (#S7024, Selleck); PP2, 10 μM (#S7008, Selleck); dasatinib, 0.3 μM (#S1021, Selleck); PF573228, 10 μM (#S2013, Selleck); LY294002, 10 μM (#S1105, Selleck); RGD, 0.5 mg/ml (Selleck); zVf3 or zVf5 blocking antibody, 0.1 mg/ml (Merck Millipore).

**QUANTIFICATION AND STATISTICAL ANALYSIS**

GraphPad Prism 5.0 software was used for all experimental result analysis. All data were represented as the mean ± standard error of the mean (SEM). All n-values per group are reported in the figure legends. Statistical significance is represented in figures as
follows: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 and n.s. indicates not significant. Statistical significance was analyzed by the Student's two-tailed t test for two groups or analysis of variance (ANOVA) analysis for more groups. A p value that was less than 0.05 was considered statistically significant. For each quantification by immunofluorescence, at least 6 macroscopic fields per biological replicates and at least 3 biological replicates per group were analyzed. In the study, no statistical methods were used to pre-determine sample size, and the investigators were not blinded to allocation during experiments and result analysis.

**DATA AND CODE AVAILABILITY**

This study did not generate new datasets or code.