Tenascin-C Produced by Intestinal Myofibroblasts Promotes Colitis-associated Cancer Development Through Angiogenesis

Takafumi Kawamura, MD,* Masayoshi Yamamoto, MD, PhD,* Katsunori Suzuki, MD,* Yuhi Suzuki, MD,* Megumu Kamishima, MD,* Mayu Sakata, MD,* Kiyotaka Kurachi, MD, PhD,* Mitsutoshi Setoh, MD, PhD,† Hiroyuki Konno, MD, PhD,* and Hiroya Takeuchi, MD, PhD* 

INTRODUCTION

Colitis-associated cancer (CAC) is one of the major prognostic factors of inflammatory bowel disease (IBD). Approximately 15% of IBD-related deaths are thought to be associated with colorectal cancer (CRC), which is a major problem to overcome. The carcinogenesis of CAC is traditionally considered to be closely related to inflammation, and it has been reported that anti-inflammatory drugs, such as 5-aminosalicylic acid, reduce the risk of developing CAC. In contrast, some reports indicated that 5-aminosalicylic acid was not protective against CAC, and the usefulness of anti-inflammatory drugs is still controversial. To date, only surveillance colonoscopy has been recommended for the early detection of CAC.

The development of CAC is associated with the duration of the disease and the degree of mucosal inflammation. With the recent development of new drugs such as immunomodulators and biologic agents, a number of IBD patients are now able to avoid surgery. However, some patients eventually need to undergo surgery, and the disease duration is increasing in such cases. Consequently, the frequency of CAC will increase, and novel, preventive therapies for CAC are required.

The development of cancer is markedly influenced by the tumor microenvironment (TME), which is composed of the extracellular matrix (ECM) and a variety of stromal cells such as fibroblasts, immune cells, and endothelial cells. Fibroblasts are one of the most important cell types in the TME. In response to TGF-β and IL-6 signals, fibroblasts transform into myofibroblasts, a hyperactivated type of fibroblasts that are known to be predominant in areas of inflammation and remodeling tissues during wound healing. Intestinal myofibroblasts (IMFs) are important cells of the intestinal lamina propria that maintain intestinal homeostasis and can play a role in disease development. Intestinal myofibroblasts are involved in the development of CAC through the production of hepatocyte growth factor (HGF), which enhances carcinogenesis. Aberrant HGF secretion from IMFs maintains colon cancer stem cell fate and consequently regulates epithelial Wnt genes.

Background: Colitis-associated cancer (CAC) is one of the prognostic factors in inflammatory bowel disease (IBD), and prevention of CAC is a critical concern for patients with IBD. Component cells of the microenvironment, especially myofibroblasts, are known to affect tumor development, but the role of intestinal myofibroblasts (IMFs) in CAC has not been clarified. Here, we explored the role of IMFs in CAC and sought to identify candidate genes as novel therapeutic targets for the prevention of CAC.

Methods: We used the azoxymethane (AOM)/dextran sodium sulfate (DSS) model for dysplasia and CAC. Flow cytometry and RNA sequencing (RNA-seq) were performed to obtain an unbiased gene expression profile of IMFs. The transcriptome of significantly differentially expressed genes was analyzed by RNA-seq, quantitative reverse transcriptase polymerase chain reaction, and immunohistochemistry.

Results: Comparison of normal intestinal fibroblasts and IMFs revealed 1045 genes with significantly differential expression. Among them, we focused on tenascin-C (TNC; q = 0.00232, Log2(Fold Change) = 3.87). Tenascin-C gene expression was markedly increased in the dysplasia model compared with control and CAC model (P < 0.05). Tenascin-C protein was barely expressed in normal and nondysplastic mucosa but strongly expressed in the stroma around dysplastic lesions. Moreover, TNC surrounded and enclosed integrin αvβ3-positive microvessels. Administration of ATN-161, an antagonist of αvβ3-integrin, significantly suppressed tumorigenesis of CAC through inhibition of angiogenesis (P < 0.05).

Conclusions: In the early stages of CAC, TNC produced by IMFs affects tumor development via integrin αvβ3-mediated angiogenesis. Intestinal myofibroblasts might be a novel therapeutic target for preventing CAC.

Key Words: colitis-associated cancer, intestinal myofibroblasts, tenascin-C, angiogenesis, ATN-161
signaling through regulation of the MAP3 kinase Tp2. Wnt signaling plays a pivotal role in cellular developmental processes and CRC carcinogenesis; however, the involvement of IMFs in CAC development has not been fully elucidated.

In this study, we explored the role of IMFs in CAC development using RNA sequence (RNA-seq) analysis and sought to identify candidate genes that might be targeted to prevent CAC.

**MATERIALS AND METHODS**

**Animals**

Seven-week old C57Bl/6 WT mice (Japan SLC, Hamamatsu, Japan) were purchased. The method of induction of CAC is described previously. Briefly, mice were injected intraperitoneally with 10 mg/kg mutagenic azoxymethane (AOM; Sigma-Aldrich, Milan, Italy). Seven days later, 2.5% dextran sodium sulfate (DSS; MW: 5000 D; Wako, Osaka, Japan) was added to the drinking water for 7 days; this treatment was repeated for 2 or 3 additional cycles, with an interval of 14 days between each cycle. Control cohorts were given regular drinking water. At day 49 or 70, the colon was removed. Experimental chronic colitis was induced by sole administration of DSS without AOM following the same schedule as the AOM/DSS protocol described previously. The colon species were harvested at day 49.

**Inhibitory Effect of ATN-161 on CAC**

Starting at the time of the initial dose of DSS in the AOM/DSS protocol, 1 mg/kg ATN-161 (Ac-PHSCN-NH2 D; CAS 262438-43-7, MedKoo Biosciences, North Carolina, USA) or phosphate buffer saline (PBS), as a control, was administered by intraperitoneal injection 3 times a week for a 6-week period. Macroscopically visible tumors were counted with the aid of a Zeiss Discovery V8 stereomicroscope (Carl Zeiss, Jena, Germany) and sliding caliper. All mouse studies were performed according to protocols approved by the Hamamatsu University School of Medicine Animal Care Committees at the Center Animal Care facility (approval number: H27-069, H30-001).

**Flow Cytometry**

Flow cytometry was performed using a BD FACS Aria SORP flow cytometer (BD Bioscience, San Jose, CA), and analysis was performed using Kaluza Analysis software version 1.3. The distal colon was removed and extensively washed with 5 mM EDTA in Hank’s Balanced Salt Solution (HBSS; Invitrogen, Eugene, OR). The tissue was digested in 20 mL of RPMI-1640 (Invitrogen), 10 U of dispase (Life Technology, Waltham, MA), and 2000 U of collagenase D (Roche, Basel, Switzerland) for 60 minutes at 37°C. The supernatant was passed through a 70-µm cell strainer and incubated in ACK lysis buffer (Thermo Fisher Science, Rockford, IL) for 5 minutes at 47°C. The single-cell suspension was stained using the following antibodies: anti-CD45 tagged with PE-Cy7, and anti-PDGFRα tagged with PE, 7-AAD (eBioscience, San Diego, CA). The labeled cells were sorted, and total RNA was immediately extracted.

**Quantitative Real-Time PCR**

Total RNA was harvested with NucleoSpin RNA XS (TaKaRa, Shiga, Japan) according to the manufacturer’s instructions. cDNA was generated using PrimeScript RT reagent kit (TaKaRa). Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) was performed on a Thermal Cycler Dice Real Time System (TaKaRa) using TB Green Premix Ex Taq (TaKaRa). Primers used for qRT-PCR analysis are shown in Table S1. Results of quantitative polymerase chain reaction were normalized against the reference gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

**RNA Sequencing**

Using GeneData Expressionist for Genomic Profiling v9.1.4a, we established a bioinformatics pipeline for the detection of specific differentially expressed genes. Sequence reads from each sample were aligned to the reference mouse genome (UCSC mm10). Gene expression was normalized by the fragments per kilobase of exon per million mapped fragments (FPKM) method and filtered as previously described. Differentially expressed genes with statistical significance were identified through the following filtering steps: the default threshold was Log2(FoldChange) ≥1 and q value <0.05 (false discovery rate was adjusted with Benjamini-Hochberg method). KEGG pathway analyses were performed with DAVID databases. Raw data analysis was performed by TaKaRa, Inc.

**Immunohistochemical Staining**

Mouse colon specimens were fixed in formalin and embedded in paraffin. Paraffin-embedded sections (3-µm thick) were deparaffinized and rehydrated. For evaluation of TNC, sections were incubated with α-tenascin-C antibody (1:200, Abcam108930, Cambridge, UK) for 2 hours at 4°C.
room temperature (RT). Dako Envision+ System (Agilent Technologies) was used for detection. For CD31 visualization, sections were incubated with anti-CD31 antibody (1:50, Abcam28364) for 20 minutes at RT, and the Dako Envision+ System was used for detection. For integrin αvβ3 evaluation, sections were incubated with anti-Integrin αvβ3 antibody (sc-7312, Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C, and secondary antibody staining and detection were performed using the Histofine Mouse Stain Kit (Nichirei, Tokyo, Japan). Images were taken with an Olympus BX51 microscope (Olympus, Tokyo, Japan).

**Histological Assessment**

Sections were stained with hematoxylin and eosin (H&E). The histological score of inflammation and criteria of dysplasia/carcinoma were evaluated as described in a previous study.\(^1^5\)

Protein expression was quantified using Image J software after appropriate thresholding at subtracted RGB image of immunohistochemical (IHC)-3,3'-diaminobenzidine slides in 10 randomly selected fields at 1000× magnification.

For assessment of CD31 and integrin αvβ3, 15 fields with the greatest density of positively stained vessels were measured at 400× magnification in mice of the CAC model treated with PBS or ATN-161.

**Statistical Analysis**

Statistical analyses were performed using BellCurve for Excel (Social Survey Research Information Co., Ltd. Tokyo, Japan). Student t test was used to compare differences between two groups for normally distributed data. The size distribution of tumors in CAC model mice treated with ATN-161 or PBS was analyzed by the nonparametric Mann-Whitney U test. All results are presented as the mean ± SEM. Statistical significance was set as follows: *P < 0.05, **P < 0.01, ***P < 0.001.

**RESULTS**

**AOM/DSS Treatment Induced Dysplasia and Colitis-Associated Cancer in Distal Colon**

We investigated tumor formation in the various cycles of DSS administration as shown in Fig. 1A. After 2 cycles of DSS administration, a few macroscopic tumors developed. Histopathological examination of these tumors revealed colonic aberrant crypt foci (ACF) and dysplasia, which are putative precursor lesions of cancer (Fig. 1B, C), but no obvious carcinoma lesion was detected. However, after 3 cycles of DSS administration, macroscopic tumors developed predominantly in the distal colon, and these tumors were histologically shown to be carcinoma (Fig. 1B, C). Thus, we defined 3 cycles...
of DSS administration as the “CAC model,” and 2 cycles of DSS administration as the “dysplasia model.”

Colitis Induced an Increase in the Number and Activity of Intestinal Fibroblasts

We isolated intestinal fibroblasts from colon specimens by flow cytometry using a robust fibroblasts marker, PDGFRα. We used EpCAM, F4/80, and CD45 to eliminate epithelial cells, macrophages, and leukocytes, respectively (Fig. 2A). We assessed the purity of EpCAM+CD45-F4/80-PDGFRα- cells (epithelial cells) and EpCAM-CD45-F4/80-PDGFRα+ cells (fibroblasts) by qRT-PCR. The PDGFRα+ cells showed high gene expression of α-Sma, another fibroblast marker, whereas EpCAM+ cells did not. In contrast, EpCAM+ cells showed high expression of the E-cadherin gene, which represented epithelial cells, but PDGFRα+ cells did not (Fig. 2B). Based on

![Image](https://academic.oup.com/ibdjournal/article-figures/25/4/732/5230928)

**FIGURE 2.** Colitis induced an increase in the number and activity of intestinal fibroblasts. A, Cells were gated on 7-AAD−, CD45−, F4/80−, and EpCAM− to exclude dead, hematopoietic, macrophage, and epithelial cells, respectively. PDGFRα was used as a fibroblast marker, and PDGFRα+ cells were used for RNA extraction. B, Purity of EpCAM+ or PDGFRα+ cells was assessed by real-time RT-PCR for α-SMA (fibroblasts) and E-cadherin (epithelial cells). Results were normalized to GAPDH (n = 3). C, Quantification of the number of intestinal fibroblasts. Percentage of total cells in the mucous membrane of no treatment, AOM only, chronic colitis model, dysplasia model, and CAC model are shown. Data are presented as mean ± SE (n = 3). D, α-SMA mRNA expression in intestinal fibroblasts determined by qRT-PCR. Results were normalized to GAPDH. Data are presented as mean ± SE (n = 3).
these data, the purity of extracted intestinal fibroblasts was considered sufficiently high for the subsequent experiments. First, we analyzed the abundance of intestinal fibroblasts during the course of CAC development, from normal tissue to carcinoma, by flow cytometry. Administration of DSS without AOM was used as a chronic colitis model. The number of intestinal fibroblasts was significantly increased in colitis induced by DSS (chronic colitis, dysplasia, and CAC) but not in noncolitis (no treatment and AOM only) (Fig. 2C). Moreover, expression of αSMA-activated fibroblast marker was significantly elevated in colitis, especially in dysplasia and CAC (Fig. 2D). These results indicated that activated fibroblasts were increased by inflammation during the course of CAC development and strongly affected tumor promotion.

**Gene Expression Profiling Revealed TNC Was Overexpressed in Intestinal Myofibroblasts**

We analyzed gene expression profiling of intestinal fibroblasts from the dysplasia model to identify target genes with the potential to prevent CAC development. Injection of AOM only was used as a control. We used αSMA, fibroblast activation protein (FAP), PDGFRα, PDGFRβ, CXCL12, and vimentin as myofibroblast markers. In line with previous reports, expressions of αSMA, vimentin, CXCL12, and PDGFRα were significantly elevated in intestinal fibroblasts from the dysplasia model, whereas expressions of PDGFRβ and FAP were unchanged (Fig. 3A). These results indicated that intestinal fibroblasts of the dysplasia model are hyperactivated and transformed into myofibroblasts; we used this cell fraction as a source of IMFs in

![Gene Expression Profiling Revealed TNC Was Overexpressed in IMFs](https://academic.oup.com/ibdjournal/article/25/4/732/5230928)
the subsequent study. We performed gene expression profiling of IMFs compared with normal intestinal fibroblasts by RNA-seq analysis and found that the expression of 1045 genes was significantly changed (Fig. 3B). In KEGG pathway analysis, focal adhesion, ECM-receptor interaction, and PI3K-Akt signaling were highly ranked and significantly changed pathways (Fig. 3C). Among several candidate genes common to these 3 pathways (Fig. 3D), we focused on TNC, which encodes one of the extracellular matrix (ECM) proteins and is known to be involved in tumor progression and intractable inflammatory diseases.17

**High Tenascin-C Expression Was Detected in the Stroma of Dysplasia**

Tenascin-C gene expression of IMFs in the dysplasia model was 9-fold higher than that in the controls (Fig. 4A). IHC analysis revealed that TNC protein was expressed predominantly in the stroma around ACF and dysplastic lesions (Fig. 4B) but barely expressed in normal control and nondysplastic lesions (Fig. 4C). Tenascin-C protein surrounded and enclosed tumor cells in ACF and dysplasia. In contrast, TNC deposition was only detected at the surface of epithelia and above the basement membrane in carcinoma of the CAC model (Fig. 4D). Tenascin-C gene expression was markedly decreased in IMFs of the CAC model compared with the dysplasia model (Fig. 4A). These results implied that TNC gene expression and TNC protein localization were spatiotemporally different in distinct tumor stages and that TNC produced by IMFs may have a role in the early stage of CAC development.

**Coexpression of Tenascin-C and Integrin αvβ3—Positive Vessels in Dysplasia**

Because some studies have reported a potential role of TNC in tumor angiogenesis,18,19 we hypothesized that TNC promotes angiogenesis and accelerates CAC formation. To investigate whether TNC was involved in angiogenesis, we performed TNC and CD31 immunostaining. Mucosal microvessels were abundantly observed in the TNC-positive stroma of both dysplasia and carcinoma (Fig. 5A). Tenascin-C is known to interact with endothelial cells in part through integrin α2β1 and αvβ3.20 In particular, integrin αvβ3 is critical for angiogenesis and is upregulated in the angiogenic blood vessels of glioblastoma that are embedded in a TNC-rich matrix.21 To confirm the expression of integrin αvβ3 in tumor vessels in our mice, we performed IHC staining of integrin αvβ3. Vessels in TNC-expressing stroma of dysplasia and carcinoma lesions expressed high levels of integrin αvβ3, whereas the vessels of normal stroma did not (Fig. 5B). These results suggested that TNC might affect tumor angiogenesis through interaction with integrin αvβ3 during CAC progression.

**ATN-161 Administration Decreased CAC Development**

To investigate the role of TNC in tumor development, we used ATN-161, an inhibitor of α5β1 and αvβ3 integrins that was previously shown to block angiogenesis and metastasis in breast cancer.22 Because TNC protein and integrin αvβ3-positive vessels were expressed in dysplasia, which is known to be a precancerous lesion (Fig. 5B), we speculated that ATN-161 might prevent CAC development. To assess the inhibitory effect, we administered ATN-161 for 6 weeks to block TNC function during the early stages of CAC development (Fig. 6A). Administration of PBS was used as a control. Weight loss (Fig. 6B), colon length (Fig. 6C), and histological score (Fig. 6D) did not show any differences compared with
the control, indicating that the extent of inflammation was not changed. However, macroscopic tumor size dramatically decreased after ATN-161 administration (Fig. 6E). Tumor number (Fig. 6F) and tumor burden (Fig. 6G) were significantly reduced, and ATN-161 also reduced the size of tumors (Fig. 6H). These results indicated that ATN-161 suppressed tumor development, and this inhibitory effect was not due to suppression of inflammation.

**ATN-161 Decreased the Number of Integrin αvβ3-Positive Tumor Vessels and Suppressed Angiogenesis in CAC**

To examine whether ATN-161 affects angiogenesis, we analyzed microvessel density (MVD) and the number of integrin αvβ3-positive vessels in CAC. Tumor MVD significantly decreased in the ATN-161–treated compared with the controls (Fig. 7A, B). Integrin αvβ3-positive vessels were also markedly reduced in tumors treated with ATN-161 (Fig. 7C, D). These results suggested that ATN-161 suppressed the signaling of TNC/integrin αvβ3-mediated angiogenesis and consequently prevented CAC formation.

**DISCUSSION**

Inflammatory bowel disease ranks in the top 3 high-risk conditions for the development of CRC, and affected individuals have 10-fold greater risk of colon cancer compared with age-matched controls. Colitis-associated cancer is a critical pathological condition in IBD patients, and there is a compelling need for preventive therapies targeting CAC. In this study, we focused on ACF and dysplasia because intervention for CAC prevention would be most effective when initiated before the promotion of carcinoma.

Inflammation is assumed to be a main component in the etiology of CAC. Stromal cells such as IMFs are profoundly affected by the recurrent and persistent inflammation that influences CAC development. Recently, contradictory results have been reported in terms of the involvement of stromal cells in CAC development. In general, IKKβ-dependent activation of NF-κB in epithelial and cancer cells promotes tumorigenesis in many types of cancer, including CAC. Mesenchymal cell-specific genetic deletion of IKKβ reduces epithelial cell proliferation and decreases the innate immune responses that inhibit CAC formation. In contrast, fibroblast-specific IKKβ deletion promotes epithelial cell proliferation and enhances accumulation of CD4+Foxp3+ regulatory T cells that promote CAC tumorigenesis. These contradictory reports may reflect the plasticity and complex nature of stromal cells. Among the diverse stromal cells, we focused on IMFs because they are one of the central components of stromal cells and have been shown to have an important role in tumorigenesis. However, the transcriptome signature of IMFs remains largely unknown.

To elucidate a signature of IMFs involved in CAC development, we performed unbiased gene expression profiling of IMFs using flow cytometry and RNA-seq. Although some previous studies performed cell culture before RNA extraction to obtain an adequate sample for analysis, gene expression could be affected during cell culture. Therefore, we extracted RNA immediately after sorting by flow cytometry and analyzed transcriptomic differences by RNA-seq, which enables analysis of gene expression from a small amount of RNA. In KEGG pathway analysis, pathways in cancer, the Wnt signaling pathway, and the Hippo signaling pathway were extracted as candidate pathways (Fig. 3C). Wnt signaling is crucial for stem cell maintenance and tissue homeostasis in intestinal crypts, and aberrant Wnt activation is frequently observed in CRC. Hippo signaling also affects cell survival, proliferation, and regeneration in the intestine, and interactions with Wnt signaling have been reported to promote intestinal tumorigenesis. These gene
expression profiles indicate that IMFs have an integral role in tumor development; therefore, we attempted to identify target genes with the potential for preventing CAC development.

In this study, we focused on TNC among several candidate genes because it is known to be involved in several cancers and colitis. It has been reported that TNC is expressed at high levels during development and in response to injury and plays an important role in various cancers such as glioblastoma, breast cancer, prostate cancer, lung cancer, and CRC. In IBD patients, TNC serum level correlated with clinical and histological parameters of disease activity. Tenascin-C was recently identified as a gene associated with IBD in a genome-wide association study of African Americans with IBD. Mucosal expression of TNC was increased and contributed to modulation of intestinal inflammation in DSS-induced acute colitis and SAMP1/Yit mice, a model of spontaneous Crohn’s disease. The association between malignant potential and TNC expression in CRC has already been demonstrated. Recent studies revealed that TNC is a biomarker for metastasis of CRC. These findings suggested that TNC affects the late stage of cancer development. The role of TNC in CAC promotion, especially in the early stages, has not been previously

FIGURE 6. The peptide ATN-161 suppressed CAC development. A, Schematic presentation of PBS or ATN-161 treatment schedule in AOM/DSS mice. B–D, Body weight (B), colon length (C), and histological damage (D) were measured in CAC model mice treated with PBS or ATN-161 at day 70. Data are presented as mean ± SE (n = 5, 7). Abbreviations: NS, nonsignificant. E–G, Macroscopic view of colon (E), number of macroscopic tumors (F), and tumor burden (G). H, Size distribution of tumors in CAC model mice. Data are presented as mean ± SE (n = 5, 7).
demonstrated. Our data revealed overexpression of TNC mRNA in IMFs of dysplasia but not in CAC (Fig. 4A). In immunohistochemical analysis, TNC protein was more strongly expressed in ACF and dysplastic lesions compared with carcinoma (Fig. 4D). Tenascin-C deposition in precursor lesions of CAC suggests that stromal TNC produced by IMFs may affect the early stages of CAC development.

TNC has several potential roles supporting tumorigenesis, including tumor growth, metastasis, angiogenesis, and immune suppression. A recent report suggested that Ephrin-B2 induced by TNC acts as a pro-angiogenic molecule, whereas YAP, which is repressed by TNC in endothelial cells, enhances anti-angiogenic signaling via downregulation of pro-angiogenic proteins such as CTGF and Cyr61 in glioblastoma. Therefore, TNC has multiple roles in tumor angiogenesis and has been established as a specific marker of tumor vasculature. The binding of TNC to specific cell adhesion receptors induces intracellular signals that regulate cell behavior. Tenascin-C binds to a diverse array of receptors, including Syndecan-4, EDNRA/EGFR, and Annexin. Integrins are the best-known receptors associated with TNC; in particular, integrin heterodimers of \( \alpha_2/7/8/9\beta_1 \) and \( \alpha_v\beta_3/1/3/6 \) are known to play a role in TNC signaling. Integrin \( \alpha_v\beta_3 \) is highly expressed on activated endothelial cells and seems to be the most important integrin in angiogenesis. Integrin \( \alpha_v\beta_3 \) has been shown to induce angiogenesis by promoting endothelial cell migration, proliferation, and survival in malignant gliomas embedded in a TNC-rich matrix. Integrin \( \alpha_v\beta_3 \)-positive vessels have been detected in malignant glioma but not in normal brain and consequently have received attention as targets for anti-angiogenic therapy of cancers. We showed that integrin \( \alpha_v\beta_3 \)-positive vessels were present in dysplasia and CAC but not in normal mucosa (Fig. 5B). These results suggest that integrin \( \alpha_v\beta_3 \) in endothelial cells has an impact on tumor progression and angiogenesis through TNC. What is important in this discussion is that these experimental results are contradictory to previous reports in the context of TNC in CRC development. This contradictory result can be explained by previous observations that the developmental mechanism of CAC is different from that of sporadic CRC. Repeated and persistent inflammation is one of the most important effectors in CAC development and the most exclusive process of CAC. Islam et al reported that TNC expression was increased in severely inflamed areas of DSS-induced colitis. Moreover, Park et al reported that angiogenesis was induced in the early phases of hepatocellular carcinoma, which is closely associated with continuous inflammation and has been shown to have a relationship with TNC expression. These observations suggest that TNC expression and angiogenesis are closely associated with inflammation-induced cancer development in the early stages. We speculated that early-phase angiogenesis induced by TNC is the most important and distinctive feature of CAC pathogenesis.

The effects of ATN-161 administration have been reported in several animal models. The peptide ATN-161 blocked breast cancer growth and skeletal metastasis, with decreased phosphorylated mitogen-activated protein kinase signaling, microvessel density, and cell proliferation. Administration of ATN-161 in combination with 5-FU reduced growth and tumor cell proliferation in a CRC animal model and consequently suppressed liver metastasis and improved survival. Thus, ATN-161 has been suggested as a cancer therapeutic drug that might inhibit tumor angiogenesis, growth, and metastasis in several cancers associated with TNC expression. In the present study, we administered ATN-161 during the early stages of CAC progression and angiogenesis when TNC was highly expressed. The ATN-161 inhibited CAC promotion through suppression of angiogenesis with a significant decrease in integrin \( \alpha_v\beta_3 \)-positive tumor vessels (Figs. 6, 7). Considering these observations, we speculate that
ATN-161 inhibits angiogenesis by blocking the TNC-integrin αvβ3 interaction and thus suppresses the promotion of CAC.

There are some limitations in the current study. At present, there is no marker that can exclusively define myofibroblasts, and it is a very demanding task to discriminate myofibroblasts from other mesenchymal cells. We confirmed that most of the extracted cells were myofibroblasts that express several myofibroblast markers, including α-SMA, the most typical myofibroblast marker. However, it is possible that there were other intestinal mesenchymal cells in the clusters of analyzed myofibroblasts. Also, we did not show direct evidence of TNC involvement in CAC development. Because there are currently no useful direct inhibitors of TNC,35 we used a modulator of a TNC receptor to suppress the function of TNC in this study. Therefore, further studies to confirm the mechanism of TNC in CAC development are needed.

CONCLUSION

Our data highlight a role of IMFs in CAC development and show that TNC produced by IMFs is involved in the progression of CAC through integrin αvβ3-mediated angiogenesis. Targeting IMFs and TNC could be a novel therapeutic approach to the prevention of CAC.

SUPPLEMENTARY DATA

Supplementary data is available at Inflammatory Bowel Diseases online.

ACKNOWLEDGMENTS

We thank Ryo Horiguchi, Aya Kitamoto, and Yayoi Kawanaga for their expert technical advice.

REFERENCES