Abstract

Compared with prostaglandin E2, which has an established role in cancer, the role of the COX metabolite prostaglandin D2 (PGD2) in chronic inflammation leading to tumorigenesis is uncertain. In this study, we investigated the role of PGD2 in colitis and colitis-associated colon cancer (CAC) using genetically modified mice and an established model of inflammatory colon carcinogenesis. Systemic genetic deficiency in hematopoietic PGD synthase (H-PGDS) aggravated colitis and accelerated tumor formation in a manner associated with increased TNFα expression. Treatment with a TNFα receptor antagonist attenuated colitis regardless of genotype. Histologic analysis revealed that infiltrated mast cells strongly expressed H-PGDS in inflamed colon. Mast cell–specific H-PGDS deficiency also aggravated colitis and accelerated CAC. In contrast, treatment with a PGD2 receptor agonist inhibited colitis and CAC. Together, our results identified mast cell–derived PGD2 as an inhibitor of colitis and CAC, with implications for its potential use in preventing or treating colon cancer. Cancer Res; 74(11); 3011–9. ©2014 AACR.

Introduction

Colorectal cancer is the cause of death for 600 million people per year in the world and patient numbers are still increasing. Inherited factors and/or daily habits such as smoking or alcohol consumption, as well as bacterial/viral infections, cause bowel inflammation, the prolongation of which leads to tumorigenesis. Epidemiologic studies showed that patients with inflammatory bowel disease (IBD) have a predisposition for colorectal cancer and that the cancer risk is highly correlated with the duration and severity of inflammation (1).

In the chronically inflamed colon, infiltration of various types of immune cells such as macrophages, T lymphocytes, and mast cells is consistently observed (2). These immune cells produce a large quantity of inflammatory cytokines, chemokines, and prostaglandins (PG) that further promote inflammation. In the mouse colorectal cancer model, TNFα and one of the major prostaglandins, PGE2, induce tumorigenesis via an aberrant activation of NF-κB or Wnt/β-catenin signaling in epithelial cells (3, 4). Consistently, clinical studies have shown that treatment with an anti-TNFα antibody or a COX inhibitor is effective against the development of human colorectal cancer (5, 6). However, each COX metabolite PG or TNFα-induced cytokine possesses a pro- or an anti-inflammatory role and acts coordinately to govern inflammatory responses, eliminate foreign objects, and remodel tissues. Inhibitions of these upstream signaling potentially cause broad and serious side effects such as an increase in the susceptibility to infectious diseases or a loss of mucosal healing (7, 8). To develop a better treatment with fewer side effects to control chronic inflammation and subsequent tumorigenesis, it is indispensable to understand the pathophysiologic contribution of each immunomodulating factor.

PGD2 is one PG produced by activation of COX and PGD synthase. Mast cells, macrophages, and helper T (Th2)-type lymphocytes are reported to express hematopoietic PGD synthase (H-PGDS) and potentially produce PGD2. There is controversy over the pathophysiologic roles of PGD2. Several groups reported its pro-inflammatory properties, whereas PGD2 was shown to enhance the chemotactic activity of immune cells and to accumulate them in inflamed sites, causing the aggravation of OVA-induced asthma in mice or OVA-induced rhinitis in guinea pigs (9, 10). In contrast, other groups reported that PGD2 exerted anti-inflammatory properties, where it attenuated 2,4,6-trinitrochlorobenzene–induced contact hypersensitivity and zymosan-induced peritonitis in mice (11, 12). Our group also showed that PGD2 inhibits vascular permeability and inflammation in acute lung inflammation and implanted tumor models (13, 14). Thus, the role of PGD2 appears to vary with disease type and stage, and its contribution in chronic inflammation remains virtually unknown. It is indispensable to identify what types of cells produce and receive PGD2 in each disease model to understand its pathogenesis and develop a new therapeutic approach.
We focused here on the contribution of PGD₂ in persistent colitis and the subsequent development of colorectal cancer and found that tissue-infiltrating mast cell–derived PGD₂ has anti-inflammatory and antitumorigenic effects.

Materials and Methods

Materials
The following reagents were used: azoxymethane (AOM), collagenase (Wako); dextran sulfate sodium salt (DSS, 36–50 kDa, MP Biomedical, LLC); rabbit anti-H-PGDS antibody BW245C (Cayman Chemicals); anti-TNFα antibody (R&D Systems); interleukin (IL)-3 (PROSPEC); 4',6-diamidino-2-phenylindole (DAPI; Sigma); FBS (Nichirei Biosciences); random RT-primer, ReverTra Ace (Toyobo Engineering); WP9QY (Medical & Biological Laboratories); Platinum SYBR Green qPCR SuperMix-UDG and dispase (Invitrogen); anti-β-catenin and anti-Gr-1 antibodies (BD Biosciences); anti-histone H1 antibody (Santa Cruz Biotechnology); anti-CD68 antibody (Serotech); anti-CD4, anti-CD8, and anti-FcεRI antibodies (Biologrend); anti-c-kit antibody (eBioscience); and anti-F4/80 antibody (BMA Biomedicals).

Animals
H-PGDS–deficient (H-PGDS−/−) mice on a C57BL/6j background were generated and bred as previously described (15). Mast cell–deficient mice (KitW−/−) were kindly gifted by RIKEN BRC. The animal care and treatments were performed in accordance with the guidelines outlined within the Guide to Animal Use and Care from The University of Tokyo (Tokyo, Japan). All experimental procedures in this study were performed in accordance with the Animal Use and Care from The University of Tokyo (Tokyo, Japan). All experimental procedures in this study were approved by the institutional Animal Care and Use Committee at the University of Tokyo (P11-576 and P08-258).

Colitis and colitis-associated colon cancer
Eight- to 10-week-old mice received a single injection of the large intestine–specific carcinogen AOM [12 mg/kg body weight, intraperitoneally (i.p.)]. Five days later, mice were treated with DSS [2% (w/v) in drinking water ad libitum] for 4 days, followed by a 17-day-off period. This treatment cycle was repeated three times (Fig. 1A). The body weight and disease activity index (DAI) were measured twice a week. The DAI was determined as follows: 0, normal stool; 1, soft but formed; 2, very soft; 3, diarrhea; 4, dysenteric diarrhea. The colon was excised at each time point and used for the experiments (Supplementary Table S1). In some experiments, mice were injected with WP9QY (40 mg/kg, subcutaneously) or with BW245C (150 mg/L, i.p.) twice a day for each experimental period (Figs. 3 and 7).

Measurement of urinary tetranor-PGDM
The urinary concentration of a degraded product of PGD₂, tetranor-PGDM, was measured as previously described (13). Briefly, the collected urine samples were mixed with HCl to give a final pH of 3.0 and 5 ng of tetranor PGDM-d6 in ethanol (pH 2.0) was added as an internal control. Then, the samples were purified by solid-phase extraction and separated by high-performance liquid chromatography. An LCMS-TQ8030 triple-quadruple tandem mass spectrometer (Shimadzu) was used for the measurement. The results are expressed as nanogram of tetranor-PGDM per milligram of creatinine.

Hematoxylin and eosin and chloroacetate esterase staining
Colon tissue was fixed in 4% paraformaldehyde for 24 hours and embedded in paraffin, which was then cut into 4-μm-thick sections for each staining. To identify mast cells, chloroacetate esterase (CAE) staining was performed using naphthol-AS-D chloroacetate and counterstained with hematoxylin. The cell number was counted under a microscope (Nikon ACT-1C for DXM1200; Nikon) at 400-fold magnification in four randomly selected fields.

Analysis of nuclear β-catenin level
Colon tissue was treated with a low-salt homogenizing buffer containing 10 mmol/L KCl, 10 mmol/L HEPES, 0.1 mmol/L EDTA, 0.1 mmol/L EGTA, 0.5 mmol/L phenylmethylsulfonyl fluoride, 0.5 mmol/L dithiothreitol, 3 μg/mL aprotinin, 3 μg/mL leupeptin, and 1% Nonidet P-40 substituted and homogenized. After centrifugation, the supernatant (cytosol compartment) was removed. The pellet was treated with a high-salt homogenizing buffer, made by adding 400 mmol/L NaCl and 20 mmol/L HEPES to the other components of the low-salt buffer, and sonicated. After centrifugation, the supernatant (nuclear compartment) was collected. The protein samples were separated by 10% SDS-PAGE and transferred to polyvinylidene difluoride membranes. The membranes were labeled with mouse anti-histone H1 antibody (1:200) or mouse anti-β-catenin antibody (1:200) and β-actin antibody (1:1000) in blocking solution (5% nonfat dry milk, 3% bovine serum albumin, 0.2% Triton X-100) overnight at 4°C. After washing three times with washing buffer, the membranes were incubated with horseradish peroxidase–labeled antibody (1:2000) for 1 hour at room temperature. After thorough washing, the protein bands were visualized using the chemiluminescent reagent (Amersham Bioscience, Piscataway, NJ). The bands were normalized with β-actin and quantified using the densitometry software (ImageJ 1.37).
anti-β-catenin antibody (1:1,000). After secondary antibody labeling with goat anti-mouse IgG IRdye 800 antibody, the bands were detected by an Odyssey system (LI-COR Biosciences).

Reverse transcription PCR

Total RNA extraction was conducted using TRI reagent according to the manufacturer’s protocol. The first strand of cDNA was reverse-transcribed using random 9-mer oligonucleotide primers and ReverTra Ace. Quantitative PCR was performed on a LightCycler Nano system (Roche Diagnostics) with Platinum SYBR Green qPCR SuperMix-UDG and specific primers for each gene (Supplementary Table S2).

Immunohistochemistry

Paraffin section. Deparaffinized 4-μm-thick sections were incubated with 1% hydrogen peroxide in methanol for 30 minutes and then treated with 0.1% Triton X-100 and 3% bovine serum albumin (BSA) in PBS for 15 minutes. Primary antibody was treated overnight at 4°C. After washing, secondary antibody was treated for 3 hours, followed by reaction with an avidin–biotin peroxidase complex. After the reaction, sections were treated with 0.02% 3,3’-diaminobenzidine tetrahydrochloride solution containing 0.033% hydrogen peroxide. Then, counterstaining by hematoxylin was performed. The cell number was counted under a microscope (Nikon ACT-1C) at 400-fold magnification in four randomly selected fields.

Cryosection. The fixed colon was dehydrated in 30% sucrose for 24 hours and then frozen in Tissue-Tek O.C.T. Compound 4583 (Sakura Finetechnical Co. Ltd.). The 4-μm-thick cryosection was incubated with 0.05% Triton X-100 and 3% BSA in PBS for 30 minutes. Then, the primary antibody was treated overnight at 4°C. After washing, treatment with secondary antibody for 3 hours was carried out. Nuclei were labeled with DAPI (1 μg/mL) for 5 minutes. The images were photographed using an Eclipse E800 fluorescence microscope (Nikon). The Gr–1–positive neutrophil or F4/80 macrophage number was counted at 400-fold magnification in four randomly selected fields.

Bone marrow transplantation

Bone marrow cells were isolated from the humerus, femur, and tibia of either H-PGDS−/− or wild-type (WT) mice. Five-week-old recipient mice were subjected to 9 Gy of γ-irradiation and injected intravenously with 2.0 × 10⁶ bone marrow cells. Mice were used for experiments 6 weeks after reconstitution. Then, AOM/DSS treatment was started.

Isolation and adoptive transfer of bone marrow–derived mast cell

Bone marrow cells were obtained as described above. The cells were cultured in RPMI-1640 medium supplemented with 10% FBS, 100 U/mL of penicillin, 100 μg/mL of streptomycin, 125 ng/mL of amphotericin B, 100 μmol/L of 2-mercaptoethanol, and 10 ng/mL of recombinant mouse IL-3. Half of the medium was changed twice a week. After a 4-week incubation, more than 90% of the cells were stained positively with toluidine blue. The cells cultured for 6 to 10 weeks were used for bone marrow–derived mast cell (BMMC) injection. The 5-week-old Kitw-sh/w-sh mice were pre-injected intravenously with 5.0 × 10⁶ BMMCs. At 6 weeks after pre-injection, the mast cells were redistributed to the colon mucosa of the Kitw-sh/w-sh mice. Then, AOM/DSS treatment was started. In addition, 1.0 × 10⁶ BMMCs were injected intravenously every 1 week after AOM treatment.

Flow cytometric analysis

Colon tissues were cut into pieces and incubated in RPMI-1640 containing 2 mg/mL of collagenase and 0.3 mg/mL of dispase for 60 minutes at 37°C. Digested tissues were resuspended in 30% Percoll solution and centrifuged for 15 minutes at 1,300 rpm to remove undigested tissue. The resulting cell pellet was washed and resuspended in staining medium (PBS supplemented with 5% FBS) with fluorochrome-conjugated mAbs (1:100 each). After washing, fluorescence intensities were examined by flow cytometry (BD Accuri C6 Flow Cytometer, BD Biosciences). Colon-infiltrating neutrophils, macrophages, and mast cells were identified as CD45+ Gr-1−, CD45+ F4/80+−, and CD45+ FcεRI− c-kit+ cells, respectively.

Statistical analysis

Results are expressed as the mean ± SEM. Survival curves were evaluated by the log-rank test. The DAI was assessed by the Mann–Whitney test for comparison between two groups and by the Kruskal–Wallis test, followed by Dunn post hoc test for comparison between more than two groups. Other data evaluations were conducted using the unpaired Student t test for comparison between two groups and by one-way ANOVA, followed by Tukey test for comparison between more than two groups. P < 0.05 was regarded as significant.

Results

Hematopoietic prostaglandin D synthase deficiency exacerbates colitis

We first confirmed that there was no significant difference in morphologic appearance of colon tissue between H-PGDS naïve (WT) and H-PGDS−/− mice (Supplementary Fig. S1). Few WT mice died with the AOM/DSS treatment, whereas H-PGDS−/− mice exhibited a lower survival rate. This was obvious especially just after the second (days 21–25) or third (days 42–46) treatments of DSS (Fig. 1B).

After each DSS treatment, H-PGDS−/− but not WT mice presented with significant weight loss, and elevated DAI was observed in both genotype (Fig. 1C and D). At each time point, H-PGDS−/− mice presented greater DAI scores than did WT mice.

Hematopoietic prostaglandin D synthase deficiency exacerbates colitis-associated colon cancer

After the third round of DSS treatment, tumor had formed in both WT and H-PGDS−/− mice. Few WT mice died with the AOM/DSS treatment, whereas H-PGDS−/− mice exhibited a lower survival rate. This was obvious especially just after the second (days 21–25) or third (days 42–46) treatments of DSS (Fig. 1B).

After each DSS treatment, H-PGDS−/− but not WT mice presented with significant weight loss, and elevated DAI was observed in both genotype (Fig. 1C and D). At each time point, H-PGDS−/− mice presented greater DAI scores than did WT mice.
the colon length of H-PGDS−/− mice was about 15% shorter than that of WT mice. In WT mice, a few tumors were detected only at the end point of treatment (day 56; Fig. 2C). In H-PGDS−/− mice, several tumors had formed by day 42 and the tumor numbers increased toward the end of the study (Fig. 2C). Of interest, the tumor sizes at day 56 in both mice lines were comparable (WT: 1.97 ± 0.12 mm; H-PGDS−/− mice: 2.03 ± 0.13 mm; n = 6 each).

Given that PGD2 is produced in response to injurious stimuli and is quickly metabolized in vivo (half-life: ~1.5 minutes; ref. 16), it is difficult to accurately measure its content in tissues. We previously showed that the urinary concentration of a PGD2 metabolite, tetranor-PGDM, can be a stable index of PGD2 production in tissues. AOM/DSS treatment significantly increased the excretion of tetranor-PGDM in WT mice urine (Fig. 2D). In contrast, little tetranor-PGDM was detected in H-PGDS−/− mice even after the treatment. These observations suggest that PGD2 is produced during the disease progression and is dependent on H-PGDS activity.

Hematopoietic prostaglandin D synthase deficiency stimulates carcinogenic signaling

Aberrant activation of Wnt/β-catenin signaling is often observed in patients with colitis-associated colon cancer (CAC) and in CAC experimental models (17). Upon activation of Wnt signaling, β-catenin translocates into the nucleus and stimulates gene transcription, leading to carcinogenesis. Immunostaining showed that the DSS treatment stimulated nuclear translocation of β-catenin in colon epithelial cells (day 56) and H-PGDS deficiency accelerated it (Supplementary Fig. S2A). In Western blotting, there were no differences detected in the levels of nuclear β-catenin protein in the colons of WT and H-PGDS−/− at day 26 (n = 5–7, data not shown). However, its level in the colons of H-PGDS−/− mice, several tumors had formed by day 42 and the tumor numbers increased toward the end of the study (Fig. 2C). Of interest, the tumor sizes at day 56 in both mice lines were comparable (WT: 1.97 ± 0.12 mm; H-PGDS−/− mice: 2.03 ± 0.13 mm; n = 6 each).

Given that PGD2 is produced in response to injurious stimuli and is quickly metabolized in vivo (half-life: ~1.5 minutes; ref. 16), it is difficult to accurately measure its content in tissues. We previously showed that the urinary concentration of a PGD2 metabolite, tetranor-PGDM, can be a stable index of PGD2 production in tissues. AOM/DSS treatment significantly increased the excretion of tetranor-PGDM in WT mice urine (Fig. 2D). In contrast, little tetranor-PGDM was detected in H-PGDS−/− mice even after the treatment. These observations suggest that PGD2 is produced during the disease progression and is dependent on H-PGDS activity.

Hematopoietic prostaglandin D synthase deficiency stimulates carcinogenic signaling

Aberrant activation of Wnt/β-catenin signaling is often observed in patients with colitis-associated colon cancer (CAC) and in CAC experimental models (17). Upon activation of Wnt signaling, β-catenin translocates into the nucleus and stimulates gene transcription, leading to carcinogenesis. Immunostaining showed that the DSS treatment stimulated nuclear translocation of β-catenin in colon epithelial cells (day 56) and H-PGDS deficiency accelerated it (Supplementary Fig. S2A). In Western blotting, there were no differences detected in the levels of nuclear β-catenin protein in the colons of WT and H-PGDS−/− at day 26 (n = 5–7, data not shown). However, its level in the colons

Figure 2. H-PGDS deficiency exacerbates AOM/DSS-induced tumor formation. A, macroscopic view of a whole colon at day 49 in AOM/DSS-treated mice. The black line indicates the edge of the tumor. Scale bar, 1 cm. B, quantitative analysis of colon length. #, P < 0.05 compared with naïve WT mice; †, P < 0.05 compared with naïve H-PGDS−/−; §, P < 0.05 compared with WT mice at day 56. C, summarized data of the tumor number in the colon at days 42, 49, and 56 (n = 8–11). †, P < 0.05; ‡, P < 0.01 compared with WT mice. D, tetranor-PGDM level in urine. The results are expressed as nanogram per milligram creatinine (n = 5). * , P < 0.05 compared with WT mice at day 0.

Figure 3. Increased secretion of TNFα worsened the colitis in H-PGDS−/− mice. A, representative histopathologic sections (hematoxylin and eosin staining) of the colon in mice at day 26. Black arrow, loss of epithelial layer; #, area of severe immune cell infiltration. Scale bar, 200 μm. B, quantitative analysis of cytokines and COX-2 mRNA expression in the colon. Total RNA was extracted at days 0 and 26. The ratio of the cytokines and COX-2 expression to that of 18S rRNA is calculated, and results are expressed as a value relative to the ratio of WT mice (n = 6). †, P < 0.05; ‡, P < 0.01 compared with WT mice at day 26. C, typical pictures of the cross-section of the colon at day 4. Cross-sections were stained with hematoxylin and eosin. Scale bar, 100 μm. D, DAI at day 4. The TNFα receptor I antagonist WP9QY (40 mg/kg, s.c.) was treated twice a day (n = 8). †, P < 0.05 compared with vehicle-treated H-PGDS−/− mice.
of H-PGDS−/− was about 1.5-fold higher than that in WT mice at day 56 (Supplementary Fig. S2B). Previous studies showed that treatment with PGE2 accelerated the nuclear translocation of β-catenin in the human colon cancer cell line, LS-174T (18). In contrast, treatment with PGD2 (1 μmol/L for 2 hours) did not influence the nuclear β-catenin protein level in the human colon cancer cell line Caco-2 (β-catenin expression; no treatment, 0.57 ± 0.15; PGD2, 0.51 ± 0.07; n = 6 each). Other mediators hypersecreted under absence of H-PGDS are likely to stimulate these signaling processes secondarily.

Hypermethylation of tumor-suppressed genes by DNA methyltransferases (DNMT) is also implicated in carcinogenesis (19). There are three types of DNMTs, DNMT1, DNMT3a, and DNMT3b, which contribute to inflammation-induced carcinogenesis (20). As expected, the mRNA expressions of these three genes in the H-PGDS−/− colon were higher than those of the WT colon on day 26 (Supplementary Fig. S2C). These results suggest that H-PGDS deficiency stimulates tumor formation via stimulation of Wnt/β-catenin signaling and DNMTs.

**Increased secretion of TNFα worsens the colitis in hematopoietic prostaglandin D synthase–deficient mice**

After the second round of DSS treatment, the morphology of the inflamed colon was examined. In WT mice, infiltration of some immune cells into the lamina propria, damage of the epithelium, shortened crypts, and lack of epithelial cells were observed (Fig. 3A). H-PGDS deficiency worsened these manifestations (the black arrow indicates the loss of epithelium and # shows the immune cell infiltration; Fig. 3A). Next, mRNA expression levels of the pro-inflammatory cytokines IL-1β, TNFα, monocyte chemotactic protein-1 (MCP-1), and COX-2 were confirmed by reverse transcription PCR (RT-PCR). AOM/DSS treatment increased the mRNA expressions of these genes in inflamed colon tissues of both lines of mice (at day 26). As expected, H-PGDS deficiency elevated their expressions (for IL-1α = 0.13). In particular, the expression of TNFα in the H-PGDS−/− colon was elevated up to 4.2-fold that of WT.

TNFα signaling is crucial for AOM/DSS-induced colitis and tumor formation (3). It is possible that PGD2 attenuates the colitis and subsequent tumor formation by inhibiting TNFα signaling. Next, we explored the effect of a TNFα receptor 1 antagonist, WP9QY, on the colitis. Treatment with WP9QY (40 mg/kg s.c., twice a day) decreased the DAI at day 4 in both lines of mice (Fig. 5D; in WT mice, the score was decreased but not significant, P = 0.08). The scores for H-PGDS−/− mice were decreased to the similar level of WT mice. Consistently, histologic study revealed that WP9QY treatment ameliorated the immune cell infiltration to the lamina propria or the inflammation-induced thickened muscularis mucosa in H-PGDS−/− mice (day 4, Fig. 3C). These results indicate that the aggravated inflammation observed under H-PGDS–deficient condition is partly through hyperactivation of the TNFα signaling.

**Mast cells express hematopoietic prostaglandin D synthase**

H-PGDS is known to be expressed mainly in hematopoietic lineage cells. Indeed, hematopoietic lineage–specific H-PGDS deficiency aggravated the colitis (DAI, Fig. 4A; colon length, Fig. 4B) and tumor formation (Fig. 4C) in WT mice, and in contrast, hematopoietic lineage–specific reconstitution of H-PGDS repressed the severe manifestations in H-PGDS−/− mice.

We then attempted to identify H-PGDS–expressing cells. Immunostaining showed that c-kit- or FcεRI-positive mast cells strongly expressed H-PGDS in the inflamed WT colon (day 26, Fig. 5A and B). F4/80- or CD68-positive macrophages, CD4-positive T cells, CD8-positive T cells, and Gr-1-positive neutrophils did not express H-PGDS (Fig. 5C and D). Immunostaining showed that c-kit- or FcεRI-positive mast cells strongly expressed H-PGDS in the inflamed WT colon (day 26, Fig. 5A and B). F4/80- or CD68-positive macrophages, CD4-positive T cells, CD8-positive T cells, and Gr-1-positive neutrophils did not express H-PGDS (Fig. 5C and D). In addition, c-kit–positive mast cells and CD68-positive macrophages, but not Gr-1–positive neutrophils, expressed TNFα, which was elevated in the inflamed H-PGDS−/− colon (Supplementary Fig. S4).

The number of Gr-1–positive neutrophils, F4/80-positive macrophages, and CEA-positive mast cells that infiltrated the lamina propria and muscularis mucosa were significantly bigger in H-PGDS−/− mice than that of WT mice (day 26, Fig. 5D and Supplementary Fig. S5), especially the mast cell infiltration, which increased to about 2.4-fold of WT by H-PGDS deficiency. Similar observations were obtained in flow cytometric analysis as well (day 4, Supplementary Fig. SSD).

**Hematopoietic prostaglandin D synthase deficiency in mast cells exacerbates colitis and CAC**

In CAE staining, we found abundant mast cells residing around developing tumors (Fig. 6A) implying a direct
contribution of mast cells to carcinogenesis. However, Kit<sup>−/−</sup>/w<sup>−/−</sup> mice, which lack mature mast cells because of mutation in the c-kit gene (21), showed similar sensitivities against AOM/DSS treatment as did mast cell naïve WT mice (DAI: Fig. 6B, days 4 and 25; tumor formation: Fig. 6C, day 56; colon length: Fig. 6D, day 56). Reconstitution of the WT mast cells did not influence these scores in Kit<sup>−/−</sup>/w<sup>−/−</sup> mice. In contrast, reconstitution of the H-PGDS<sup>−/−</sup> mast cells markedly increased DAI, decreased colon length, and stimulated carcinogenesis (Fig. 6B–D). These data provided direct evidence that mast cell–derived PGD<sub>2</sub> protects against colitis and CAC.

DP signaling attenuates colitis and tumor formation induced by AOM/DSS

Given that stimulation of the PGD<sub>2</sub> receptor represents anti-inflammatory reactions in acute lung inflammation and peritonitis (11, 14), it is possibly beneficial against colitis and/or CAC. Supporting this idea, treatment with a DP agonist, BW245C (150 μg/kg i.p., twice a day), suppressed the DAI (Fig. 7B, days 6 and 27) and attenuated the tumor formation in both lines of mice (Fig. 7C, day 56). Consistently, treatment with BW245C attenuated the infiltration of immune cells (Fig. 7E, day 27) and thickening of the mucosa (Fig. 7A). The TNFα expression in the inflamed colon of H-PGDS<sup>−/−</sup> but not WT
mice was strongly suppressed (Fig. 7D). The nuclear translocation of β-catenin in epithelial cells was also suppressed in both mice (Supplementary Fig. S6, day 56).

**Discussion**

We showed here that mast cell–derived PGD₂ inhibits chemical-induced colitis and CAC and that this anti-inflammatory property of PGD₂ is at least partly due to its inhibitory effect on TNFα production.

Colorectal cancer often develops as a consequence of the repeated remission and recurrence of inflammation in patients with IBD. We used the mouse CAC model made by a single carcinogen (AOM) and a good prognosis in breast cancer and prostate cancer (30, 31). Experimental studies also showed a controversial role of mast cells in tumorigenesis. Mast cell deficiency (W/W<sup>v</sup> mice) decreased the susceptibility to intestinal tumorigenesis induced by 1,2-dimethylhydrazine or H-Ras–induced skin carcinogenesis (32, 33). Other studies showed that mast cells secrete a variety of tumor-promoting mediators, including TNFα, PGE₂, and IL-6 (34). In contrast, mast cells were reported to induce tumor apoptosis in APC<sup>Min/+</sup> mice (35). Our data showed that mast cell deficiency did not influence the severity of colitis/CAC (Fig. 6B–D). Similar observations were obtained in DSS-induced colitis of mast cell–deficient W/W<sup>v</sup> mice (36). Thus, the mast cell is assumed to possess both pro- and antitumorigenic properties and its contributions vary according to the type and stage of tumor.

H-PGDS deficiency accelerated the infiltration of mast cells as well as macrophages and neutrophils into the inflamed colon (Fig. 5D), which is accompanied by abnormal expressions of MCP-1 and TNFα (Fig. 3B). Both infiltrated mast cells and macrophages expressed TNFα in the inflamed colon (Supplementary Fig. S4A and S4B). We previously showed that MCP-1 was expressed mainly in infiltrated macrophages in implanted tumor (13). Mast cell–derived PGD₂ may restrict abnormal production of these cytokines by mast cell itself and macrophage, thus governing inflammatory responses. Further investigations are required to clarify this point.

Successive treatment with the DP agonist attenuated colitis/CAC (Fig. 7). Concordantly, there is a report showing that DP agonism inhibited TNBS-induced colitis in the rat (37). DP...
agonism stimulated IL-10 secretion from dendritic cells, which is a well-known anti-inflammatory cytokine in colitis (38). A degrading product of PGG2, 15d-PGJ2, also can diminish colitis in mice and have anti-tumor effects by activating PPARγ or inhibiting NF-kB signaling (11, 39). PGD2 signaling may thus interact with such anti-inflammatory signaling and control colitis/CAC.

In conclusion, we demonstrated that mast cell–produced PGD2 inhibited prolonged colitis and subsequent tumor formation by attenuating TNFα signaling. Our findings implicate the therapeutic potential of enhancing the PGD2 signaling for IBD and CAC.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: K. Iwanaga, T. Murata
Development of methodology: Y. Urade, T. Murata
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K. Iwanaga, T. Nakamura, S. Maeda, K. Aritake, Y. Urade

References