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Celecoxib and 2,5-dimethylcelecoxib inhibit intestinal cancer growth by suppressing the Wnt/β-catenin signaling pathway

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Key words

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We previously reported that celecoxib, a selective COX-2 inhibitor, strongly inhibited human colon cancer cell proliferation by suppressing the Wnt/β-catenin signaling pathway. 2,5-Dimethylcelecoxib (DM-celecoxib), a celecoxib analog that does not inhibit COX-2, has also been reported to have an antitumor effect. In the present study, we elucidated whether DM-celecoxib inhibits intestinal cancer growth, and its underlying mechanism of action. First, we compared the effect of DM-celecoxib with that of celecoxib on the human colon cancer cell lines HCT-116 and DLD-1. 2,5-Dimethylcelecoxib suppressed cell proliferation and inhibited T-cell factor 7-like 2 expression with almost the same strength as celecoxib. 2,5-Dimethylcelecoxib also inhibited the T-cell factor-dependent transcription activity and suppressed the expression of Wnt/β-catenin target gene products cyclin D1 and survivin. Subsequently, we compared the *in vivo* effects of celecoxib and DM-celecoxib using the *Mutyh*$^{-/-}$ mouse model, in which oxidative stress induces multiple intestinal carcinomas. Serum concentrations of orally administered celecoxib and DM-celecoxib elevated to the levels enough to suppress cancer cell proliferation. Repeated treatment with celecoxib and DM-celecoxib markedly reduced the number and size of the carcinomas without showing toxicity. These results suggest that the central mechanism for the anticancer effect of celecoxib derivatives is the suppression of the Wnt/β-catenin signaling pathway but not the inhibition of COX-2, and that DM-celecoxib might be a better lead compound candidate than celecoxib for the development of novel anticancer drugs.

Several human studies have suggested that aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) could prevent colorectal cancers.[1–7] These drugs are believed to induce cell cycle arrest and/or apoptosis and to inhibit angiogenesis through COX-2-dependent or independent mechanisms.[2,3] Among those, celecoxib was originally developed as a selective COX-2 inhibitor and is now used for the treatment of various forms of arthritis and pain. Moreover, it had been used for familial adenomatous polyposis (FAP) patients to prevent colorectal adenoma and adenocarcinoma,[6] until it was reported that long-term treatment with this drug was associated with an increase in cardiovascular thrombotic events.[8,9] Indeed, celecoxib may reduce prostacyclin production in blood vessels by suppressing COX-2, thereby leading to thrombotic events.[10]

Other studies have reported that 2,5-dimethylcelecoxib (DM-celecoxib), a celecoxib analog that has lost the ability to inhibit COX-2, also shows antitumor effects as strong as celecoxib does both *in vitro* and *in vivo* (prostate cancer and Burkitt's lymphoma).[11,12] If DM-celecoxib could exert an antitumor effect on colorectal cancer without inducing thrombotic events, this celecoxib analog may be more appropriate for the treatment of malignancies. However, the underlying mechanisms for the anticancer activity of these drugs are yet to be elucidated.

The Wnt/β-catenin signaling pathway is essential for the regulation of gene transcription during embryonic development; moreover, this pathway maintains the balance between cell proliferation and differentiation in intestinal crypts throughout adult life.[13–15] However, hyperactivation of the Wnt/β-catenin pathway triggered by the functional loss of adenomatous polyposis coli (APC) or over-accumulation of β-catenin can lead to cancer development.[16,17] This is owing to the fact that a number of β-catenin targeted genes are proto-oncogenes.[18–20] Indeed, most colorectal cancers have somatic mutations in APC or β-catenin.[13,21,22]

Non-steroidal anti-inflammatory drugs have been shown to inhibit the Wnt/β-catenin signaling pathways.[23–28] We reported that celecoxib strongly inhibited the proliferation of human colon cancer cells by suppressing the Wnt/β-catenin signaling pathway through enhanced degradation of T-cell factor 7-like 2 (TCF7L2),[26,27] a key transcription factor.[13,19]

Here, using *in vitro* and *in vivo* systems, we explored the potential application of celecoxib or DM-celecoxib for the treatment of colon cancer. First, we compared the antitumor effects of celecoxib and DM-celecoxib on the human colon cancer cell lines HCT-116 and DLD-1, in which the Wnt/β-catenin signaling pathway is constitutively active, and analyzed downstream mediators of the aforementioned pathway. Subsequently, we examined the effect of these compounds on intestinal cancer development in *Mutyh*-deficient (*Mutyh*$^{-/-}$) mice, which lack the mammalian DNA glycosylase MutY homolog (MUTYH) and develop multiple intestinal cancers in the presence of oxidative stress (likely caused by over-activation of the Wnt/β-catenin signaling pathway).[29–33]

Materials and Methods

Compounds and antibodies. Celecoxib (MW 381.37) was kindly provided by Pfizer Inc. (New York, NY, USA). 2,5-Dimethylcelecoxib (MW 395.40) was synthesized as previously described.[12,28] MG132 was purchased from the Peptide Institute (Osaka, Japan). The monoclonal anti-TCF7L2 antibody was purchased from Merck Millipore (Billerica, MA, USA). The monoclonal anti-cyclin D1 antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The polyclonal anti-survivin antibody was purchased from R&D Systems (Minneapolis, MN, USA). The monoclonal anti-GAPDH antibody was purchased from Abcam (Cambridge, UK). The monoclonal anti-β-tubulin antibody was purchased from Calbiochem (Darmstadt, Germany).

Cell culture. Human colon cancer cell lines HCT-116 (expressing wild-type APC and mutant β-catenin) and DLD-1 (expressing mutant APC and wild-type β-catenin) were cultured in DMEM (Sigma, St. Louis, MO, USA) supplemented with 10% FBS, 100 U/mL penicillin G, and 0.1 μg/mL streptomycin. HCT-116 cells were obtained from Riken BRC (Tsukuba, Japan) and DLD-1 cells were from JCRB cell bank (Osaka, Japan).

Cell proliferation assay. HCT-116 or DLD-1 cells were seeded into 24-well plates (5×10^4 cells/well) and treated with various concentrations of celecoxib or DM-celecoxib for the indicated periods. Cells were harvested by addition of trypsin/EDTA and counted using an automated cell counter (TC10; Bio-Rad, Tokyo, Japan).

Caspase-3 activity assay. Caspase-3 activity was assayed using a cysteine protease protein 32/caspase-3 colorimetric protease assay kit (Medical and Biological Laboratories, Nagoya, Japan), following manufacturer's instructions.

Western blot analysis. Samples were separated with 12% SDS-PAGE, and then transferred to a PVDF membrane using a semidry transfer system (1 h at 12 V). Proteins of interest were detected after incubation with primary and secondary antibodies, and were visualized using a detection reagent (LumiGLO; Cell Signaling Technology, Danvers, MA, USA). Densitometric analysis was carried out using ImageJ software (version 1.49; NIH, Bethesda, MD, USA).

Luciferase reporter assay. TOPFlash (a TCF reporter plasmid) and FOPFlash (a TOPflash negative control) were purchased from Upstate Biotechnology (Lake Placid, NY, USA). Cells were cotransfected with luciferase reporter plasmids and pRL-SV40, a *Renilla* luciferase expression plasmid (transfection efficiency control), using Lipofectamine Plus reagent (Invitrogen, Rockville, MD, USA). After 24 h, cells were stimulated with celecoxib or DM-celecoxib for the indicated periods. Luciferase activity was determined with a luminometer (Lumat LB 9507; Berthold Technologies, Barsinghausen, Germany) and normalized against *Renilla* luciferase activity.

Measurement of celecoxib and DM-celecoxib plasma concentrations. Murine blood samples were collected by cardiac puncture at the indicated times and plasma was isolated by centrifugation at 500*g* for 15 min. The plasma concentrations of celecoxib and DM-celecoxib were determined by a reverse phase HPLC system (2695; Waters, Milford, MA, USA), as previously described[34] with a slight modification. Briefly, the plasma samples (200 μL) containing 500 ng caffeine (Wako Pure Chemical Industries Ltd., Osaka, Japan) as an internal standard were mixed with 200 μL chloroform. After mixing, the solution was centrifuged at 13 000*g* for 5 min, and the organic phase was then separated and evaporated. The obtained residue was dissolved in 80 μL mobile phase (methanol:water = 72:28, v/v) and an aliquot (50 μL) was then injected into a column (TSKgel ODS-80Ts; Tosoh, Tokyo, Japan) for separation. The running time was 10 min, and the flow rate 1.0 mL/min. Samples were measured with a UV detector operating at 254 nm. A calibration curve was prepared by plotting the ratios of celecoxib or DM-celecoxib areas normalized to that of the internal standard.

Intestinal tumor model. Intestinal tumors (adenomas and carcinomas) were induced in *Mutyh*$^{-/-}$ mice by a method previously reported.[29,35,36] Briefly, $KBrO_3$ dissolved in water at a concentration of 2 g/L was given to 4-week-old mice for

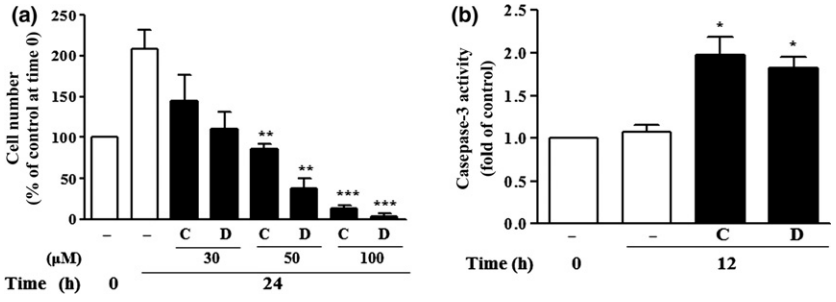

Fig. 1. Celecoxib and 2,5-dimethylcelecoxib (DM-celecoxib) inhibit proliferation and induce apoptosis in the human colon cancer cell line HCT-116. (a) Cell proliferation assay. HCT-116 cells were seeded onto a 24-well plate and treated with different concentrations of celecoxib (C) or DM-celecoxib (D) for 24 h. Cell numbers were enumerated and are shown as the percentage of the control level at time 0. The results represent the mean ± SEM of three independent experiments. (b) Caspase-3 activity. Cells were treated with or without celecoxib (100 μM) or DM-celecoxib (50 μM) for 12 h before caspase-3 activity was tested. Data are represented as percentage of the control level at time 0. Values are expressed as mean ± SEM of three independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ *versus* control.

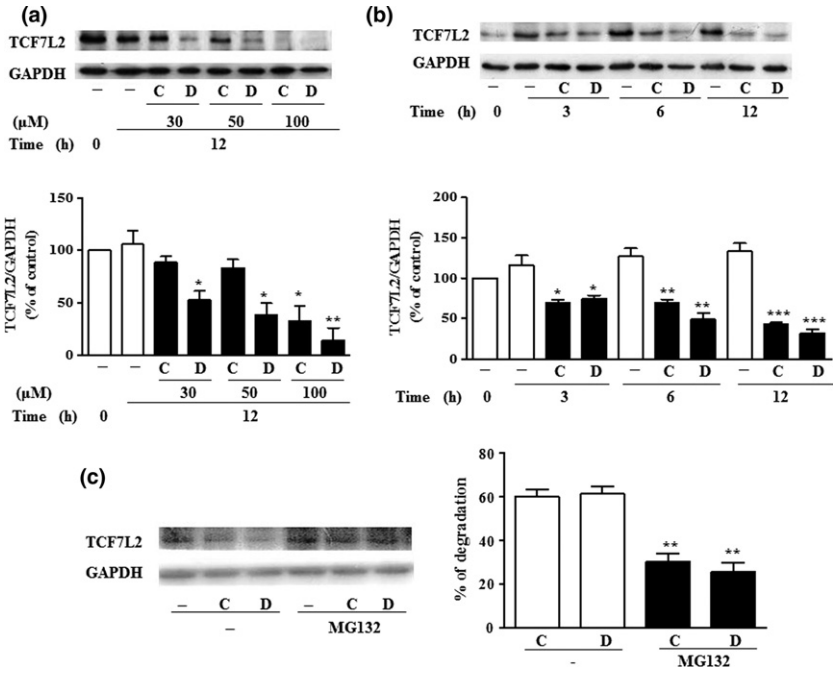

Fig. 2. Celecoxib and 2,5-dimethylcelecoxib (DM-celecoxib) induce T-cell factor 7-like 2 (TCF7L2) degradation in HCT-116 cells. (a) Dose dependency. Cells were treated with the indicated concentrations of celecoxib (C) or DM-celecoxib (D) for 12 h. (b) Time course. Cells were treated with or without celecoxib (100 μM) or DM-celecoxib (50 μM) for the indicated periods. Samples were subjected to Western blot analysis using anti-TCF7L2 and anti-GAPDH antibodies. Protein bands were quantified and are shown as percentage of the control at time 0. (c) Effect of proteasome inhibitor MG132. Cells pretreated with 10 μM MG132 for 1 h were incubated with or without celecoxib (100 μM) or DM-celecoxib (50 μM) for 6 h. Protein bands were quantified and are shown as the percentages of the degraded amounts. Data are expressed as the mean ± SEM of three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *versus* control.

12 weeks. At 16 weeks of age, mice were randomly divided into five groups (male:female = 1:1). The indicated amounts of celecoxib or DM-celecoxib suspended in a 0.25% methylcellulose solution were given orally to mice in the test groups for 5 days/week over 4 weeks. Control mice received the vehicle only (methylcellulose). The body weight of the mice was monitored weekly. At 20 weeks of age, all mice were killed and blood and intestinal samples were collected. Blood cell counts were determined by a Celltac-α MEK-6358 (Nihon Kohden, Tokyo, Japan). Intestines were fixed in 4% formaldehyde, and the tumors were scrutinized under a microscope. Images of the tumors were analyzed using ImageJ software. For immunohistochemical analysis, tumors of 1.0–2.0 mm diameter that had developed 3.0–5.0 cm distal from the pylorus, were resected from formaldehyde-fixed intestines. Samples were embedded in paraffin and subjected to immunohistochemical staining. Briefly, the sections were incubated with the anti-TCF7L2 antibody (1:1000 dilution), the anti-cyclin D1 antibody (1:100 dilution), or anti-survivin antibody (1:100 dilution) overnight at 4°C, followed by incubation with the secondary antibody

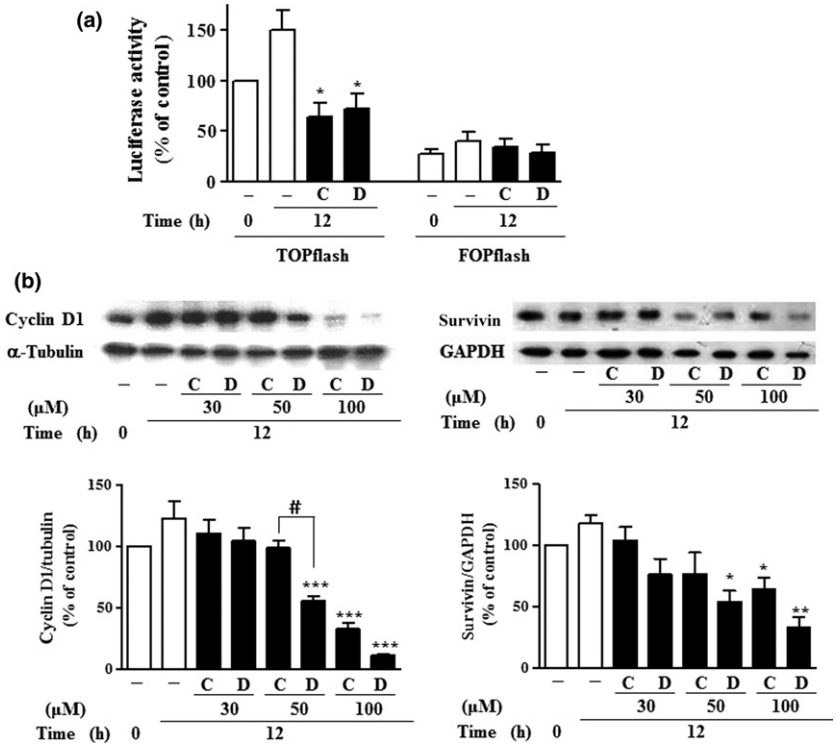

Fig. 3. Celecoxib and 2,5-dimethylcelecoxib (DM-celecoxib) suppress T-cell factor-dependent transcription in HCT-116 colon cancer cells. (a) Effects of celecoxib (C) and DM-celecoxib (D) on T-cell factor-dependent transcriptional activity. TOPFlash or FOPFlash plasmids were cotransfected with pRL-SV40 into HCT-116 cells. After 24 h of incubation, cells were stimulated with celecoxib (100 μM) or DM-celecoxib (50 μM) for 12 h. Luciferase activity is shown as the percentage of the control level at time 0. (b) Effects of celecoxib and DM-celecoxib on the expression of Wnt/β-catenin signaling pathway target gene products. Cells were treated with or without celecoxib (100 μM) or DM-celecoxib (50 μM) for 12 h. Protein samples were subjected to Western blot analysis using anti-cyclin D1 (left panel) or anti-survivin (right panel) and anti-GAPDH antibodies. Protein bands were quantified and are shown as the percentage of the control level at time 0. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *versus* control. $\#P < 0.05$.

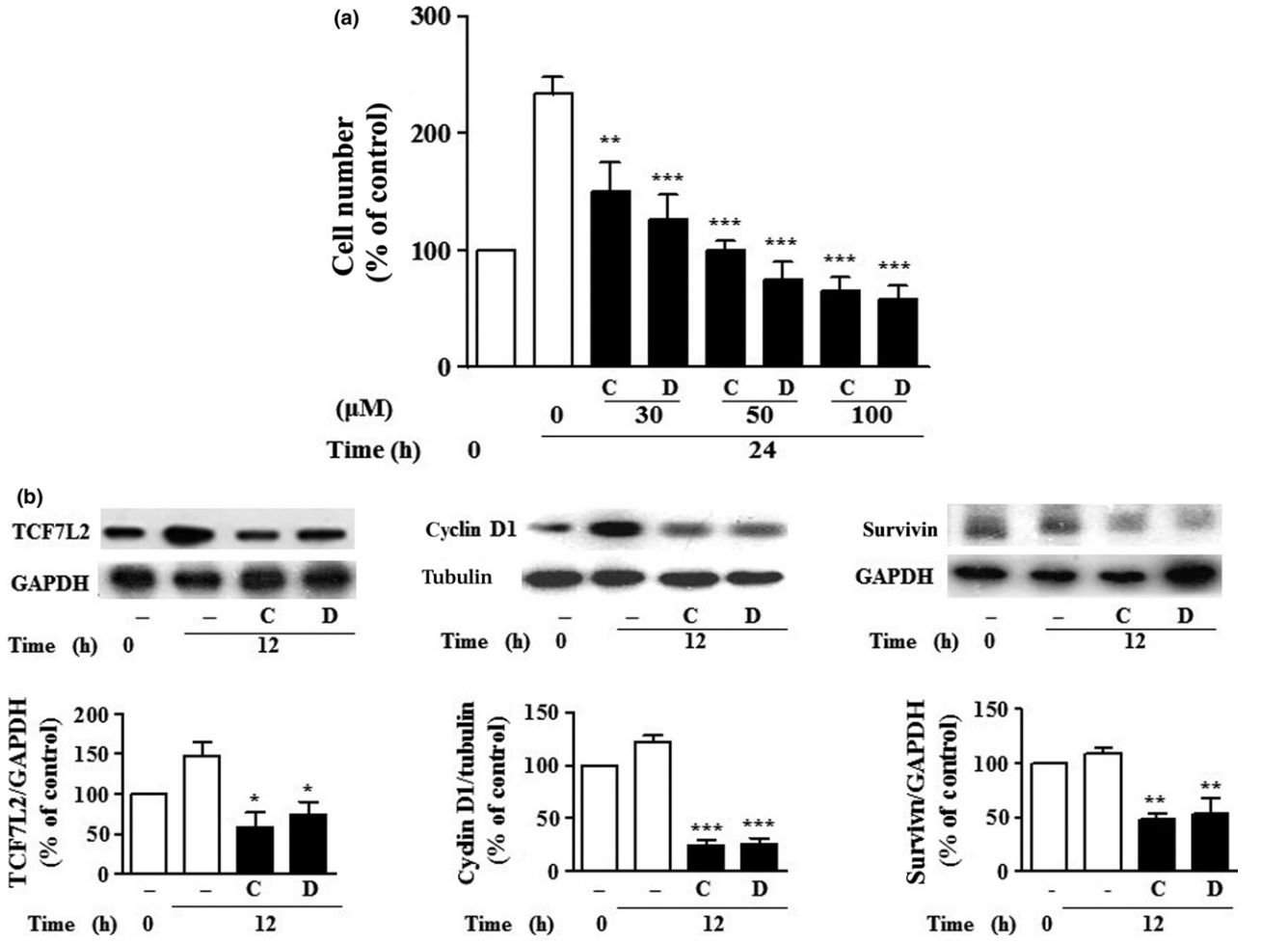

Fig. 4. Effect of celecoxib and 2,5-dimethylcelecoxib (DM-celecoxib) in DLD-1 colon cancer cells. (a) Cell proliferation assay. DLD-1 cells were treated with the indicated concentrations of celecoxib (C) or DM-celecoxib (D) for 24 h. Cells were counted and are shown as a percentage of the control level at time 0. (b) Effect of celecoxib and DM-celecoxib on the Wnt/β-catenin signaling pathway related proteins. DLD-1 cells were treated with celecoxib (100 μM) or DM-celecoxib (50 μM) for 12 h. Protein samples were subjected to Western blot analysis using anti-T-cell factor 7-like 2 (TCF7L2) (left), anti-cyclin D1 (center), or anti-survivin (right) antibodies. Protein bands were quantified and are shown as the percentage of the control at time 0. Values are expressed as mean ± SEM of three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *versus* control.

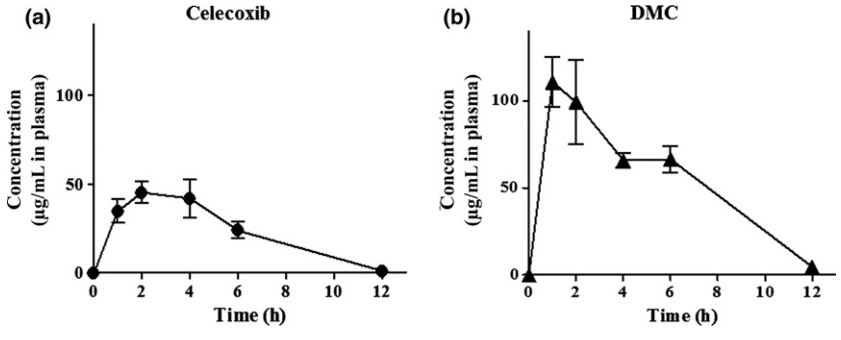

Fig. 5. Plasma concentrations of celecoxib and 2,5-dimethylcelecoxib (DM-celecoxib). Celecoxib (a) or DM-celecoxib (DMC) (b) were given to mice orally (150 mg/kg). Blood samples were collected at the indicated times and prepared for HPLC analysis. Values are expressed as means ± SEM (n = 3).

(Histofine; Nichirei, Tokyo, Japan) for 1 h. The sections were then analyzed with a Biozero microscope (Keyence, Osaka, Japan). For Western blot analysis, mucosal strips (2 cm to the bottom, from the pylorus) were homogenized in Laemmli's sample buffer immediately after resection. The same amount of proteins underwent electrophoresis and Western blotting.

Statistical analyses. Statistical analyses were carried out using ANOVA with Tukey's multiple comparison test (GraphPad Prism 5.0; GraphPad Software, La Jolla, CA, USA). P-values of <0.05 were considered statistically significant.

Ethics statement. This study protocol was approved by the Committee of Ethics on Animal Experiments at Kyushu University (Fukuoka, Japan; permit no. A22-046-0). Animal handling and procedures were in compliance with the Guidelines for Animal Experiments, Kyushu University, the Law (no. 105), and Notification (no. 6) of the Japanese Government and

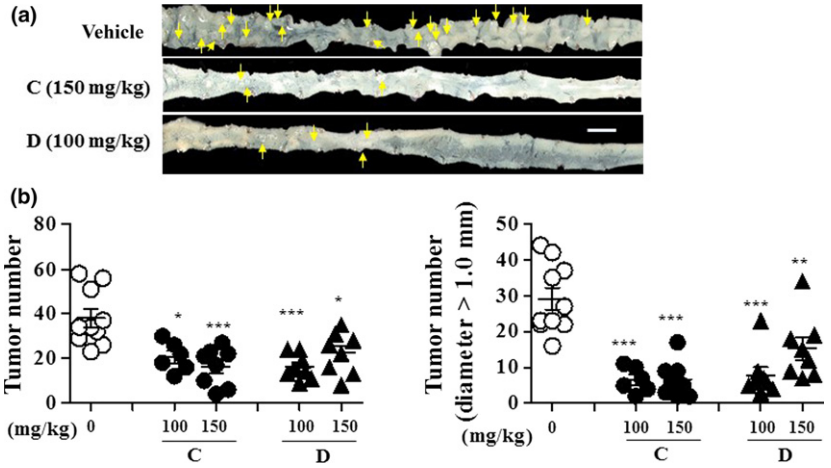

Fig. 6. Effect of celecoxib and 2,5-dimethylcelecoxib (DM-celecoxib) on intestinal tumor development induced by $KBrO_3$ in *Mutyh*$^{-/-}$ mice. (a) Proximal region of the small intestine. Arrows indicate tumors >1.0 mm in diameter. Scale bar = 1 cm. (b) Tumor number. Total tumor number (left) and number of tumors >1.0 mm in diameter (right) were plotted for each mouse treated with either vehicle, celecoxib (C), or DM-celecoxib (D). Horizontal bars indicate the mean of each group. *P < 0.05, **P < 0.01, ***P < 0.001 versus vehicle-treated mice.

Table 1. Effects of celecoxib and 2,5-dimethylcelecoxib (DM-celecoxib) on blood cell counts and body weight in mice treated for 4 weeks

	WBC ($\times 10^2$ cells/μL)	RBC ($\times 10^4$ cells/μL)	Hb (g/dL)	Plt ($\times 10^4$ cells/μL)	ΔBW (g)
Vehicle (n = 10)	54.9 ± 10.3	675.1 ± 62.1	10.6 ± 0.7	66.4 ± 4.9	7.22 ± 0.51
Celecoxib (mg/kg)					
100 (n = 6)	43.2 ± 9.5	914.7 ± 94.3*	13.6 ± 1.5*	67.7 ± 3.4	5.83 ± 0.75
150 (n = 8)	46.2 ± 9.0	903.3 ± 27.8*	13.2 ± 0.5	64.6 ± 3.0	7.39 ± 0.67
DM-celecoxib (mg/kg)					
100 (n = 8)	66.2 ± 10.1	805.9 ± 44.3	12.2 ± 0.8	58.0 ± 5.5	5.45 ± 0.89
150 (n = 8)	47.4 ± 6.4	769.8 ± 62.9	11.1 ± 0.6	67.7 ± 4.0	6.93 ± 0.45

*P < 0.05 *versus* vehicle-treated mice. Celecoxib, DM-celecoxib, or vehicle (methylcellulose) were given orally for 4 weeks. Data represent means ± SEM. Statistical significance was determined by one-way ANOVA with Dunnett's multiple comparison test. ΔBW, body weight change from 16 to 20 weeks; Hb, haemoglobin; Plt, platelets; RBC, red blood cells; WBC, white blood cells.

NIH guidelines (Guide for the Care and Use of Laboratory Animals). All surgical operations were undertaken under inhaled isoflurane anesthesia and all efforts were made to minimize animal suffering.

Results

Celecoxib and DM-celecoxib inhibit proliferation and induced apoptosis in human colon cancer cell line HCT-116. First, we examined the effect of celecoxib and DM-celecoxib on the proliferation and apoptosis of the human colon cancer cell line HCT-116, which expresses the wild-type APC and a mutant form of β-catenin. Both compounds inhibited HTC-116 proliferation in a dose-dependent manner (Fig. 1a). As we previously reported,[26] celecoxib induces apoptosis, assessed by caspase-3 activity measurement. As shown in Figure 1(b), DM-celecoxib was also able to significantly elevate caspase-3 activity.

Celecoxib and DM-celecoxib accelerate TCF7L2 degradation in HCT-116 cells. We previously reported that celecoxib induced degradation of TCF7L2, a key transcription factor in the Wnt/β-catenin signaling pathway, in HCT-116 cells.[26,27] As shown in Figure 2(a,b), not only celecoxib but also DM-celecoxib suppressed TCF7L2 expression in dose- and time-dependent manners. As the effect of 50 μM DM-celecoxib was almost comparable with 100 μM celecoxib, we mainly used these concentrations in the following experiments. Next, we examined the effect of the proteasome inhibitor MG132 on celecoxib- or DM-celecoxib-induced TCF7L2 reduction. Cells were treated with or without 10 μM MG132 for 1 h and then incubated in the presence or absence of 100 μM celecoxib or 50 μM DM-celecoxib for 6 h. As shown in Figure 2(c), pretreatment with MG132 significantly attenuated the effects of celecoxib and DM-celecoxib, indicating that both compounds accelerated the proteasome-dependent degradation of TCF7L2.

Celecoxib and DM-celecoxib inhibit TCF-dependent transcription in HCT-116 cells. As celecoxib and DM-celecoxib induced TCF7L2 degradation in HCT-116 cells, we examined the effects of celecoxib and DM-celecoxib on TCF-dependent transcription activity using the TOPflash assay. Celecoxib strongly inhibited TOPflash activity without affecting FOPflash (negative control) activity, confirming our previous results.[26] 2,5-Dimethylcelecoxib also showed a similar response (Fig. 3a). These results suggest that not only celecoxib but also DM-celecoxib inhibit the transcription activity of the Wnt/β-catenin signaling pathway target genes through TCF7L2 degradation.

Indeed, both celecoxib and DM-celecoxib suppressed the expressions of cyclin D1 and survivin (Fig. 3b), two representative Wnt/β-catenin signaling pathway target gene products, in a dose-dependent manner, whereas the effect of DM-celecoxib on cyclin D1 was slightly stronger than that of celecoxib.

Effects of celecoxib and DM-celecoxib on DLD-1 cells. We next examined whether celecoxib and DM-celecoxib were able to inhibit cell proliferation in DLD-1 cells, expressing wild-type β-catenin and a mutant form of APC. Both compounds clearly suppressed cell proliferation in a dose-dependent manner (Fig. 4a), similar to that observed in HCT-116 cells. Moreover, similar to their effects on HCT-116 cells, both compounds markedly reduced the expression levels of TCF7L2, cyclin D1, and survivin, in DLD-1 cells (Fig. 4b).

Effect of celecoxib and DM-celecoxib on oxidative stress-induced cancers in Mutyh$^{-/-}$ mice. We then carried out *in vivo* experiments. First, we examined whether, after oral administration, the plasma concentrations of celecoxib and

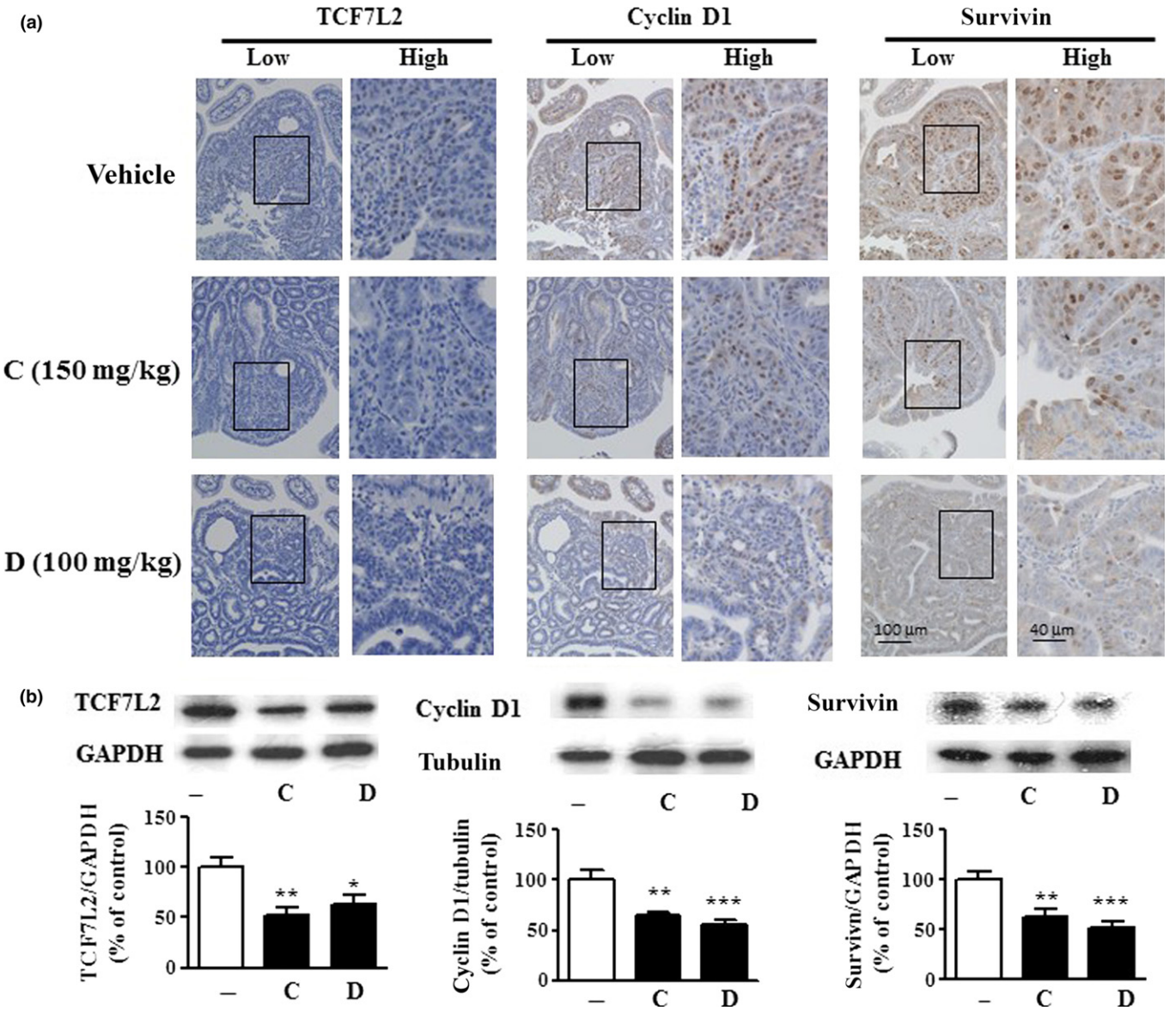

Fig. 7. Immunohistochemical and Western blot analyses of intestinal tumors induced by $KBrO_3$ in *Mutyh*$^{-/-}$ mice. (a) Immunostaining of intestinal tumors. Tumor samples prepared from the small intestines of mice treated with vehicle, celecoxib (C; 150 mg/kg), or 2,5-dimethylcelecoxib (DM-celecoxib [D]; 100 mg/kg) were stained with anti-T-cell factor 7-like 2 (TCF7L2), anti-cyclin D1, or anti-survivin antibodies. High magnification pictures correspond to the areas surrounded by black squares in low magnification pictures. Scale bar = 100 μm (low magnification picture) and 40 μm (high magnification picture). (b) Western blot analysis. Protein samples prepared from the small intestines of mice treated with vehicle, celecoxib (150 mg/kg), or DM-celecoxib (100 mg/kg) (n = 6, each group) were analyzed by Western blotting. Band densities are shown as percentages of the controls (vehicle). Values are represented as the mean ± SEM of individual samples. *P < 0.05, **P < 0.01, ***P < 0.001 *versus* vehicle.

DM-celecoxib reached levels to show antiproliferative effects on tumor cells. We measured the drug concentrations in wild-type C57BL/6J mice using an HPLC system. The concentration of celecoxib reached the maximal level (45.3 ± 6.3 μg/mL; n = 3) 2 h after treatment; the concentration of DM-celecoxib reached the maximal level (110.7 ± 14.3 μg/mL; n = 3) more rapidly, within 1 h of treatment (Fig. 5). Because the EC_{50} values of celecoxib and DM-celecoxib calculated from the *in vitro* antiproliferative assay (Figs 1a,4a) were approximately 15–19 μg/mL and 12–16 μg/mL, respectively, we hypothesized that the plasma concentrations of these compounds may be high enough to induce antiproliferative effects *in vivo*.

To investigate the *in vivo* effects of celecoxib and DM-celecoxib, we used mice deficient for MUTYH (*Mutyh*$^{-/-}$), an enzyme that prevents the formation of oxidative stress-induced DNA damage. MUTYH deficiency has been associated with the development of colorectal adenomas and carcinomas in humans.[30–32] We previously reported that the occurrence of oxidative stress-induced carcinomas in the small intestine was dramatically increased in *Mutyh*$^{-/-}$ mice compared to normal mice.[29,35,36] Twelve weeks of treatment with 0.2% $KBrO_3$, a strong oxidant, induced the development of numerous intestinal carcinomas in *Mutyh*$^{-/-}$ mice.[30,35,36] To evaluate the effect of the two compounds on these carcinomas, celecoxib, DM-celecoxib, or the vehicle were given orally to *Mutyh*$^{-/-}$ mice for 4 weeks. Treatment with celecoxib or DM-celecoxib markedly reduced the number of intestinal carcinomas (Fig. 6), and this was more pronounced in the case of large carcinomas with a diameter >1.0 mm (Fig. 6b).

We also examined whether long-term treatment with celecoxib and DM-celecoxib showed adverse effects on general animal condition. For this purpose, we measured mice body weight and peripheral blood cell counts, and we observed their general appearance and activity. Treatment with celecoxib or DM-celecoxib did not affect their appearance, activity, white blood cell count, platelet numbers, or body weight of mice. Red blood cell count and hemoglobin concentrations tended to be elevated after celecoxib or DM-celecoxib treatment, but the changes were not statistically significant (Table 1).

Immunohistochemical analysis showed that treatment with celecoxib or DM-celecoxib significantly decreased the protein expression levels of TCF7L2, cyclin D1, and survivin, consistent with the *in vitro* experiments (Fig. 7a) and these results were confirmed by Western blot analyses of tumor samples (Fig. 7b).

Discussion

Celecoxib was originally developed as a selective COX-2 inhibitor in an attempt to decrease adverse reactions caused by existing NSAIDs acting on COX-1, and it is used for the treatment of rheumatoid arthritis and other types of inflammatory diseases. Moreover, celecoxib was once approved as an antitumor agent for FAP patients, as this compound was found to be effective in colorectal adenoma and carcinoma.[4,6] However, celecoxib is not currently used for the treatment of FAP patients, because coxib derivatives (particularly rofecoxib) were reported to increase the risk of developing thromboembolic disorders.[37,38]

As for the mechanism responsible for the antitumor effect of celecoxib, our earlier investigations showed that celecoxib induced TCF7L2 degradation, thereby preventing TCF-mediated transcription of several genes (e.g., cyclin D1 and survivin in colorectal cancer cell lines),[26,27] although the mechanism for celecoxib-induced degradation of TCF7L2 remains to be determined. Interestingly, it has been reported that DM-celecoxib, a celecoxib analog unable to interact with COX-2, also has antitumor activity, suggesting that COX-2 inhibition is not involved in the antitumor mechanisms of these drugs.[12] Moreover, we reported that both celecoxib and DM-celecoxib were able to suppress TCF7L2 expression in the myocardium of mice with dilated cardiomyopathy in which the Wnt/β-catenin signaling pathway is activated due to upregulation of TCF7L2 expression.[28]

Given the above information, in the present study we aimed to compare the anticancer effects of celecoxib and DM-celecoxib in a model of intestinal cancer. Both celecoxib and DM-celecoxib inhibited cell proliferation, induced apoptosis, and accelerated TCF7L2 degradation with similar potencies in two different types of human colon cancer cell lines, HCT-116 and DLD-1, in which the Wnt/β-catenin signaling pathway is constitutively active due to mutations in the CTNNB1 or *APC* gene, respectively. We also showed that both compounds inhibited oxidative stress-induced intestinal cancer growth *in vivo*.

The mammalian DNA glycosylase MUTYH initiates the base excision repair process by excising the adenine opposite 8-oxoguanine and the 2-hydroxyadenine opposite guanine, thereby preventing G:C to T:A transversion mutations caused by oxidative stress.[39–41] A biallelic germline mutation in the *MUTYH* gene has been found in humans; the carriers of this mutation tend to develop multiple adenomatous colon polyps and have an increased risk of colorectal cancers together with an increased incidence of G:C to T:A somatic mutations in the *APC* gene (MUTYH-associated polyposis).[29,30] We recently reported that 87.1% of oxidative stress-induced intestinal tumors developed in MUTYH-deficient mice had a mutation in either the *APC* or CTNNB1 genes and that almost all mutations were caused by G:C to T:A transversion.[33] Therefore, the animal model we used in the present study may indeed be suitable for evaluating the efficacy and potential use of celecoxib and DM-celecoxib in a clinical setting.

Our results indicate that both celecoxib and DM-celecoxib inhibited proliferation and induced apoptosis in HCT-116 cells, independent of their actions on COX-2. Thus, the results obtained from the *in vitro* and *in vivo* experiments clearly show that the inhibition of COX-2 is not essential for the antitumor effect of celecoxib, and that not only celecoxib but also DM-celecoxib can inhibit tumor growth, even under the Wnt/β-catenin signaling pathway activated condition, because they directly inhibit TCF7L2-mediated transcription. Although it appeared that DM-celecoxib showed slightly stronger effects compared with celecoxib *in vitro* and celecoxib showed slightly stronger effects *in vivo*, the effects resulting from the same concentration of these drugs were not significantly different, except for the effect on cyclin D1 expression *in vitro* (Fig. 3b). Therefore, the structural difference between celecoxib and DM-celecoxib may not have significant influence on the ability of these drugs to inhibit the Wnt/β-catenin signaling pathway by the degradation of TCF7L2. Further studies are required to elucidate the mechanism by which these drugs induce the degradation of TCF7L2.

Although celecoxib appears to be useful as an anticancer agent, its clinical use may be faced with resistance, as several clinical studies reported an increase rate of cardiovascular events after long-term treatment with celecoxib, particularly when given at high doses (≥400 mg/day).[37,42–44] However, other studies have shown that celecoxib did not significantly increase cardiovascular risks compared to other NSAIDs such as diclofenac or etodolac.[38,45] Further basic and clinical studies are therefore needed to develop a novel anticancer agent based on the effect of celecoxib derivatives.

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Disclosure Statement

The authors have no conflict of interest.

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