Downregulation of PRMT1, a histone arginine methyltransferase, by sodium propionate induces cell apoptosis in colon cancer

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Abstract. The microbiota and bacterial metabolites in the colon are regarded as alternative targets for colon cancer prevention and therapy. Among these metabolites, short-chain fatty acids (SCFAs) exhibit anticancer effects and suppress inflammation in the colon. However, the molecular mechanisms and target development of SCFAs require additional study. In the present study, using RNA-seq results from colon cancer samples derived from the Cancer Genome Atlas (TCGA) portal, overexpressed epigenetic modifiers were identified and RT-PCR and qRT-PCR analysis was performed to select target genes that responded to treatment with propionate in HCT116 cells. Downregulation of protein arginine methyltransferase 1 (PRMT1), a histone arginine methyltransferase, was observed after sodium propionate (SP) treatment. Moreover, phospho-array analysis demonstrated that the mTOR pathway was involved in propionate and siPRMT1 treatment, and regulation of this pathway was associated with apoptosis in HCT116 cells. The present study, to the best of our knowledge, was the first to demonstrate that PRMT1 levels were reduced by propionate treatment in HCT116 cells and that downregulation of PRMT1 induced cell apoptosis. Thus, this novel mechanism of sodium propionate treatment for colon cancer therapy may indicate more effective approaches, such as dietary therapy, for CRC patients.

Introduction

Colorectal cancer (CRC; colon and rectal cancer) is the most common cancer worldwide. To overcome the challenges of CRC treatment, recent studies of microbiota and metabolites derived from the microbiome in the colon, have investigated alternative methods for colon cancer prevention and treatment (1). Among these metabolites, short-chain fatty acids (SCFAs; butyrate, acetate and propionate) are produced from dietary fiber in the colon via bacterial fermentation to maintain host health (2). Moreover, in a disease context, SCFAs are related to colonic inflammation, insulin resistance and high-fat-diet-induced obesity (3). The signaling components activated by SCFAs have been divided into two main pathways: a histone deacetylase (HDAC) inhibitor pathway and a G-protein coupled receptor (GPCR) activation pathway (2). In colon cancer, treatment with SCFAs was revealed to induce hyperacetylation and suppress colon cancer proliferation and metastasis (4-7). Specifically, butyrate induced apoptosis in HCT116 cells by regulating p38 MAPK. Additionally, in an in vivo study, SCFA mixtures suppressed azoxymethane/dextran sodium sulfate (AOM/DSS)-induced tumors by inhibiting COX-2 expression (7-10). In addition, propionate treatment was revealed to reduce free fatty acid receptor 2 (FFAR2; also known as GPR43) expression in leukemic cell lines and to reduce leukemic cell proliferation (11). However, despite studies on the roles of SCFAs in the colon, their molecular mechanisms and target development require further investigation. Moreover, since SCFAs studies have focused mainly on butyrate treatment, the relationship between propionate and colon cancer was examined in this study.

SCFA treatment in colon cancer has been revealed to induce hyper-acetylation by inhibiting HDAC activity, suggesting that SCFAs can act as an HDAC inhibitor. Although major studies of the anticancer effects of SCFAs (mainly butyrate) have focused on inhibiting HDAC activity, the molecular mechanism of propionate in colon cancer remains unclear. Epigenetic modifiers, such as histone methyltransferase, acetylase and DNA methyltransferase, have become important target genes for developing anticancer...
drugs for colon cancer (12,13). Among these genes, protein arginine methyltransferase 1 (PRMT1) is a histone arginine methyltransferase that mainly mono- and dimethylates histone H4 arginine 3, which is an activation site for gene expression (14,15). In colon cancer, PRMT1 was revealed to be involved in epidermal growth factor (EGF) receptor methylation during resistance to cetuximab treatment, and methylation of methyl-DNA binding domain 2 (MBD2) by PRMT1 inhibited binding to methyl-CG DNA in colon cancer (16,17). Moreover, PRMT1 overexpression was revealed to be clearly associated with poor prognosis in colon cancer cohorts. Although the relationship between colon cancer and PRMT1 has been studied, regulation of PRMT1 expression in colon cancer is unclear.

Therefore, in this study, the HCT116 cell line was treated with sodium propionate (SP) and cellular apoptosis was observed upon downregulation of PRMT1 expression. This is the first study, to the best of our knowledge, revealing that downregulation of PRMT1 expression by propionate, induced apoptosis by inhibiting p70 S6 kinase phosphorylation. Thus, PRMT1 inhibitors and propionate treatment are expected to reveal synergistic anticancer effects which may be used in the treatment of CRC patients.

Materials and methods

Cell culture and reagents. The human colon cancer cell lines HCT116 and SW480 were purchased from the Korean Cell Line Bank (Seoul, South Korea) and cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin in a humidified atmosphere with 5% CO₂ at 37°C. Sodium propionate (product no. P5436) was purchased from Sigma-Aldrich; Merck KGaA (Darmstadt, Germany).

CRC patients in TCGA data. The mRNA expression (RNA-Seq) data of 572 colon-related samples (51 normal samples and 521 tumor samples) was obtained from TCGA data portal (http://cancergenome.nih.gov). The RNA-Seq quantification data (HTSeq-FPKM) was downloaded and the mean value for the expression levels of each gene across samples was calculated. These mean values represented the expression each gene in normal and tumor samples.

Cell viability assay. Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Rockville, MD, USA) was used to conduct cell viability assays. Cells were seeded in 6-well plates starting at 4x10⁵ cells/well and incubated for 24 h. After sodium propionate treatment and siRNA transfection for 2 days, CCK-8 solution and RPMI-1640 medium with 10% FBS mixture were added into each well and incubated with 5% CO₂ at 37°C for 5 min or 10 min. The absorbance was assessed using a microplate reader at 450 nm. For crystal violet staining, the cells were added into each well and incubated with 5% CO₂ for 5 min or 10 min. The absorbance was assessed using a microplate reader at 450 nm. For crystal violet staining, the cells were fixed with methanol for 5 min and stained with 0.1% crystal violet after sodium propionate treatment and siRNA infection for 48 h.

Human phospho-kinase array. Human phospho-kinase array was performed using Proteome Profiler Human Phospho-Kinase Array kit (cat. no. ARY003B) purchased from R&D Systems, Inc. (Minneapolis, MN, USA). HCT116 cells, treated with sodium propionate and PRMT1 siRNA, were lysed in lysis buffer. Cell lysates were centrifuged at 14,000 x g for 5 min at 4°C and diluted to 400 µg per array set. Cell lysates were incubated with membranes overnight at 4°C on a rocking platform shaker. Then, the membranes were incubated with detection antibody cocktail for 2 h and Streptavidin-HRP for another 30 min at room temperature. The signal was detected by incubating the membranes with Chemiluminescent substrate and exposing to film.

FACS analysis. After treatment with SP or knockdown of PRMT1 for 2 days, the cells were collected and incubated with the MUSE Annexin V and Dead Cell Assay kit (cat. no. MCH100105; Merck KGaA) for 20 min at room temperature. After incubation, ~5x10⁵ cells were analyzed using a Muse Cell analyzer (Merck KGaA) (18). The FACS result was analyzed using Muse 1.5 Analysis (Merck KGaA).

siRNA transfection. siRNA duplexes against PRMT1 (siPRMT1; 5'-GAGUUCACAAGCGGUCACATT-3'; 5'-UGUGCCACGGUGUGAACCTT-3') were purchased from ST Pharm Co., Ltd. (Seoul, South Korea). Negative control siRNA (siCont; 5'-AUGAACCUGAAUUGCUAAATTT-3'; 5'-UGAGCGAUUCACGUACTT-3') was used as a control (19). The siRNAs (100 nM) were transfected into cancer cell lines using RNAiMax (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) for 48 h.

Semi-quantitative reverse transcription PCR and quantitative real-time PCR. Total RNA was isolated from the indicated cell lines using a Qiagen RNeasy Mini kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's instructions. RNA aliquots of 1 µg were then reverse-transcribed using the iScript™ cDNA synthesis kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA), according to the standard protocols provided by the manufacturer. For semi-quantitative RT-PCR, cDNA was used as a template for PCR using AccuPower® HotStart PCR PreMix (Bioneer, Daejeon, South Korea). For quantitative RT-PCR, PCR reactions (PRMT1: annealing temperature 55°C, 30 cycles; ACTB: annealing temperature 55°C, 28 cycles) were performed using the SimpliAmp Thermal Cycler (Applied Biosystems; Thermo Fisher Scientific, Inc.) following the manufacturer's instructions. Quantitative real-time PCR was performed on cDNA samples using Brilliant III Ultra-Fast SYBR® Green QPCR Master Mix and the signal was detected using AriaMx Real-Time PCR System (both from Agilent Technologies, Inc., Santa Clara, CA, USA). The fluorescence threshold value was calculated using Agilent Aria 1.6 software (Agilent Technologies, Inc.) (20,21). The PCR primers were as follows: PRMT1 forward, 5'-GGGCTACTGCTTCTTTACGAGTC-3' and reverse, 5'-GTCTTTGTACTGCCGGTCCTCG-3'; and ACTB forward, 5'-ACTCTTCCACGCTCTCTCC-3' and reverse, 5'-CAATGCACGGGTACATGGGT-3'.

Western blot analysis. The cells were washed once with phosphate-buffered saline (PBS) and then lysed in cold lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 1 mM EDTA, 1 mM Na3VO₄, 1 mM
NaF and 1X protease inhibitor cocktail). Cell lysates were centrifuged at 14,000 x g for 15 min at 4°C and then boiled in 5X sample buffer following protein determination (BSA, cat. no. 23208; Thermo Fisher Scientific, Inc.). The protein samples were subjected to western blot analysis. For western blot analysis, nitrocellulose membranes (cat. no. 1620145; Bio-Rad Laboratories) and blocking reagent (5% skim milk, 1 h at room temperature), 4-20% precasting gel (cat. no. 456-1094; Bio-Rad Laboratories) were used with the indicated antibodies at a 1:1,000 dilution ratio (22). The samples were stained with the following antibodies: anti-PRMT1 (cat. no. 2449), PARP (cat. no. 9542), caspase-3 (cat. no. 9662), phospho-p70 S6 kinase (Thr389) (cat. no. 9234), p70 S6 kinase (cat. no. 9202) all from Cell Signaling Technology (Danvers, MA, USA) and PRMT1 (cat. no. sc-166963) and ACTB (cat. no. sc-47778) from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Secondary antibodies (rabbit; cat. no. SC-2357, mouse; cat. no. SC-2031; Santa Cruz Biotechnology) were incubated at room temperature for 1 h, and ECL solution (cat. no. 170-5060; Bio-Rad Laboratories) was used for visualization. Chemiluminescence imaging system (Mini HD9; UVitec, Cambridge, UK) was used for imaging.

Statistical analysis. Results were expressed as the mean ± SD (error bars) of three independent experiments. One-way analysis of variance (ANOVA) with Bonferroni post hoc test was performed using Graphpad Prism 5.0 (Graphpad Software, Inc., La Jolla, CA, USA). The cut-off for significance was P<0.05.

Results

**SP suppresses the proliferation of the HCT116 and SW480 cell lines.** First, to verify the anticancer activity of propionate in colon cancer, a cell growth assay and CCK-8 analysis were performed. Following treatment with SP, a significant SP dose-dependent reduction of the number of cells and growth rates was observed compared to those of the control (Figs. 1A and B and 2A and B). In addition, in western blot analysis after treatment with SP using anti-PARP and anti-caspase-3 antibodies, ACTB was used as the internal control. (D) FACS analysis using Annexin V staining was performed after treatment with SP. The lower right and upper right quadrants indicate early apoptosis and late apoptosis, respectively. SP, sodium propionate, ACTB, β-actin.
Figure 2. Induction of cell apoptosis of SW480 cells by SP treatment. (A and B) Cell growth assay. (A) After treatment with SP (0 and 10 mM), cell fixation with 100% methanol and cell staining with crystal violet were performed. ‘1’ and ‘2’ are results of two independent experiments after treatment with SP. The cells were imaged under a microscope. Scale bar, 50 µm. (B) CCK-8 solution was added to the culture medium, and an incubation step was performed for 5 min at 37˚C. The intensity of cell growth was assessed by a microplate reader (450 nm). P-values were calculated using Student’s t-tests (***P<0.001). (C) FACS analysis using Annexin V staining was performed after treatment with SP. The lower right and upper right quadrants indicate early apoptosis and late apoptosis, respectively. SP, sodium propionate.

Figure 3. PRMT1 expression is downregulated by SP treatment. (A) Heat map of gene expression related to PRMT genes, sorted by fold change of the CRC/normal FPKM value. (B and C) RT-PCR and qRT-PCR analysis of PRMT1. ACTB was used as an internal control. P-values were calculated using one-way ANOVA (**P<0.01). (D) Western blot analysis of PRMT1 after treatment with SP. ACTB was used as the internal control. PRMT1, protein arginine methyltransferase 1; SP, sodium propionate; CRC, colorectal cancer; ACTB, β-actin.
several types of cancer (23). Therefore, in the present study, the identification of the novel epigenetic modifiers controlled by propionate treatment in colon cancer, was attempted. To investigate the relationship between epigenetic modifiers and propionate treatment in colon cancer, an *in silico* epigenetic panel (61 histone methyltransferases/demethylases, 28 histone acetylases/deacetylases and 7 DNA methyltransferases/ demethylases) was established to select epigenetic modifiers overexpressed in colon cancer (data not shown). To examine the expression levels of epigenetic modifiers, the RNA-seq results for colon cancer (n=521) and normal samples (n=51) derived from the TCGA portal were evaluated, and PRMT1, which is a histone arginine methyltransferase 1 (>2-fold change), was selected from the PRMTs (Fig. 3A). Since the knockdown of PRMT1 was revealed to suppress cell proliferation in hepatocellular carcinoma (HCC), lung cancer and bladder cancer (15,24), RT-PCR and qRT-PCR analysis after treatment with SP in the HCT116 cell line was performed to assess the relationship between propionate treatment and PRMT1 expression. As observed in Fig. 3B and C, the expression level of PRMT1 was decreased by SP treatment in a dose-dependent manner. Concurrently, the reduction of PRMT1 expression by SP treatment using western blot analysis was confirmed (Fig. 3D). Collectively, these data revealed the negative regulation of PRMT1 expression by propionate treatment in colon cancer, indicating that PRMT1 downregulation induced by SP treatment may lead to anticancer effects via the induction of cell apoptosis.

**Overexpression of PRMT1 in colon cancer.** GENT2 analysis (http://gent2.appex.kr/gent2/) revealed that PRMT1 was overexpressed in several types of cancer, including colon cancer (Fig. 4A), and dot blot graph revealed that the expression levels of PRMT1 were significantly increased in colon cancer, with data derived from the TCGA portal (Fig. 4B). Additionally, in Oncomine data (https://www.oncomine.org), the expression level of PRMT1 in colon cancer samples was higher than that in normal colon and rectum samples (Fig. 4C). Thus, in colon cancer, PRMT1 was overexpressed, and the functions of PRMT1 in colon cancer may be involved in colon cancer proliferation. These results indicated that overexpressed PRMT1 may become a useful molecular target for propionate treatment.

**Knockdown of PRMT1 suppresses cell proliferation via the apoptosis pathway.** To determine whether overexpressed PRMT1 is related to colon cancer proliferation, a PRMT1-specific siRNA was designed and PRMT1 knockdown...
analysis was performed. In RT-PCR and qRT-PCR analysis, PRMT1 expression was significantly decreased by treatment with siPRMT1 compared to that in cells treated with siCont (Fig. 5A). In cell growth analysis, cell staining with crystal violet was performed, '1' and '2' are results of two independent experiments after treatment with siRNAs. The cells were imaged under a microscope. Scale bar, 50 µm. (C) In addition, CCK-8 solution was added to culture medium, and an incubation step was performed for 5 min at 37˚C. The intensity of cell growth was assessed by a microplate reader (450 nm). P-values were calculated using Student's t-tests (**P<0.01). (D) Western blot analysis after PRMT1 knockdown using anti-PRMT1, anti-PARP and anti-caspase-3 antibodies. ACTB was used as the internal control. (E) FACS analysis using Annexin V staining was performed after treatment with siCont and siPRMT1. The lower right and upper right quadrants indicate early apoptosis and late apoptosis, respectively. PRMT1, protein arginine methyltransferase 1.

Figure 6. Phosphorylation of p70 S6 kinase is regulated by SP and PRMT1 knockdown. (A) Phospho-array with lysates from SP-treated and PRMT1-knockdown cells. The phospho-array was purchased from R&D Systems. Approximately 400 µg of cell lysate was used. Green boxes revealed blots of p-p70 S6 kinase (Thr389). P-values were calculated using Student's t-tests (**P<0.01). (B) Western blot analysis after treatment with SP and siPRMT1 using anti-PRMT1 and anti-phospho-p70 S6 kinase antibodies. ACTB was used as the internal control. (C) Schematic summary of the effects of SP and PRMT1 on colon cancer. PRMT1, protein arginine methyltransferase 1; SP, sodium propionate; ACTB, β-actin.
violet revealed that the number of stained cells was reduced by PRMT1 knockdown (Fig. 5B). In addition, the proliferation of HCT116 cells was clearly suppressed after the knockdown of PRMT1 using CCK-8 analysis (Fig. 5C). Additionally, as revealed in Fig. 1D, PRMT1 knockdown induced cleaved PARP and caspase-3 compared to the levels in cells treated with siCont; moreover, in FACS analysis, the proportion of apoptosis was increased in PRMT1 knockdown samples (Fig. 5D and E). Thus, it was demonstrated that PRMT1 expression was associated with colon cancer proliferation, and cell apoptosis induced by propionate treatment was linked to the PRMT1-related apoptosis pathway.

**p70 S6 kinase phosphorylation status is regulated by SP treatment and PRMT1 knockdown.** To assess the pathway for propionate/PRMT1-induced apoptosis, a phosho-array was purchased from R&D Systems. Fig. 6A revealed that the intensity of positive spots after treatment with 0 mM propionate was similar to that of the positive spots after treatment with 5 mM propionate; however, the p70 S6 kinase (Thr389) phosphorylation status was reduced by 5 mM SP treatment compared to that of the control. Tang et al reported that the phosphorylation of mTOR, 4E-BP1 and p70 S6 kinase was reduced depending on the concentration of propionate and the incubation time, and the mTOR pathway was involved in the suppression of colon cancer proliferation and the stimulation of cell apoptosis (9,25,26). Notably, the results of the phosho-array using cell lysates from cells subjected to PRMT1 knockdown also demonstrated that the phosphorylation of p70 S6 kinase was reduced compared to that of cells subjected to SP treatment (Fig. 6A). To confirm the phosho-array results, western blot analysis was carried out using a phospho-antibody. After treatment of HCT116 cells with SP, the downregulation of PRMT1 expression was clearly detected, and the phosphorylation of p70 S6 kinase was reduced after both PRMT1 knockdown and 5 mM SP treatment compared to that of cells treated with siCont and 0 mM SP (Fig. 6B). Collectively, these results indicated that treatment with SP induced cell apoptosis via the inhibition of the mTOR signaling pathway by PRMT1 downregulation in colon cancer (Fig. 6C). Therefore, this novel PRMT1-related mechanism induced by propionate treatment in colon cancer may help to increase our understanding of the use of propionate treatment for colon cancer patients.

**Discussion**

Epigenetic modifiers [histone methyltransferase (HMT)/demethylase (HDM), histone acetyltransferase (HAT)/deacetylase (HDAC) and DNA methyltransferase/demethylase] have been investigated as therapeutic targets for developing anticancer drugs in several cancers. In colon cancer, overexpression of epigenetic modifiers plays an important role in proliferation and metastasis. For example, EZH2, SMYD3, EHMT2 and LSD1 have been linked with colon cancer proliferation. In addition, p300 and HDAC are important factors in colon cancer (27-30). Thus, studies on reducing epigenetic modifier expression are required to treat colon cancer.

In the present study, it was demonstrated that PRMT1 is overexpressed in several cancers, including colon cancer, using GENT2, the TCGA portal and Oncomine data (Fig. 4). Generally, benign and malignant tumors are the main tumor types. In malignant tumors, carcinoma (carcinoma, squamous cell carcinoma and adenocarcinoma), sarcoma and undifferentiated carcinoma are divided. In colon cancer progression, several steps occur (Polyp >> precancer [adenoma] >> Stage I-IV). Thus, using Oncomine data, we focused on the high PRMT1 expression in several tumor types including malignant tumors and adenoma compared with the normal colon and rectum. In Fig. 4C, PRMT1 was overexpressed in colon adenoma, carcinoma and adenocarcinoma according to Oncomine data. Thus, it was indicated that PRMT1 was overexpressed in early colon cancer stages, and its overexpression may help to acquire malignant characteristics for colon cancer progression. In addition, it was clearly demonstrated that propionate treatment downregulated PRMT1 in the HCT116 cell line (Fig. 3). Subsequently, PRMT1 downregulation induced apoptosis by inhibiting phospho-p70 S6 kinase as revealed by siPRMT1 treatment (Figs. 5 and 6), however, to confirm the anticancer effect of SP, cell growth assays with crystal violet were performed after SP treatment (0 and 5 mM) in normal enterocytes and the HCT116 cell line. Notably, the effect of SP treatment could not be detected in normal enterocytes (data not shown). Therefore, it is suggested that SP may play a role as an anticancer agent for colon cancer treatment.

Recently, our group reported that PRMT1 could serve as a prognostic and therapeutic marker in hepatocellular carcinoma (HCC), and PRMT1 overexpression downregulated CDKN1A to promote HCC proliferation (15). Additionally, Mathioudaki et al reported that high PRMT1 expression was associated with poor survival rates in patients with colon cancer (31). Therefore, PRMT1 should be recognized as a useful therapeutic marker for CRC treatment, and developing novel methods for downregulating PRMT1 expression or activity is important for CRC therapy. AMI-1 is a pan-PRMT inhibitor, and treatment with this agent suppressed cell proliferation by reducing arginine methylation. However, problems have been reported regarding the specificity and adverse effects of PRMT inhibitors (32). Thus, downregulation of PRMT1 by propionate treatment should be recognized as a safe procedure for CRC treatment since propionate is a bacterial fermentative product in the colonic epithelium and a safe compound authorized in the EU for food use. Therefore, developing PRMT1 inhibitors and establishing guidelines for co-treatment with propionate and PRMT1 are required to increase the curative value of this approach in CRC treatment.

As revealed in Fig. 3, although propionate treatment decreased PRMT1 expression, the definite mechanisms that link propionate and PRMT1 regulation remain unknown. Similar to butyrate, propionate was also revealed to inhibit HDAC (5). First, it was hypothesized that the reduction in HDAC activity by propionate would decrease PRMT1 expression. However, as hyperacetylation by propionate is associated with reconstructing the euchromatin structure from heterochromatin to induce gene expression, PRMT1 downregulation by propionate may not be involved in the hyperacetylation mechanism. However, upregulation of unknown regulators by propionate-induced hyperacetylation may affect the suppression of PRMT1 expression. Thus,
the relationship between PRMT1 and propionate should be investigated, and the status of H4R3me2 after SP treatment should be validated in further studies.

SCFA treatment induced apoptosis in colon cancer via mitochondrial defects and mTOR pathway inhibition (25,26,33). Additionally, the cAMP/PKA/CREB pathway is controlled by SCFAs for dysregulating inflammatory suppressors in the colonic epithelium (34). In the present study, to ascertain the common signaling pathway for cellular apoptosis used by propionate and PRMT1 knockdown, phospho-array analysis was performed after treatment with propionate and siPRMT1 and a reduction in p70 S6 kinase phosphorylation after separate treatments with propionate and siPRMT1 was observed (Fig. 6A). This is the first study, to the best of our knowledge, which revealed PRMT1-related mTOR signaling pathway effects in colon cancer. Liao et al demonstrated that overexpressed PRMT1 methylated the EGF receptor to increase EGFR signaling activation. Subsequently, the ERK and AKT pathways were activated for survival during cetuximab treatment (16). Thus, it is suggested that: i) the AKT pathway, which is regulated by PRMT1, may affect the mTOR pathway; or ii) the epigenetic mechanism induced by PRMT1 may regulate the mTOR pathway. Therefore, this study was the first to report PRMT1 downregulation by propionate treatment in the HCT116 cell line. Suppressed cell growth via mTOR signaling pathway inhibition was also observed. Thus, this mode-of-action (MOA) study of propionate treatment in colon cancer may provide a basis for new guidelines, such as a dietary therapy, for CRC patients.

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Availability of data and materials
The datasets used during the present study are available from the corresponding author upon reasonable request.

Authors’ contributions
DSK and HSC were involved in the conception and design of the study, TYR, TSH and KK were involved in the development of the methodology. DSK, MYS and JKM were involved in the analysis and interpretation of the data (e.g., statistical analysis, biostatistics and computational analysis). KK, TYR, JK, DSK, MYS, JKM and HSC were involved in the writing, reviewing and/or revision of the manuscript. JK and JKM were responsible for administrative, technical or material support. DSK and HSC supervised the study. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate
Not applicable.

Patient consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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