A gut butyrate-producing bacterium *Butyricicoccus pullicaecorum* regulates short-chain fatty acid transporter and receptor to reduce the progression of 1,2-dimethylhydrazine-associated colorectal cancer

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Abstract. Gut microbes influence tumor development and progression in the intestines and may provide a novel paradigm for the treatment of colorectal cancer (CRC). Gut dysbiosis may be associated with the development and progression of CRC. Identifying the interactions between the colonic tract and gut microbiota may provide novel information relevant to CRC prevention. The present study examined the effects of butyrate-producing *Butyricicoccus pullicaecorum* (*B. pullicaecorum*) on mice with 1,2-dimethylhydrazine (DMH)-induced CRC and the microbial metabolite of *B. pullicaecorum* on CRC cells. Immunohistochemical staining of the mouse colon tissues and reverse transcription PCR of CRC cells were used to determine the protein and mRNA expression levels of the short-chain fatty acid (SCFA) transporter solute carrier family 5 member 8 (SLC5A8) and G-protein-coupled receptor 43 (GPR43). In CRC-bearing mice fed *B. pullicaecorum*, DMH-induced CRC regressed, body weight increased and serum carcinoembryonic antigen levels decreased. Notably, SLC5A8 and GPR43 were diffusely and moderately to strongly expressed in the neoplastic epithelial cells and underlying muscularis propria in the colons of the mice. In conclusion, administration of *B. pullicaecorum* or its metabolites improved the clinical outcome of CRC by activating the SCFA transporter and/or receptor. These results indicated that *B. pullicaecorum* was a probiotic with anti-CRC potential.

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Abbreviations: CRC, colorectal cancer; *B. pullicaecorum*, *Butyricicoccus pullicaecorum*; DMH, 1,2-dimethylhydrazine; SCFA, short-chain fatty acids; CEA, carcinoembryonic antigen

Key words: Butyricicoccus pullicaecorum, butyrate, colorectal cancer, 1,2-dimethylhydrazine

Introduction

An understanding of the mechanisms by which gut microbes affect tumor development and progression in the intestine may provide a novel paradigm for colorectal cancer (CRC) therapy (1). As one of the leading causes of death among patients with cancer worldwide in recent years with an age-standardized mortality rate of 8.9 per 100,000 patients, CRC is a heterogeneous disease characterized by diversions of multiple molecular pathways throughout its evolutionary process, such as reduction in the apoptotic rate and development of metastasis (2,3). Numerous contributing factors to CRC, including genes and microbiota, have been identified (4-6).

Certain fecal genes have been demonstrated to serve important molecular roles in cancer biology and molecular medicine (7-9). Improving clinical outcomes depends on identifying and understanding these genes and applying this knowledge to CRC detection and chemotherapy (4,10,11). By stimulating CRC cells, which exhibit high chemokine expression, the gut microbiota recruit beneficial T cells into colonic tumor lesions (12). Biomaterials in the stool, including nucleic acids or gut microbiota, are associated with the risk of CRC (1,13-15). Furthermore, gut dysbiosis is associated

with the development and progression of CRC through DNA damage, activation of oncogenic signaling pathways, production of tumor-promoting metabolites and suppression of antitumor immunity (1).

Gut microbes affect gene expression in colonic cells and may alter the progression of CRC (16-18). Short-chain fatty acids (SCFAs), which are derived from microbial metabolism in the gut, serve multiple roles in host homeostasis (19). Certain reports have indicated that decreased SCFA production is associated with increased CRC risk and may have applications in CRC therapy (20). Butyrate is a type of SCFA with various molecular effects on intestinal cells, including anticancer formation and cell immunity (21-23). SCFA transporters and receptors act as molecular links between the gut and microbes (24). For instance, butyrate was demonstrated to alleviate gut inflammation by coupling with a cell-surface G-protein-coupled receptor 43 (GPR43) (25,26) and regulate intestinal tight junction proteins through a transporter, solute carrier family 5 member 8 (SLC5A8) (27). Furthermore, butyrate-producing *Butyricicoccus pullicaecorum* (*B. pullicaecorum*) has been revealed to prevent necrotic enteritis and reduce pathogen abundance in the cecum and ileum (28,29). Therefore, this next-generation probiotic has been reported to be safe in a human intervention trial (28). The present study observed a trend towards decreasing the abundance of *B. pullicaecorum* in the stool of patients with late-stage CRC (28).

An understanding of the role of *B. pullicaecorum* or its metabolites in CRC is crucial prior to considering *B. pullicaecorum* a probiotic. However, the anti-CRC effects of *B. pullicaecorum* or its metabolites, particularly butyrate, on butyrate transporters and receptors have not been directly confirmed. The aims of the present study were to examine the effects of providing a supernatant of *B. pullicaecorum* culture to investigate the growth of CRC cells and evaluate 1,2-dimethylhydrazine (DMH)/dextran sulfate sodium (DSS)-induced colonic tumors of mice with *B. pullicaecorum* administration.

Materials and methods

Gut bacterium, mice and CRC induction. The gut bacterium, *B. pullicaecorum* (BCRC-81109), was purchased from the Bioresource Collection and Research Center and was grown in a modified peptone yeast extract broth (PY-X broth: 0.5 g peptone from meat; 0.5 g trypticase peptone; 1.0 g yeast extract; 0.1 mg resazurin; 50 mg L-cysteine-HCI.H_2O; 0.5 g D-glucose; 10 mg $CaCl_2.2H_2O$; 20 mg $MgS0_4.7H_2O$; 40 mg K_2HP0_4; 40 mg KH_2P0_4; 0.4 g $NaHC0_3$; 0.8 mg NaCl; 0.5% xylan in 100 ml distilled water; pH 7.0; DSMZ GmbH) for 3 days under anaerobic conditions at 37˚C. The conditioned medium from *B. pullicaecorum* cultures was harvested by centrifugation (1,000 x g, 15 min) at room temperature to remove the bacteria and sterilized by filtration with a 0.22 μm syringe filter (Thermo Fisher Scientific, Inc.).

BALB/cByJNarl male mice (age, 4-6 weeks; weight range, 20-25 g) were provided by the National Laboratory Animal Center, National Applied Research Laboratories, Taipei, Taiwan. All animal experiments complied with the Animal Research: Reporting of *In Vivo* Experiments guidelines (30). All protocols were approved by the Institutional Animal Care and Use Committees at Cathay General Hospital, Taipei, Taiwan and followed the '3Rs' (Reduction, Refinement and Replacement) (31). All efforts were made to minimize the number of animals and their suffering. The body weight of mice was monitored every week and mice were euthanized when they exhibited weakness and rapid weight loss (15-20% of their body weight) within a few days (32). A total of 3-5 were housed per plastic cage under pathogen-free conditions (humidity, 50±10%; light/dark cycle, 12 h; temperature, 23±2˚C) in an individually ventilated cage rack system (Tecniplast S.p.A).

To evaluate the effect of *B. pullicaecorum* ($3.125x10^7$ colony-forming units in 100 μl) on CRC formation, CRC was induced with DMH (40 mg/kg; cat. no. D0741; TCI America; Tokyo Chemical Industry)/DSS (2%; cat. no. D5144; TCI America; Tokyo Chemical Industry), as reported by De Robertis *et al* (33) and as described in the legend of Fig. 1A. A total of 17 mice were used and randomly divided into the following groups to study the induction of CRC by DMH and the *in vivo* effect of *B. pullicaecorum*: i) Control group (CG; n=5), no DMH treatment or *B. pullicaecorum* administration; ii) DMH mice (n=6), administered DMH by intraperitoneal injection and DSS in drinking water and no *B. pullicaecorum* administration; and iii) DMH/*B. pullicaecorum* (BP) mice (n=6), administered DMH, DSS, and *B. pullicaecorum* by oral gavage. Anuses of mice were imaged on days 29, 32 and 36 following initial *B. pullicaecorum* administration. Mice were sacrificed 8-9 weeks later with CO_2 euthanasia in a cage. The CO_2 flow rate was set to displace 30% of the cage volume/minute. The following parameters were used to confirm death: i) immobility; and ii) lack of spontaneous breathing for >2 min. During the experiments, the clinical characteristics of mice, including anal bleeding, body weight and serum carcinoembryonic antigen (CEA) levels were recorded.

CRC cell lines, cell culture and cell counts. SW480 colon cancer cell line (ATCC CRL-1459; AJCC stage II) and SW620 lymph node metastatic derivative cell line (ATCC CRL-1831; AJCC stage III) from the same patient were purchased from American Type Culture Collection. The two cell lines were expanded in complete medium [Leibovitz's L-15 medium (Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (NQBB International Biological Co., Ltd.)] under 100% atmospheric air (without CO_2) in a 37˚C humidified incubator. To evaluate the effects of *B. pullicaecorum* on CRC cell proliferation, the numbers of SW480 and SW620 cells were respectively counted at days 1 and 5. Briefly, cells incubated with or without 10% conditioned medium from *B. pullicaecorum* cultures in complete medium were imaged under an Olympus BX41 light microscope (Olympus Corporation) and analyzed using ImageJ software (version 1.52; National Institutes of Health) (34).

Reverse transcription PCR. Total RNA from SW480 and SW620 cells, which were untreated or treated with 5 mM sodium butyrate (NaB) for 48 h as reported in our previous study (21) was independently extracted using a RNeasy Mini kit (Qiagen, Inc.), according to the manufacturer's protocol. Total RNA (1 μM) was reverse-transcribed to single-stranded cDNA using a high-capacity cDNA Reverse Transcription kit

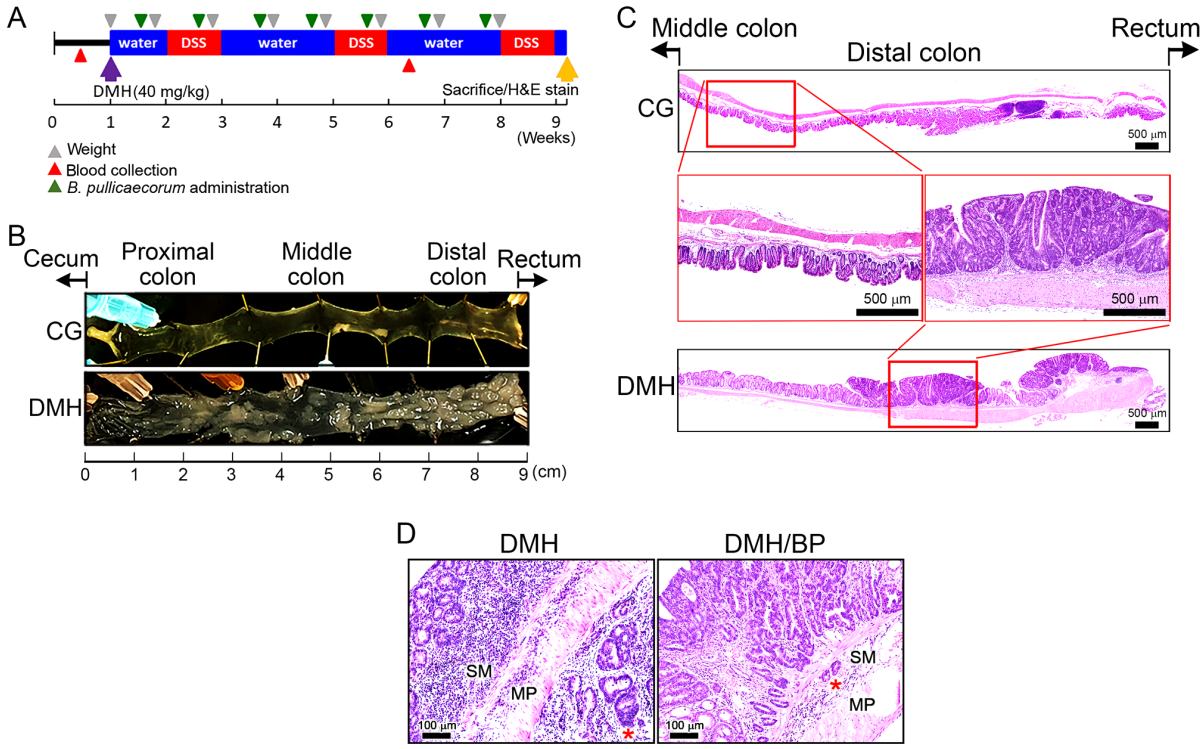

Figure 1. Reduction in DMH/DSS-induced CRC formation following *B. pullicaecorum* administration. (A) Timing of DMH and DSS induction of CRC and *B. pullicaecorum* administration. Purple arrow, subcutaneous injection of DMH 40 mg/kg body weight. Yellow arrow, sacrifice and colon sampling of H&E staining; blue box, water drinking; red box, DSS drinking; gray triangle, recording body weight; red triangle, blood collection; green triangle, *B. pullicaecorum* administration. (B) Inner layer of the colon of mice. (C) Histopathological examination of the colon of mice. Red square, higher magnification. Scale bar, 500 μm. (D) The effect of *B. pullicaecorum* administration on DMH-induced CRC. Scale bar, 100 μm. DMH, 1,2-dimethylhydrazine; DSS, dextran sulfate sodium; CRC, colorectal cancer; CG, control group; BP, *B. pullicaecorum*; H&E, hematoxylin and eosin; SM, submucosa; MP, muscularis propria; *, the maximal depth of tumor invasion.

(cat. no. 4368813; Thermo Fisher Scientific, Inc.), as previously described (35). To quantify the expression of GPR43, the reaction mixture containing the cDNA sample, QuantiTect SYBR-Green PCR Master mix (Qiagen GmbH), QuantiTect Primer assay (cat. no. Hs_FFAR2_1_SG; Qiagen GmbH) and RNase-free water was amplified using the following cycling program: 10 min at 95˚C, followed by 40 cycles at 95˚C for 15 sec and at 60˚C for 1 min. To quantify the expression of SLC5A8, the reaction mixture containing forward (5'-TCTTCC TCCCGGTGTTCTAC-3') and reverse primers (5'-GAACAC ATTTGTTAAATCGAAGTTCT-3'), no. 48 universal probe (Roche Diagnostics GmbH), LightCycler TaqMan Master (Roche Diagnostics GmbH) and RNase-free water was amplified by a program (10 min at 95˚C, proceeding with 60 cycles at 95˚C for 10 sec and at 60˚C for 20 sec). The expression of GAPDH (no. 60 universal probe (Roche Diagnostics GmbH); forward primer, 5'-CTCTGCTCCTCCTGTTCGAC-3'; reverse primer, 5'-ACGACCAAATCCGTTGACTC-3') was used as an internal control for calibration. For control purposes, the human reference cDNA (Clontech Laboratories) was used as a positive control to estimate the relative expression levels in the cells and the non-template reaction was a negative control. All RT-PCR reactions were run in a LightCycler 96 (Roche Diagnostics GmbH) and the data were analyzed using the $2^{-\Delta\Delta Cq}$ method (36).

Histological and immunohistochemical staining of mouse tissues. Colon tissues were obtained immediately following sacrifice and death and embedded in paraffin. Hematoxylin and eosin (H&E) were added to the paraffin section (thickness, 3-5 μm) to identify non-CRC and CRC areas using the method reported by Fischer *et al* (37). Immunohistochemistry (IHC) was performed as described by Huang *et al* (9). Briefly, colon tissue sections were hybridized with anti-GPR43 (1:100 in blocking solution; cat. no. BS-13536R) and anti-SLC5A8 (1:100 in blocking solution; cat. no. BS-6106R) polyclonal antibodies obtained from Bioss Antibodies. GPR43 and SLC5A8 were visualized using 3,3¢-diaminobenzidine (Vector Laboratories, Inc.) as the substrate. A pathologist viewed and categorized the H&E and IHC-stained sections.

Measurement of serum CEA levels. Blood samples (100 μl) were collected from a tail incision in each mouse. Sample was centrifuged at 1,000 x g for 15 min at room temperature to isolate the serum. Serum CEA levels were measured using a commercial ELISA kit (cat. no. E-EL-M0232; Elabscience), according to the manufacturer's protocol.

Statistical analysis. One-way ANOVA with Fisher's least significant difference (LSD) post-hoc test was performed to identify significant differences between groups. Differences in gene expression were compared using Student's t-test. Statistical analyses were performed using the SPSS statistics software (version 22.0; IBM Corp.). Data for cell numbers, body weight and serum CEA levels are presented for a >3 mice or ≥3 experiments with similar results. Data are presented as

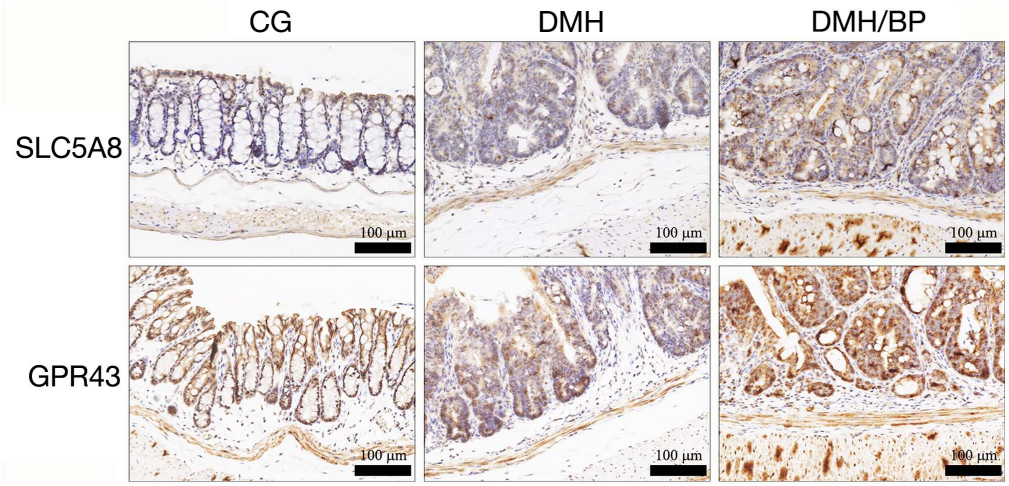

Figure 2. IHC staining of SLC5A8 and GPR43 in the colons of mice. SLC5A8 and GPR43 were examined by IHC staining of the colon of mice. CG: Control group, no DMH/DSS treatment or *B. pullicaecorum* administration. Scale bar, 100 μm. SLC5A8, solute carrier family 5 member 8; GPR43, G-protein-coupled receptor 43; IHC, immunohistochemistry; CG, control group; DMH, 1,2-dimethylhydrazine; DSS, dextran sulfate sodium; BP, *B. pullicaecorum*.

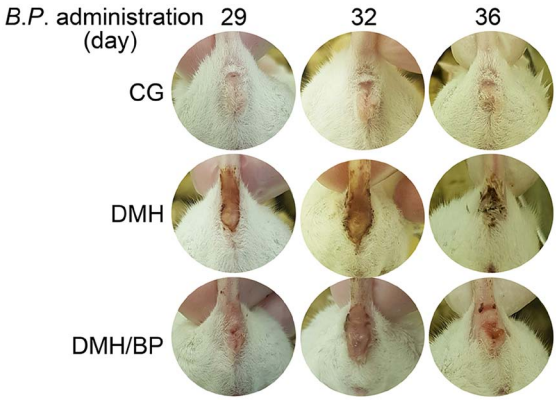

Figure 3. Bleeding images of the anuses of mice. Anuses were imaged at days 29, 32 and 36 following initial *B. pullicaecorum* administration. BP, *B. pullicaecorum*; CG, control group; DMH, 1,2-dimethylhydrazine; DSS, dextran sulfate sodium.

mean ± SEM. P<0.05 was considered to indicate a statistically significant difference.

Results

Reduction in DMH/DSS-induced CRC formation following B. pullicaecorum administration. Fig. 1A presented the timing of DMH and DSS induction of CRC and *B. pullicaecorum* administration. Numerous irregular and different sized crypt foci were observed in the inner layer of the colon of the DMH group, particularly in the distal colon and near the rectum (Fig. 1B). The histopathological examination demonstrated no foci in the colons of CG mice and the intestinal tract had an intact mucosal epithelium without any significant dysplastic changes (Fig. 1C, top panel). Conversely, aberrant crypt foci and multiple exophytic tumors in the mucosal layer were observed in the intestinal tract of DMH mice (Fig. 1C, bottom panel).

Visualization at higher magnification detected no specific histological changes in the intestinal mucosa, submucosa or muscular layers of CG mice (Fig. 1C, left middle panel). Furthermore, the neoplastic glands of DMH mice exhibited a complex or fused glandular pattern and notable nuclear atypia (Fig. 1C, right middle panel).

Fig. 1D demonstrated the regressive effect of *B. pullicaecorum* administration on DMH-induced CRC. In DMH/BP mice, tumors appeared to be downstaged histopathologically. Invasive carcinoma was limited to the submucosa with narrow maximal tumor invasion depth and fewer inflammatory cells infiltrates were observed in the periglandular area. The colonic tumors of DMH mice exhibited an early stage of the carcinogenesis process, which ranged from *in situ* to minimally invasive adenocarcinoma.

Conditional induction of SLC5A8 and GPR43 following B. pullicaecorum administration. Levels of SCFA transporter (SLC5A8) and receptor (GPR43) were examined by IHC staining of the colon of mice. As shown in Fig. 2, SLC5A8 and GPR43 were detected mainly in the superficial portion of the crypt glands in CG mice, while faint and randomly distributed reactivity was observed in DMH mice. Notably, transporters and receptors were diffusely and moderately to strongly expressed in neoplastic epithelial cells in DMH/BP mice. In addition to the epithelial cell populations, patchy areas with variable cytoplasmic staining for SLC5A8 and GPR43 were observed in the underlying muscular layer.

Characteristics of DMH-injected mice administered B. pullicaecorum. Phenotypic abnormalities and clinical characteristics of DMH and DMH/BP mice were analyzed. Compared with CG mice, anal bleeding was worse in the DMH mice; however, this was improved in DMH/BP mice (Fig. 3). Body weights and serum CEA levels of mice were measured. As shown in Fig. 4A, the mean body weight increased significantly from 19.2 to 30.0 g in 8 weeks in CG mice and no mice died during this time. Mice in the DMH/BP group exhibited significant weight loss, which was previously reported by Kim *et al* (38). The rate of weight gain was slower in mice in the groups administered DMH (DMH and DMH/BP mice) compared with CG mice. DMH mice (initial n=6, two

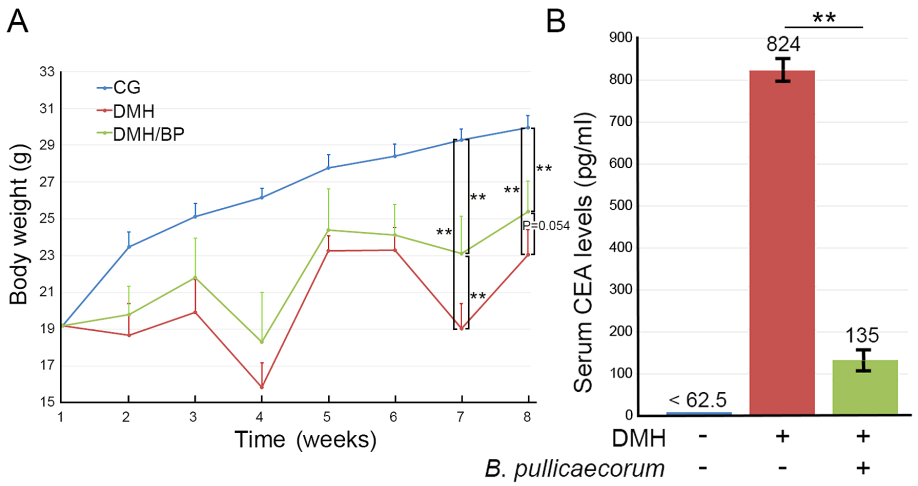

Figure 4. Effects of *B. pullicaecorum* on mice with DMH-induced CRC. (A) Body weights. Each mouse was weighed once a week. (B) Serum CEA levels. Sera were collected from tail incision of mice on the week for sacrifice. Levels of CEA in sera were measured using a commercial ELISA kit. One-way ANOVA with Fisher's least significant fifference (LSD) post-hoc test was performed to identify significant differences between groups. Data are presented as mean ± SEM. **P<0.01. DMH, 1,2-dimethylhydrazine; CRC, colorectal cancer; CEA, carcinoembryonic antigen; CG, control group; BP, *B. pullicaecorum*.

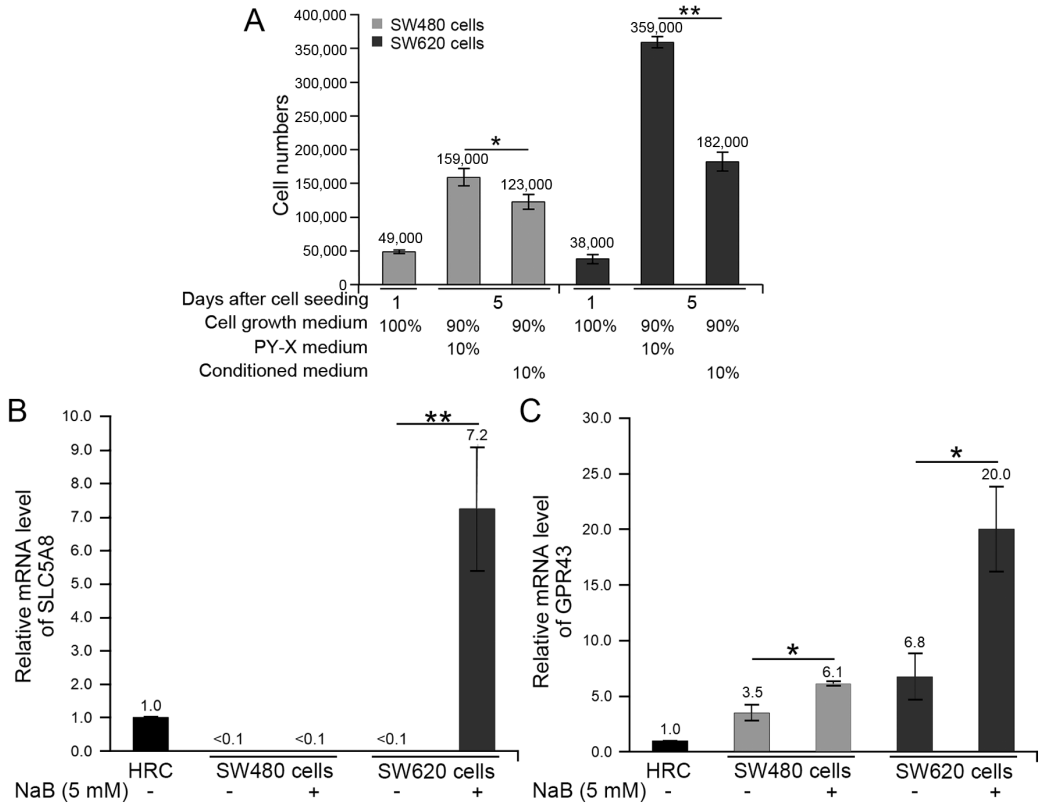

Figure 5. Effects of butyrate on CRC cells. (A) Effect of conditioned medium of *B. pullicaecorum* cultivation on CRC cell proliferation. Numbers of SW480 and SW620 cells were counted at days 1 and 5 following cell seeding. One-way ANOVA with Fisher's least significant difference (LSD) post-hoc test was performed to identify significant differences between groups. (B) Relative mRNA levels of SLC5A8. (C) Relative mRNA levels of GPR43. Gene quantitation was performed from SW480 cells and SW620 cells treated with 5 mM NaB for 48 h. Differences in gene expressions were compared using Student's t-test. Data are presented as mean ± SEM. *P<0.05. **P<0.01. CRC, colorectal cancer; NaB, sodium butyrate; SLC5A8, solute carrier family 5 member 8; GPR43, G-protein-coupled receptor 43; PY-X, peptone yeast extract broth; HRC, human reference cDNA.

died during the experiment) exhibited the lowest weight. DMH/BP mice (initial n=6, one died during the experiment) lost less weight than DMH mice. Briefly, statistical significance was observed at week 7 (P<0.01) and near significance was observed at week 8 (P=0.054) when comparing the body weights of DMH mice and DMH/BP mice. Overall, three mice (two DMH mice and one DMH/BP mouse) died during the experiments due to colon tumors. The rest of the mice were euthanized at the humane endpoint. DMH/BP mice exhibited longer survival rates (83.3±5.2%; Fig. S1), reduced body

weight loss and lower serum CEA levels compared with DMH mice (Fig. 4B).

Effects of butyrate on CRC cells. Gas chromatography-mass spectrometry analysis demonstrated that 3.05 mM butyrate was the predominant metabolite in the medium following cultivation of *B. pullicaecorum* (Table SI). The changes in SW480 and SW620 cells following treatment with the butyrate-containing supernatant from *B. pullicaecorum* cultures were evaluated using ImageJ software (Fig. 5A). On day 5, SW480 and SW620 cell numbers were lower in cells cultured in 90% cell growth medium + 10% filter-sterilized conditioned medium compared with cells cultured with the control medium (90% cell growth medium + 10% PY-X broth). However, the growth of SW620 cells was more sensitive than SW480 cells to cultivation with 10% filter-sterilized conditioned medium. In the cultures of SW480 and SW620 cells with 10% supernatant from *B. pullicaecorum*, SW620 cell growth was reduced to 50.7% (from 359,000 cells to 182,000 cells) and SW480 cells to 77.4% (from 159,000 cells to 123,000 cells). This inhibitory effect on the CRC cell growth following butyrate treatment was also reported in our previous study (21). SLC5A8 and GPR43 expressions in butyrate-treated CRC cells were quantified (Fig. 5B and C for SLC5A8 and GPR43, respectively). In comparison with untreated cells, the NaB-treated SW480 cells only presented higher GPR43 ($P<0.05$), but the NaB-treated SW620 cells expressed the most SLC5A8 ($P<0.01$) and GPR43 ($P<0.05$).

Discussion

A greater understanding of gut microbes may lead to different use of medicine in CRC. Gut microbe-based therapeutics are actively used for cancer treatment or prevention (39). Numerous studies have reported on the use of microbial markers, including microbes and metabolites, to target CRC (40,41). Wang *et al* (18) reported that a target microbe affected CRC or reduced the occurrence in an animal model. The present study demonstrated that *B. pullicaecorum* administration following DMH/DSS-induced tumorigenesis led to CRC regression in mice. Gao *et al* (41) revealed that gut microbe-mediated CRC suppression may occur through the production of histamine, a specific metabolite of *Lactobacillus reuteri*.

In a previous study, *Butyricicoccus* spp. was observed in the stools of patients with CRC (21). The species of *Butyricicoccus*, *B. pullicaecorum*, was originally isolated from the cecal content of a broiler chicken and is an anaerobic and butyrate-producing bacterium that may protect chickens from harmful microorganisms and necrotic enteritis (29,42). Patients with infectious gastroenteritis, including inflammatory bowel disease (IBD), exhibit fewer *B. pullicaecorum* in their stools (43,44). Butyrate has been reported to be involved in CRC cell function, including the regulation of gene expression (45,46), cell signaling (47) and repression of cell growth (48).

The present study demonstrated that SLC5A8 and GPR43 were upregulated in neoplastic epithelial cells in the underlying muscular layer of the colon following *B. pullicaecorum* administration. SLC5A8 and GPR43 interact with butyrate in the lumen of the colon (49,50). Their upregulation may be associated with the production of butyrate, a major metabolite of *B. pullicaecorum*. Furthermore, SLC5A8 and GPR43 are known to serve as tumor suppressors (51,52). As reviewed by Zaiss *et al* (53), mice lacking SLC5A8 develop CRC. Additionally, previous studies reported that GPR43 activation prevented colon inflammation and carcinogenesis (52,53). The high signal intensities of SLC5A8 and GPR43 staining observed in the current study indicated that *B. pullicaecorum* administration may be associated with CRC prognosis. For instance, the present and other previous studies have observed that butyrate reduced histone deacetylase (HDAC) activity and acted as an HDAC inhibitor (21,54). SLC5A8-dependent inhibition of HDAC activity can be caused by the production of butyrate (55). These results indicated that the reduction of HDAC activity caused by butyrate may be due to SLC5A8-dependent inhibition. This hypothesis is supported by our previous studies concerning *in vivo* animal and *in vitro* cell models.

The results of the animal experiments indicated that *B. pullicaecorum* exhibited potential anti-CRC activity by increasing the expression of SLC5A8 and GPR43 in an animal model of DMH/DSS tumorigenesis. In the *in vitro* cell model, conditioned medium from cultured *B. pullicaecorum* affected the growth of SW480 and SW620 cells. There was a minor difference between the two cell lines: The SW480 and SW620 cells were derived from primary tumor and lymph-node metastasis, respectively, from the same patient. SW620 cells exhibit a short doubling time (~25 h) and grow faster than SW480 cells (50 h doubling time) under regular incubation conditions (56,57). Growth reduction was found in SW480 cells and SW620 cells, indicating that a metabolite of *B. pullicaecorum*, butyrate, served a potential role in the inhibition of CRC cell growth. Notably, the growth inhibition effect was more apparent for cells from an advanced-stage of CRC, such as in SW620 cells. The present study further revealed that butyrate upregulated the expression of SLC5A8 and GPR43 in CRC cells. Additionally, SLC5A8 has been reported to mediate the concentrative entry of butyrate from the lumen into colonocytes (58). Moreover, advanced CRC cells, including SW620 cells, have been demonstrated to express very low GPR43 (59,60). Therefore, the changes in the expression of SLC5A8 and GPR43 in CRC cells needs to be elucidated.

The histopathological and other results of the present study demonstrated that DMH/BP mice exhibited decreased colon tumor progression. These findings indicated positive effects of *B. pullicaecorum* in CRC-bearing mice. CRC-bearing mice that were not treated with *B. pullicaecorum* exhibited advanced CRC tumors. Additionally, the butyrate-induced upregulated expression of SLC5A8 and GPR43 may alter the progression of CRC cells *in vitro* or *in vivo*.

The importance of probiotics in the prevention and treatment of CRC has been studied (61) and there is increasing evidence of the clinical significance of butyrate-producing gut microbes (44,62,63). For instance, another butyrate-producing probiotic in the same family of *B. pullicaecorum*, *Clostridium butyricum*, has been demonstrated to prevent tumor development in the intestinal barrier (64,65). Other families (e.g. *Lachnospiraceae* and *Ruminococcaceae*) also include a number of butyrate-producing microbes (63). Therefore, one limitation

of the present study was that studying *B. pullicaecorum* alone cannot fully reflect all the butyrate-producing probiotics in CRC.

B. pullicaecorum is considered to be a potential next-generation probiotic that is safe following oral administration (28). The microbial metabolite SCFAs, including butyrate, are involved in the regulation of intestinal homeostasis and IBD pathogenesis (66). Dysbiosis leading to reduction in SCFA levels is associated with numerous human diseases, such as stroke and non-small-cell lung cancer (62,63,67). Additionally, SCFAs are associated with certain physiological processes, including immune function (68), anti-inflammatory effects (69) and glucose homeostasis (70-72). SCFAs are considered to have potential as therapeutic agents against gastrointestinal cancers (73). Wang *et al* (18) reported that the four-carbon molecule butyrate may lower the risk of CRC in the absence of dysbiosis in the gut.

Animals are regarded as independent entities that coexist with microbiota in a symbiotic relationship (74,75). Understanding the interactions between the colonic tract and gut microbiota may lead to improved opportunities in the treatment of CRC (75). Accordingly, further studies are required to explore the clinical application of *B. pullicaecorum* and other butyrate-producing microbes or their major metabolite, butyrate, for patients with CRC. In conclusion, the present study demonstrated that the administration of *B. pullicaecorum* or its metabolite(s) improved the clinical outcome of CRC in a mouse model. These results indicated that *B. pullicaecorum* was a probiotic with anti-CRC potential (21,76).

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

SCC, MHS and CJH designed and conducted the experiments. SCC and MHS conceived the present study. CYL and CJH performed the pathological analyses. CMP and JMH contributed to the data interpretation. All authors read and approved the final manuscript.

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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