Nutrition and Cancer

The Effects of Short-Chain Fatty Acids on Human Colon Cancer Cell Phenotype Are Associated with Histone Hyperacetylation[1]

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ABSTRACT The short-chain fatty acid (SCFA) butyrate is produced via anaerobic bacterial fermentation within the colon and is thought to be protective in regard to colon carcinogenesis. Although butyrate (C4) is considered the most potent of the SCFA, a variety of other SCFA also exist in the colonic lumen. Butyrate is thought to exert its cellular effects through the induction of histone hyperacetylation. We sought to determine the effects of a variety of the SCFA on colon carcinoma cell growth, differentiation and apoptosis. HT-29 or HCT-116 (wild-type and p21-deleted) cells were treated with physiologically relevant concentrations of various SCFA, and histone acetylation state was assayed by acid-urea-triton-X gel electrophoresis and immunoblotting. Growth and apoptotic effects were studied by flow cytometry, and differentiation effects were assessed using transient transfections and Northern blotting. Propionate (C3) and valerate (C5) caused growth arrest and differentiation in human colon carcinoma cells. The magnitude of their effects was associated with a lesser degree of histone hyperacetylation compared with butyrate. Acetate (C2) and caproate (C6), in contrast, did not cause histone hyperacetylation and also had no appreciable effects on cell growth or differentiation. SCFA-induced transactivation of the differentiation marker gene, intestinal alkaline phosphatase (IAP), was blocked by histone deacetylase (HDAC), further supporting the critical link between SCFA and histones. Butyrate also significantly increased apoptosis, whereas the other SCFA studied did not. The growth arrest induced by the SCFA was characterized by an increase in the expression of the p21 cell-cycle inhibitor and down-regulation of cyclin B1 (CB1). In p21-deleted HCT-116 colon cancer cells, the SCFA did not alter the rate of proliferation. These data suggest that the antiproliferative, apoptotic and differentiating properties of the various SCFA are linked to the degree of induced histone hyperacetylation. Furthermore, SCFA-mediated growth arrest in colon carcinoma cells requires the p21 gene. J. Nutr. 132: 1012–1017, 2002.

KEY WORDS: • *fatty acids* • *histone hyperacetylation* • *cyclin-dependent kinase inhibitor* • *intestinal alkaline phosphatase* • *histone deacetylase*

Colon cancer is the second leading cause of cancer deaths in the United States and among the most common cancers in Western countries (1). Numerous epidemiologic studies have suggested that environmental factors strongly influence its incidence. Dietary factors such as ingested fat and secondary bile acids have been shown to act as tumor promoters, whereas fiber has been reported to exert antitumor activity (2,3). Short-chain fatty acids (SCFA)[4] are the principal by-products of fiber fermentation in the gastrointestinal tract and one SCFA, butyrate (C4), has been shown to induce growth inhibition and terminal differentiation in a variety of human colon cancer cell lines (4,5). Furthermore, in vivo studies have associated butyrate levels with a decreased incidence of colon cancer (3,6), and butyrate instilled into the colonic lumen reduced tumor production in a chemical model of colon carcinogenesis (7).

Although its precise mechanisms of action are not well understood, butyrate inhibits histone deacetylase (HDAC), resulting in a relative hyperacetylation of core histone proteins (H3 and H4) (8). Hyperacetylation of histones disrupts ionic interactions with the adjacent DNA backbone, creating less densely packed chromatin, or euchromatin, and allowing transcription factors to activate specific genes (9). This action of butyrate is likely to occur in vivo because rats fed a high fiber diet had high luminal butyrate levels, and this was associated with histone hyperacetylation and growth inhibition in colonic epithelial cells (10).

The link between histone hyperacetylation-induced transcriptional regulation and growth inhibition may lie in the effects upon specific cell cycle regulators. The eukaryotic cell cycle is regulated by protein cyclins, their catalytic partners

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[4] Abbreviations used: C2, acetate; C3, propionate; C4, butyrate; C5, valerate; C6, caproate; CB1, cyclin B1; Cdk, cyclin-dependent kinases; FACS, fluorescence-activated cell sorting; HDAC, histone deacetylase; IAP, intestinal alkaline phosphatase; p21, p21-WAF1; SCFA, short-chain fatty acids.

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(cyclin-dependent kinases, Cdk), as well as a family of Cdk inhibitor proteins (11). Two cell cycle regulators that appear to play a role in colon carcinogenesis are cyclin B1 (CB1) and the Cdk inhibitor, p21. Human colon cancers have been shown to express abnormally high CB1 levels (12) and the p53 tumor suppressor protein has been shown to inhibit CB1 function, perhaps explaining in part the mechanism by which p53 mutations lead to the development of colon cancer (13). p21 induces cell cycle arrest via inhibition of Cdk 2, 3, 4 and 6 in normal and tumor cell lines. A distinguishing feature of p21 is that it has a broad target specificity, affecting Cdk2 in complexes with cyclin A and E, as well as Cdk4 and Cdk6 in complexes with cyclins D_1, D_2 and D_3 (14,15). Butyrate induces cell cycle arrest and terminal differentiation in HT-29 cells (16,17), effects that are associated with a dramatic down-regulation of CB1 mRNA expression and induction of p21 (16,18). Furthermore, we showed recently that p21 is required for the butyrate-mediated growth arrest in colon cancer cells (19).

In addition to its effects on growth and differentiation, butyrate also significantly increases apoptosis in various cell lines (20–23). Recent evidence suggests that butyrate induces apoptosis through a histone hyperacetylation–mediated pathway, which results in the conversion of caspase-3 from its proenzyme form to the catalytically active protease, a process that is dependent on new protein synthesis (20).

Although butyrate has been studied most extensively, other SCFA are present within the colon, including acetate (C2), propionate (C3), valerate (C5) and caproate (C6), and the relative amounts of each SCFA depend largely upon the type and amount of ingested fiber (3,10). Some of the SCFA are known to modify histones in a manner similar to that seen with butyrate; however, the precise cellular effects of the different SCFA have not been well characterized. Therefore, in the present study, we examine the effects of all five of these SCFA on colon cancer cell growth, differentiation and apoptosis with specific focus on the potential role(s) of histone hyperacetylation and the cell cycle regulators.

MATERIALS AND METHODS

Cell culture. HT-29 cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA) and maintained in Dulbecco's modified Eagle's medium (Gibco, Grand Island, NY) ± 10% fetal bovine serum (v/v), 2 mmol/L L-glutamine, 1×10^5 U/L penicillin/streptomycin (Gibco) and 95%O_2/5%CO_2 at 37°C. HCT-116 (p21 +/+), and (p21 −/−) cells were kindly provided by B. Vogelstein (Johns Hopkins University) and maintained in McCoy's 5A Medium Modified (Gibco, Grand Island, NY) ± 10% fetal bovine serum (v/v), 2 mmol/L L-glutamine, 1×10^5 U/L penicillin/streptomycin (Gibco) and 95%O_2/5%CO_2 at 37°C. Each experiment was performed with the cells at ~80% confluence. The HT-29 and HCT-116 cell culture media were replenished at the beginning of each experiment with 5 mmol/L and 1 mmol/L SCFA, respectively (Sigma, St. Louis, MO). Treatment of cells with SCFA was as follows: acetic acid (C2, as acetate), propionic acid (C3, as propionate), butyrate acid (C4, as butyrate), valeric acid (C5, as valerate) or caproic acid (C6, as caproate) for 24 h, unless otherwise indicated. This time point was chosen on the basis of previous work, (16,18,19,24) which showed significant responses to butyrate for each end point studied. For control experiments, a saline solution was substituted for the SCFA. As indicated, some experiments were carried out under serum-starved conditions, i.e., after 24 h of serum deprivation.

Growth/Apoptosis assays. Cells were seeded at a density of ~2 $\times 10^6$ cells/well in 6-well cluster plates (Sigma) and appropriate treatments were given for 24 h. The medium was aspirated and discarded and the adherent cells treated with trypsin-versene mixture (Bio-Whittaker, Walkersville, MD) at 37°C until they just began to slough. Cells were then immediately washed with PBS and fixed in 70% ethanol (v/v) overnight. The next day, the collected cells were centrifuged at 800 × *g* for 10 min, resuspended in 50 mg/L propidium iodide (Sigma) in PBS and immediately subjected to flow cytometry optimized for propidium iodide using a fluorescence-activated cell sorting (FACS) Scan (Becton Dickinson, Franklin Lakes, NJ). Appropriate settings of forward and side scatter gates were used to examine 10,000 cells/experiment. Results were analyzed with Cell Quest (Becton Dickinson), and Modfit (Verity Software House, Topsham, ME) softwares.

Transient transfections. Cells were seeded at a density of ~5 $\times 10^5$ cells/well in 6-well cluster plates (Sigma). Transient transfections were accomplished using the $CaPO_4$/DNA coprecipitation technique. Luciferase reporter plasmid (10 μg) containing 2.4 kb of the human intestinal alkaline phosphatase (IAP) 5′ flanking region ($IAP_{2.4}$-Luc) was transfected into HT-29 cells with or without 5 μg histone deacetylase enzyme expression plasmid (HDAC1, provided by Stuart Schreiber, Harvard University). The total amount of DNA was kept the same for each transfection by addition of vector DNA; an empty expression vector was used as a control for HDAC1 transfection. Cells were then treated with 5 mmol/L SCFA for 24 h, harvested, and total protein was isolated and quantified. All cell culture plates received 2 μg of a CMV β-galactosidase plasmid to control for transfection efficiency, and the reported luciferase activities represent normalization with β-galactosidase activities.

Analysis of histone acetylation state. After 6 h incubation with 5 mmol/L SCFA, nuclear histones were prepared by acid extraction with 0.2 mol/L H_2SO_4 (Sigma), recovered by acetone precipitation and separated by slab gel electrophoresis using a 10-cm acid-urea-triton-X gel [5% acetic acid (v/v, Fisher, Fair Lawn, NJ), 8 mol/L urea (Sigma), 0.4% Triton X-100 (v/v, Sigma), 0.1% *N*,*N*′-methylenebis-acrylamide (v/v, Bio-Rad, Richmond, CA) and 2.1 mol/L acrylamide (Bio-Rad)] with incorporation of a 3-cm upper gel [5% acetic acid (v/v, Fisher), 10 mol/L urea (Sigma), 5% acrylamide/bis-acrylamide(v/v, Bio-Rad), and 4.2 mol/L acrylamide (Bio-Rad)]. Histones (50 μg) were incubated with the same volume of loading buffer (10 mol/L urea, 5 mol/L NH_4OH and 10 mmol/L dithiothreitol) for 5 min and 0.125 volume of 33 mmol/L pyronine G (Sigma) in glacial acetic acid was added. The mixture was then loaded onto the upper stacking gel and subjected to electrophoresis overnight in glycine (0.2 mol/L, Sigma) and acetic acid (1 mol/L, Sigma). Gels were treated with coomassie blue stain (Coomassie Brilliant Blue R-250, Sigma), destained in 40% methanol and 10% glacial acetic acid (v/v/v; Sigma), dried, and photographed.

Histone immunoblotting. Histones were prepared by acid extraction and acetate precipitation as above. A total of 30 μg of histones were incubated with the same volume of loading buffer (10 mol/L urea, 5 mol/L NH_4OH and 10 mmol/L dithiothreitol) for 5 min, and then added to 0.125 volume of 33 mmol/L pyronine in glacial acetic acid (Sigma). The mixture was loaded onto the gel, electrophoresed, and transferred onto Immobilon-P (Millipore, Bedford, MA) overnight at 40 V. Membranes were incubated with 1:1000 antiacetylated H4 antibody (Upstate Biotechnology, Lake Placid, NY), followed by 1:2000 anti-rabbit secondary antibody, and visualized by standard chemiluminescence (Amersham Life Science, Bucks, England). Analyses of reaction products were performed with Deskscan II (Hewlett-Packard, Mountain View, CA), and ImageQuan (Molecular Dynamics, Sunnyvale, CA). The resulting numerical values were normalized relative to controls, and expressed as the percentage increase in H4 hyperacetylation.

Northern blot analyses. Total RNA was extracted using the guanidium thiocyanate method (25) after 48 h of SCFA incubation. Northern blot analyses were performed by loading 20 μg of RNA/lane of an agarose-formaldehyde gel, separating through electrophoresis, transferring onto nitrocellulose membranes and baking for 2 h at 80°C. Equal loading of RNA per lane was confirmed by examination of ethidium bromide–stained gels. Complementary DNA probes were ^{32}P-radiolabled to a specific activity of ~5×10^8 cpm/μg DNA. The IAP probe was a 1.9-kb *Pst*1 fragment derived from the human IAP cDNA (26) and the p21 probe was a 1-kb *Xho*I/*Eco*RI fragment derived from the human Cip1 cDNA (15). Both the IAP and p21

cDNA were obtained from ATCC, Rockville, MD. The cyclin B1 probe was a 1.4-kb NotI/KpnI fragment derived from the human cDNA (27), and the actin probe was a 1.0-kb *Pst*1 fragment derived from the mouse β-actin cDNA (28). Hybridizations were carried out in 5X SSC/50% formamide/1% SDS at 42°C. The washing conditions were 2X SSC/0.1% SDS at 50°C. Relative changes in mRNA levels were determined by laser densitometry and normalized with respect to actin mRNA.

Statistical methods. Statistical analyses were performed using a standard one-way ANOVA with Dunnet's post-test (InStat software, GraphPad Software, San Diego, CA). Differences with $P < 0.05$ were considered significant.

RESULTS

Effects of SCFA on histone hyperacetylation. C4 induced the greatest increase (115.4%) in H4 hyperacetylation compared with untreated controls, whereas C3 and C5 produced less dramatic increases of 40.2 and 60.6%, respectively (**Fig. 1** $P < 0.05$). In contrast, C2 and C6 had no appreciable effects on the acetylation of histone H4 ($P > 0.05$). Similar results were seen in the case of histone H3 (data not shown).

SCFA effects on HT-29 cell growth. Treatment with 5 mmol/L C3, C4, or C5 decreased S-phase activity 35.5, 86.8 and 76.3%, respectively, compared with untreated controls (**Fig. 2***A*, $P < 0.005$). In contrast, cellular growth rate was not altered by treatment with either C2 or C6 ($P > 0.05$).

Effects of SCFA on the cell cycle regulators, p21 and cyclin B1. p21 mRNA expression was induced by C3, C4 and C5, but not C2 or C6 (Fig. 2*B*). A minimal increase in p21 mRNA levels was observed with C6 treatment. In contrast to the p21 induction, C3, C4 and C5 each caused dramatic reductions in CB1 mRNA levels. Just as they had no effects on p21, C2 and C6 did not alter CB1 mRNA levels. Untreated cells were used as the control. Identical time courses were seen with the different SCFA; p21 induction occurred early (within 2 h) and CB1 down-regulation occurred at 24 h (data not shown).

p21 is required for SCFA-mediated growth inhibition. Treatment of the parent HCT-116 cells (p21 +/+) with C3, C4 or C5 decreased S-phase activity 60.0, 60.4 and 62.8%, respectively, compared with untreated controls (**Fig. 3**, $P < 0.005$). As was the case in HT-29 cells, C2 and C6 did not affect the rate of HCT-116 cell growth ($P > 0.05$). In contrast to the findings in the wild-type cells, p21-deleted (−/−) HCT-116 cells treated with the various SCFA did not differ from controls in S-phase activity, indicating a requirement for p21 for the SCFA-induced growth effects. Both wild-type and p21-deleted HCT-116 cells exhibited cell cycle arrest in re-

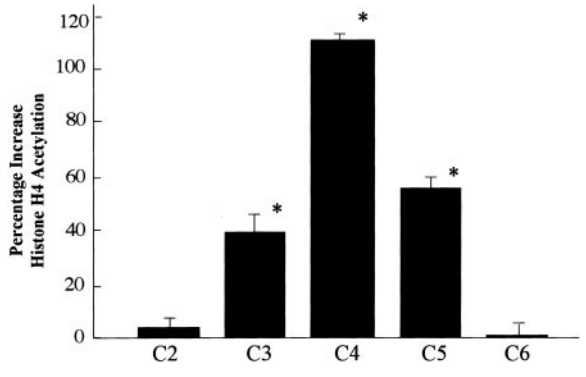

FIGURE 1 Selective induction of histone H4 hyperacetylation in the human colon cancer HT-29 cell line after 24 h incubation with 5 mmol/L short-chain fatty acids (SCFA; C2, acetate; C3, propionate; C4, butyrate; C5, valerate; C6, caproate). HT-29 cells grown under standard culture conditions were or were not treated with 5 mmol/L SCFA (C2–C6) for 6 h. Histone proteins were purified by acid extraction, acetone precipitation and then detected by acid-urea-triton-X electrophoresis followed by anti-acetyl H4 antibody chemiluminescence. Acetylation of H4 protein is depicted in arbitrary densitometric units and expressed as the percentage of increase in H4 hyperacetylation compared with untreated (saline only) control cells. Values are means ± SEM, $n = 3$. *Different from control, $P < 0.05$.

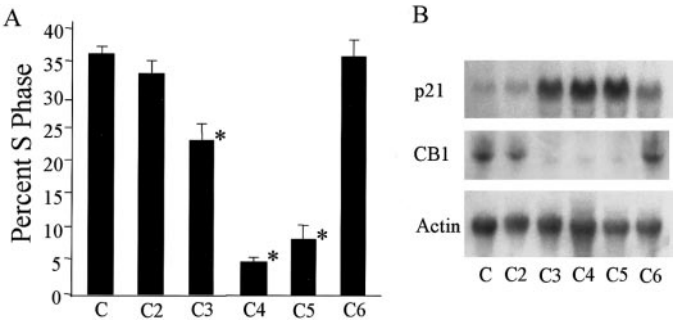

FIGURE 2 Growth inhibition, p21 and cyclin B1 (CB1) expression in the human colon cancer HT-29 cell line after 24 h incubation with 5mmol/L short-chain fatty acids (SCFA; C2, acetate; C3, propionate; C4, butyrate; C5, valerate; C6, caproate). (*A*) HT-29 cells grown under standard culture conditions were or were not treated with 5 mmol/L SCFA (C2–C6) for 24 h. The cells were then harvested and subjected to fluorescence-activated cell sorting (FACS) analysis for the percentage of S-phase. Values are means ± SEM, $n = 3$. *Different from control, $P < 0.005$). (*B*) HT-29 cells grown under standard culture conditions were or were not treated with 5 mmol/L SCFA (C2–C6) for 24 h after which the cells were harvested, total RNA extracted and purified by the guanidium thiocyanate method (22) and analyzed by Northern blotting. p21 mRNA expression was induced and CB1 mRNA expression was down-regulated by C3–C5 treatment. A representative Northern blot is depicted ($n \geq 3$) with an actin control shown in the *lower panel*.

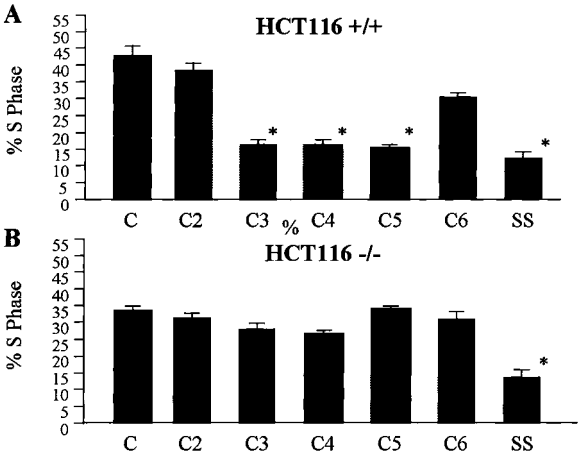

FIGURE 3 The role of p21 in short-chain fatty acid (SCFA)-induced growth arrest in the HCT-116 human colon cancer cell line. Wild-type HCT-116 (p21+/+) (*panel A*) and p21 knock out (HCT-116 p21−/−) (*panel B*) cells grown under standard culture conditions were or were not treated with 5 mmol/L SCFA (C2, acetate; C3, propionate; C4, butyrate; C5, valerate; C6, caproate) or subjected to serum starvation (SS; positive controls) for 24 h then harvested and subjected to fluorescence-activated cell sorting (FACS) analysis for the percentage of S-phase. Values are means ± SEM, $n \geq 3$. *Different from control, $P < 0.01$.

sponse to serum starvation (69.8 and 55.8% reductions in S-phase activity, respectively, Fig. 3*A*, *B*; $P < 0.01$, $n = 3$).

Effects of SCFA on HT-29 cell differentiation. Induction of the differentiation marker, IAP, was observed only with C3, C4 and C5 treatment, whereas C2 and C6 had no effects on IAP mRNA levels (**Fig. 4***A*) compared with untreated controls. As with the p21 and CB1 effects, the various SCFA induced IAP expression with identical time courses, with maximal levels at 48 h (data not shown). Similarly, HT-29 cells transfected with the IAP reporter plasmid and treated with C3, C4 and C5 had 13-, 9- and 10-fold increases in luciferase activity, respectively ($P < 0.005$). However, as seen with the endogenous IAP mRNA, treatment with C2 or C6 did not significantly alter the level of IAP reporter gene activity ($P > 0.05$). Cotransfection studies in HT-29 cells revealed that HDAC1 inhibited the C3-, C4- and C5-mediated IAP transactivation by 70, 78 and 68%, respectively, compared with cells cotransfected with the IAP reporter and empty expression vector ($P < 0.01$), confirming that histone hyperacetylation is at least part of the mechanism by which the SCFA induce IAP expression (**Fig. 5**).

Effects of SCFA on apoptosis. Of the SCFA studied, only butyrate (C4) increased apoptosis compared with untreated control cells (sevenfold, $P < 0.001$) as measured by FACS analysis. C2, C3, C5 and C6 did not affect the level of apoptosis (**Fig. 6**).

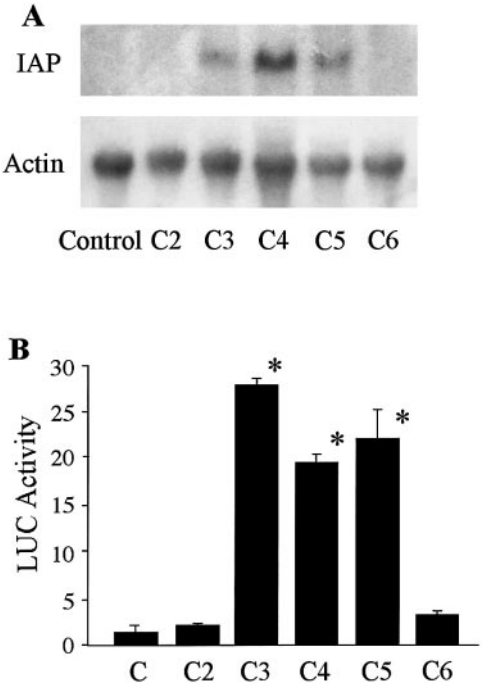

FIGURE 4 Effects of short-chain fatty acids (SCFA) on intestinal alkaline phosphatase (IAP) gene expression in the human colon cancer HT-29 cell line. (*A*) HT-29 cells grown under standard culture conditions were or were not treated with 5 mmol/L SCFA (C2, acetate; C3, propionate; C4, butyrate; C5, valerate; C6, caproate) for 24 h after which total RNA was extracted and purified by the guanidium thiocyanate method (22) and subjected to Northern blotting. A representative blot depicts IAP mRNA levels after C2-C6 treatment ($n \geq 3$). The actin control is shown in the *lower panel*. (*B*) HT-29 cells grown under standard culture conditions were transiently transfected with an IAP luciferase construct, treated or not with 5mmol/L SCFA (C2–C6) for 24 h after which the total protein was purified and assayed for luciferase activity using the luciferase gene reporter assay kit per the manufacturer's instructions (Tropix, Bedford, MA). The results are presented as absolute luciferase activity. Values are means ± SEM, $n \geq 3$. *Different from control (C), $P < 0.005$) in arbitrary luminescence units.

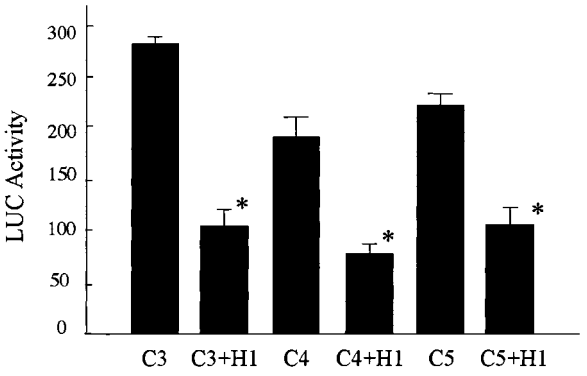

FIGURE 5 Inhibition of C3-, C4- and C5-induced intestinal alkaline phosphatase (IAP) gene transactivation by histone deacetylase (HDAC)1 (H1) overexpression in the human colon cancer HT-29 cell line. HT-29 cells grown under standard culture conditions were transiently cotransfected with an IAP luciferase reporter construct and an HDAC1 expression plasmid, treated or not with 5 mmol/L short-chain fatty acids (SCFA; C2, acetate; C3, propionate; C4, butyrate; C5, valerate; C6, caproate) for 24 h after which the total protein was purified and assayed using the luciferase gene reporter assay kit according to the manufacturer's instructions (Tropix, Bedford, MA). In addition, all culture plates received 2 μg of CMV β-galactosidase, and luciferase activities were normalized with respect to β-galactosidase activities. Empty expression vector was used as a control to keep the total amount of DNA transfected equal in all experiments. Results are absolute luciferase activities in arbitrary luminescence units. Values are means ± SEM, $n = 3$. *Different from the corresponding control, $P < 0.01$).

DISCUSSION

The present studies show that the ability of short-chain fatty acids to induce cellular growth arrest, differentiation and apoptosis in colon cancer cells appears to be dependent upon their histone hyperacetylating effects. Previously, Siavoshian et al. (29) observed that C3, C4 and C5 caused growth arrest and differentiation in colon cancer cell lines, with C4 having the most potent effects. We have extended these findings by showing that these SCFA cause varying degrees of histone acetylation, which are directly associated with the observed

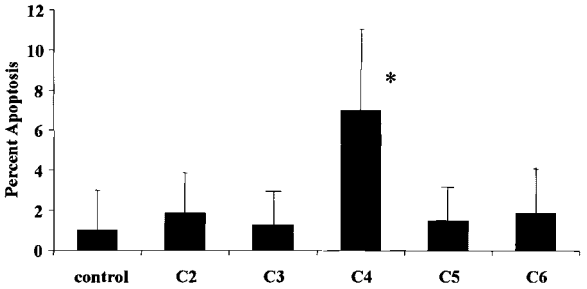

FIGURE 6 Butyrate treatment causes apoptosis in human colon cancer HT-29 cells. HT-29 cells grown under standard culture conditions were or were not treated with 5 mmol/L short-chain fatty acids (SCFA; C2, acetate; C3, propionate; C4, butyrate; C5, valerate; C6, caproate) for 24 h after which the cells were harvested, fixed in 70% ethanol (v/v), stained with propidium iodide and subjected to fluorescence-activated cell sorting (FACS) analysis for percent apoptosis. Values are means ± SEM, $n = 9$. *Different from control, $P < 0.01$).

response. In contrast, C2 and C6 had no appreciable effects on histones, nor did they induce growth arrest or differentiation in HT-29 cells. Furthermore, the present work shows that the growth arrest is dependent on alterations in the expression of the cell cycle regulators p21 and CB1. In addition, we showed a critical link between histone acetylation and SCFA-induced differentiation by demonstrating an attenuation of this response by overexpression of histone deacetylase. Finally, we examined the SCFA effects on apoptosis, and interestingly, only C4 increased the rate of programmed cell death.

Among the various SCFA, C4 caused the greatest increase in overall histone acetylation, whereas C3 and C5 resulted in more modest increases. Short-chain fatty acids inhibit histone deacetylases in vitro, leading to the hyperacetylation of selective histone proteins such as histone H4. Cousens et al. (30) demonstrated that the inhibition of calf thymus HDAC by SCFA varies among the different acids, with C4 the most potent (~ 80% inhibition), followed by C3 and C5 (~60–65% inhibition), and C6 (~ 30% inhibition). Our results in the colon cancer cells generally support these findings because C4 induced the greatest amount of tetra-acetylated histone H4, followed by C3 and C5. It is interesting to note that we found that C6 treatment did not induce H4 hyperacetylation in HT-29 cells, perhaps reflecting cell-type specificity in sensitivity to the SCFA.

Similar to the results with growth arrest, we found that the alterations in the expression of the cell cycle regulators, p21 and CB1 were seen only in the case of those SCFA (C3, C4 and C5) that induced histone hyperacetylation. Other investigators found a similar correlation with the SCFA-induced histone hyperacetylation and the modulation of the chemokines, interleukin-8 and monocyte chemoattractant protein-1 (31,32). Previous studies from our laboratory have shown that butyrate-induced growth arrest in colon cancer cells was dependent upon the p21 gene and was associated with a down-regulation of CB1 (19). The present results extend these findings to the other SCFA, demonstrating that C3 and C5 also did not induce growth arrest in the p21-deleted HCT-116 cells. p21 has been shown to induce cell cycle arrest after a wide range of stimuli, including cellular senescence, DNA damage, serum starvation and differentiation (33), and the present data further support a critical link between the p21 gene and the beneficial effects of dietary fiber in colon carcinogenesis. Interestingly, in contrast to its role in SCFA-induced growth arrest, p21 does not appear to be required for butyrate-mediated apoptosis (24). It is not clear what role p21 may play in the differentiation process.

In addition to their effects on p21 expression, the various SCFA down-regulated CB1 mRNA levels in the human colon cancer cells. Cyclin B1 appears to be critical for normal development because knockout mice die in utero (34). Furthermore, CB1 levels are high in human colon cancers (12) and CB1 has been linked to the p53 tumor suppressor, suggesting a possible role in neoplasia (13). As such, it is possible that part of the means by which SCFA exert their antitumor activity is through CB1 repression. Interestingly, Ohta et al. (35) demonstrated that CB1 and p21 coexist in a Cdk-containing complex and Choi et al. (36) showed that genestein-induced cell cycle arrest in breast cancer cells was associated with both CB1 inhibition and p21 induction. These studies along with the present data support a potentially important link between CB1 and p21 in terms of cell cycle control in cancer cells.

Recent reports have implicated chromatin remodeling via histone hyeracetylation/deacetylation in the pathogenesis of cancer. For example, Pasqualini et al. (37) showed that estrogen decreased histone acetylation in a human mammary cancer cell line, suggesting a role for histone hypoacetylation in regard to the pathogenesis of hormone-responsive breast cancer. In addition, the ability of the retinoblastoma tumor suppressor protein to mediate E2F-bound promoter repression stems from its inhibition of HDAC 1 (38,39), further supporting a role for histone hyperacetylation in the suppression of carcinogenesis. Our present findings showed that cotransfection with HDAC 1 attenuated C3-, C4- and C5-induced IAP transactivation in HT-29 cells, suggesting that IAP induction occurred via a mechanism involving histone hyperacetylation. We previously showed that HDAC1 largely inhibited butyrate-induced p21 gene transactivation (19). These results all point to the importance of histone hyperacetylation in both the growth arrest and differentiating effects of the SCFA.

Interestingly, the apoptotic effects of butyrate were not shared by the other SCFA that cause histone hyperacetylation, namely, C3 and C5. Others have found that C2, C3 and C5 can induce apoptosis in various cell lines (20–23). These studies used different assays and time points, but in general found that the increases in apoptosis were greatest with C4 and much smaller or absent with the other SCFA. It is possible that the assay used in the present studies was not sensitive enough to detect small increases in apoptosis that may have been caused by the other SCFA. Another possibility is that butyrate has a greater specificity for one of the isozymes of HDAC, which is important in the activation of the apoptotic pathway. Finally, there may be a temporal aspect to the histone hyperacetylation–induced transcriptional activation mechanism because we found recently that transient histone hyperacetylation has effects that are different from those of prolonged histone hyperacetylation (24). Further work will be required to elucidate the precise relationship between histone hyperacetylation and apoptosis in colon cancer cells. The recent cloning and molecular characterization of a family of histone deacetylases, HDAC 1, 2 and 3 (40) provide the opportunity to further define the mechanisms by which histone acetylation alters cell cycle progression, differentiation and apoptosis.

In conclusion, we have determined the relative effects of a variety of SCFA on histone acetylation, cellular growth arrest, differentiation and apoptosis in colon carcinoma cells. Our results clearly point to the importance of the histone-modifying effects of SCFA. Because different types of dietary fiber produce varying amounts of the specific SCFA (3,8), it is likely that the exact composition of fiber within the colonic lumen may determine its cellular effects, including its possible beneficial role in the prevention and/or treatment of colon cancer.

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