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Research Communications

Induction of tissue transglutaminase expression by propionate and n-butyrate in colon cancer cell lines

Kazunori Fukuda

Department of Oriental Medicine, Gifu University School of Medicine, Gifu, Japan

Short-chain fatty acids (SCFAs) have been demonstrated to induce differentiation and/or apoptosis in colon cancer cells. A close correlation between tissue transglutaminase (tTG) expression and differentiation and/or apoptosis has been suggested in many cell lineages. However, the effects of SCFAs on tTG expression in colon cancer cells have not yet been reported. In this report, the relationship between cytosolic tTG levels and differentiation state was investigated in six human colon cancer cell lines. Effects of four kinds of SCFAs (acetate, propionate, n-butyrate, and isobutyrate) on the expression of tTG then were investigated in association with their effects on apoptosis induction. High expression of tTG protein and mRNA were found in SW480 and WiDr cell lines, which exhibited well differentiated phenotypes. tTG expression was hardly detectable in the less differentiated cell lines COLO201, COLO320DM, and CW-2. However, n-butyrate and propionate significantly increased cytosolic tTG levels at concentrations above 0.5 mM in these less differentiated colon cancer cells. n-Butyrate and propionate induced growth suppression and apoptosis in these cell lines at concentrations that can induce tTG expression. Acetate and isobutyrate did not induce tTG expression or growth suppression at concentrations up to 8 mM. In conclusion, tTG induction by propionate and n-butyrate was suggested to be closely linked to their differentiation- and apoptosis-inducing effects in colon cancer cells. These findings may explain the mechanisms by which dietary fiber show preventive effects against colon carcinogenesis. (J. Nutr. Biochem. 10:397–404, 1999)

Keywords: short-chain fatty acids; n-butyrate; propionate; tissue transglutaminase; apoptosis; colon cancer cells

Introduction

Short-chain fatty acids (SCFAs) are produced in the mammalian colon as a by-product of anaerobic bacterial fermentation of dietary fiber. Acetate, propionate, and n-butyrate are quantitatively the most important SCFAs and are present in the colon in high millimolar concentrations.[1] Colonic epithelial cells arise from stem cells located near the base of the colonic glands. After exiting the proliferative compartment, the colonic epithelial cells differentiate, mature and become functional, playing important roles in absorption and secretion, and die via apoptosis at the top of the colonic glands.[2] Recent studies have revealed that SCFAs play an important physiologic role in the maintenance of the integrity of the colonic epithelium by regulating epithelial proliferation and differentiation of the colonic mucosa.[3,4] Many epidemiologic and experimental studies have found that intake of dietary fiber is closely associated with a lower incidence of colon cancer.[5] Although the mechanisms of cancer prevention by dietary fiber may be complex, the fermentation of dietary fiber and nonabsorbed starch to SCFAs by colonic bacteria appears to be a crucial component in colon cancer prevention.[6,7] By decreasing the pH level in the colonic lumen, SCFAs inhibit bacterial enzymes that are responsible for the formation of carcinogens and promoters such as secondary bile acids.[8] Numerous reports have shown that butyrate possesses antineoplastic properties against colon cancer cells in vitro by inducing differentiation and apoptosis.[9–11] Cellular differentiation is a complex

Address correspondence to Dr. Kazunori Fukuda, Department of Oriental Medicine, Gifu University School of Medicine, 40 Tsukasa-machi, Gifu 500-8705, Japan.

655 Avenue of the Americas, New York, NY 10010
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phenomenon associated with exit from the cell cycle and entry into an alternate pathway in which a more sophisticated phenotype and function are assumed.[2] Cells lose differentiated phenotype progressively during carcinogenic processes, and the progress of dedifferentiation is closely associated with malignant phenotypes of cancer cells. Although the effects of butyrate on differentiation and apoptosis in colon cancer cells have been reported by many investigators,[7,9–11] the molecular mechanisms by which butyrate induces differentiation and apoptosis in cancer cells are still not fully elucidated, and the effects of acetate and propionate in cancer cells have been less intensively studied.

Tissue transglutaminase (EC 2.3.2.13; tTG) is a GTP-binding Ca^{2+}-dependent enzyme that catalyzes an acyl transfer reaction between peptide-bound glutamine residues and primary amines including the ε-amino group of lysine residues in appropriate proteins.[12] These reactions result in the functionally significant post-translational modification of proteins either by the specific incorporation of polyamines or the crosslinking of proteins via ε-(γ-glutamyl) lysine bridges. Recent studies revealed several cellular substrates for tTG and showed that tTG-mediated post-translational modification plays an important role in various cellular functions; the involvement of tTG induction in growth arrest, differentiation, and apoptotic processes has been suggested in a number of systems.[13–15] Previous studies showed that malignant cells reduced tTG activity compared with normal cells and that reduced tTG activity is associated with cell proliferation, tumor promotion, and metastatic potential.[15,16]

Various factors have been shown to modulate tTG expression in different cell systems. For example, retinoic acid has been shown to induce tTG expression and differentiation in human promyelocytic leukemia HL60 cells and in mouse peritoneal macrophages.[13] n-Butyrate induces tTG in human lung fibroblast cells.[17] Enhanced expression of tTG by transforming growth factor (TGF)-β1 also is reported in rat hepatoma cells in close association with induction of apoptosis.[18] These reports indicate that tTG induction is closely associated with terminal differentiation and/or growth arrest in various cell types. Investigations of the molecular mechanisms by which SCFAs induce differentiation and apoptosis in colon cancer cells appear to be important for the study of preventive effects of dietary fiber against colon cancer. The effects of SCFAs on tTG expression in colon cancer cells have not yet been described. In the present study, the effects of SCFAs on cytosolic tTG levels were investigated in six human colon cancer cell lines. It is demonstrated here that less differentiated colon cancer cells have reduced tTG amounts and that the SCFAs n-butyrate and propionate significantly increased the cytosolic tTG levels in these colon cancer cells.

Materials and methods

Cell cultures and drug treatment

Six cell lines derived from human colon cancer—COLO201, COLO320DM, CW-2, WiDr, SW480, and SW620—were used in the present study. COLO201 and COLO320DM were obtained from the Japanese Cancer Research Resources Bank (Tokyo, Japan); CW-1 and WiDr were from the RIKEN Cell Bank (Saitama, Japan); and SW480 and SW620 were obtained from the American Type Culture Collection (Rockville, MD, USA). Cells were maintained at 37°C in an atmosphere of 95% air and 5% CO_2 in RPMI1640 medium supplemented with 5% heat-inactivated fetal bovine serum (FBS) and antibiotics (100 μg/mL of streptomycin and 100 units/mL of penicillin). Cells were seeded at 2×10^5/mL in polystyrene tissue culture multi-well plate (CORNING, New York NY USA). The cells then were preincubated for 24 hours before treatment to stabilize cellular functions after cell seeding. Acetic acid, propionic acid, n-butyric acid, and isobutyric acid, all of which were of analytical grade (>97% purity), were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Stock solution (1 M) of each SCFA was prepared in phosphate buffered saline (pH 7.4), and pH of each stock solution was adjusted to 7.4 by adding NaOH.

Reagents that are able to induce differentiation and/or growth suppression also were studied for their effects on tTG induction. These include TGF-β1, dexamethasone, dimethyl sulfoxide, all-trans retinoic acid, and 9-cis retinoic acid. Human recombinant TGF-β1 was obtained from King Brewing Co. Ltd. (Kakogawa, Japan). All-trans retinoic acid and 9-cis retinoic acid were obtained from Wako Pure Chemical Industries, Ltd. Dexamethasone and dimethyl sulfoxide were purchased from Sigma Chemical Co. (St. Louis, MO USA).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblot analysis

Cultured cells were dissolved directly in sodium dodecyl sulfate (SDS) sample buffer (62.5 mM Tris-HCl, pH 6.8, 10 mM EDTA, 5% β-mercaptoethanol, 2% SDS) at a density of 5×10^6 cells/mL, sonicated for 10 seconds, and then heated to 100°C for 5 minutes. Protein of lysate from 1×10^5 cells was separated on 9% SDS-polyacrylamide gel electrophoresis (PAGE) gel and electrophoretically transferred onto Immobilon-P membrane (Nippon Millipore, Tokyo, Japan). After blocking with 2% nonfat skim milk in TTBS (10 mM Tris-HCl, pH 7.5, 140 mM NaCl, 0.05% Tween 20) for 1 hour at room temperature, the membranes were incubated with primary antibody in TTBS containing 1% bovine serum albumin. The membrane then was washed with TTBS, incubated for 1 hour at room temperature with horseradish peroxidase-conjugated secondary antibodies, and then washed and developed with the Amersham ECL chemiluminescent reagent. Monoclonal antibody (MoAb) against guinea pig liver tTG (clone TG100), which reacts with human tTG, was used to detect cytosolic tTG levels in immunoblot analysis.[18] Antibodies then were stripped and the blots were reprobed with anti-actin rabbit antibody (Biomedical Technologies, Stoughton MA USA). Actin protein amount was used as an internal control for relative protein amount loaded in each lane.

Northern blot analysis

Total RNA was extracted from cultured cells by a modified guanidinium thiocyanate method using ISOGEN (Nippon Gene, Tokyo, Japan). Northern blot hybridization was carried out essentially as described previously.[18] Briefly, samples of 20 μg each of total RNA were subjected to electrophoresis on 1.2% agarose gels containing 2.2 M formaldehyde. The RNAs then were transferred to Hybond-N nylon membranes (Amersham Life Science, Tokyo, Japan) and fixed by ultraviolet (UV) irradiation using a Stratalinker UV crosslinker (Stratagene, La Jolla, CA USA). The membrane was hybridized with ^{32}P-labeled tTG cDNA probe. Cloned fragments encoding partial cDNA sequence of mouse tTG (pmTG700), which was kindly provided by Dr. Peter J.A. Davies

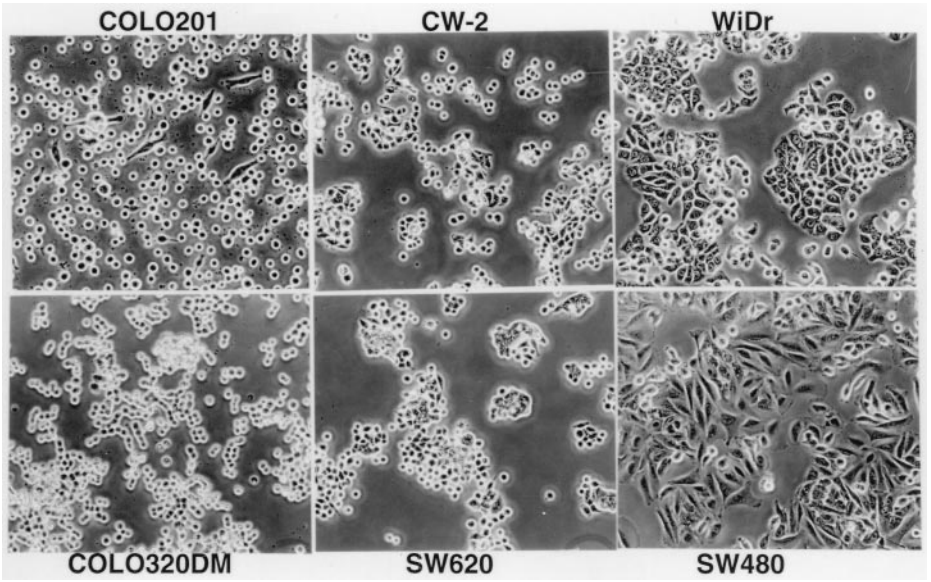

Figure 1 Morphologic features of six human colon cancer cell lines. Cells were plated onto polystyrene tissue-culture dishes (Corning) and cultured in RPMI1640 medium containing 5% fetal bovine serum. Phase-contrast photomicrographs of each cell line were taken 48 hours after plating.

(University of Texas Medical School, Houston, TX USA), were radiolabeled with ^{32}P using a multiprime DNA labeling system (Amersham). After hybridization, the membranes were washed at 55°C in solutions containing 0.1% SDS and decreasing concentrations of salt (2×, 1×, 0.5× SSC). The washed blots were autoradiographed using Kodak XAR-5 film with an intensifying screen at −70°C. The blots were stripped by washing in 0.1% SDS solution for 30 minutes at 90 to 100°C and then rehybridized with ^{32}P-labeled human glyceraldehyde 3 phosphate dehydrogenase (G3PDH) cDNA probe (Clontech, Palo Alto, CA USA). G3PDH mRNA amount was used as an internal control for RNA amount in each lane.

Growth assay

Cells were seeded at 1×10^5/mL in 96-multiwell plates (1×10^4/well) and preincubated for 24 hours before treatment. The cultures were exposed to each of SCFAs at various concentrations (two-fold dilution from 16 mM to less than 0.1 mM). After treatment for 72 hours, the percentage of cell number in treated cultures relative to control cultures was evaluated using WST-1 assay (DOJINDO Laboratories, Kumamoto, Japan) according to the procedure of the manufacturer. WST-1 is a tetrazolium compound that produces water-soluble formazan dye by reduction. Spectrophotometric measurement of formazan products reflects bioreductibility of cells, which originates from various dehydrogenase activities. The percent viability of each treatment was calculated from quadruplicate wells.

DNA fragmentation assay

DNA fragmentation is one of the biochemical markers for apoptotic cell death. Apoptotic cells contain fragmented DNA and viable cells contain intact DNA. The ratio of fragmented DNA to total (fragmented + intact) DNA reflects the ratio of apoptotic cell fraction to total cell fraction. Degree of apoptosis was evaluated by quantitative analysis of fragmented DNA following the method described previously.[19] Briefly, floating and adherent cells were collected and resuspended in 0.5 mL ice-cold lysis buffer [20 mM EDTA, 5 mM Tris-HCl, 0.5% (v/v) Triton-X 100 (pH 8)] and incubated for 30 minutes at 4°C. The samples were centrifuged at 13,000 × g for 20 minutes to separate intact chromatin in the pellet from fragmented DNA in the supernatant. Samples were heated at 90°C for 15 minutes in the presence of 1.5 N perchloric acid and centrifuged at 1,500 × g for 10 minutes to remove protein. Resulting supernatants were reacted with 0.6 volume of diphenylamine reagent for 16 hours at room temperature. Diphenylamine reagent consists of 4% diphenylamine and 0.01% paraaldehyde in glacial acetic acid. The color due to the diphenylamine-deoxypentose sugar reaction was extracted with 250 μL of amyl acetate into the organic phase. The amyl acetate layer was transferred into polypropylene 96-well plates and absorbance was measured at 600 nm with an enzyme linked immunosorbent assay (ELISA) reader. The percentage of DNA fragmentation was obtained from the ratio of fragmented DNA to total (fragmented + intact) DNA. All data are expressed as mean ± SE from quadruplicate wells. Comparisons between control and treated groups were performed with a Student's unpaired *t*-test. *P*-values of less than 0.05 were considered to be significant.

Results

Morphologic features and constitutive levels of tTG protein and mRNA in colon cancer cell lines

Figure 1 shows phase-contrast photomicrographs of six human colon cancer cell lines under control conditions. The photomicrographs demonstrate the morphologic features of cells at the maturity levels used when experimental analysis was performed. The majority of COLO201 cells floated in the medium and a few attached to the culture plate, indicating loss of cell-cell and cell-matrix interactions. COLO320DM, CW-2, and SW620 cells were attached loosely to the culture plate and had a spherical shape, indicating weak cell-cell and cell-matrix adhesion. SW480 and WiDr had a flattened polygonal shape and form a more adherent epithelial sheet. SW480 cells showed the highest adhesiveness to culture plates among the cell lines and many cells started to spread 4 hours after plating. SW620 and SW480 were derived from the same patients; SW480 was isolated from the primary tumor and SW620 was isolated from metastatic tumor in a lymph node. SW620 cells were reported to be more dedifferentiated than SW480 cells.[20] Growth activity is higher in COLO320DM and SW620 (doubling time of approximately 20 hours), and lower in CW-2 and WiDr (doubling time of approximately 40 hours) in the culture conditions used in the present

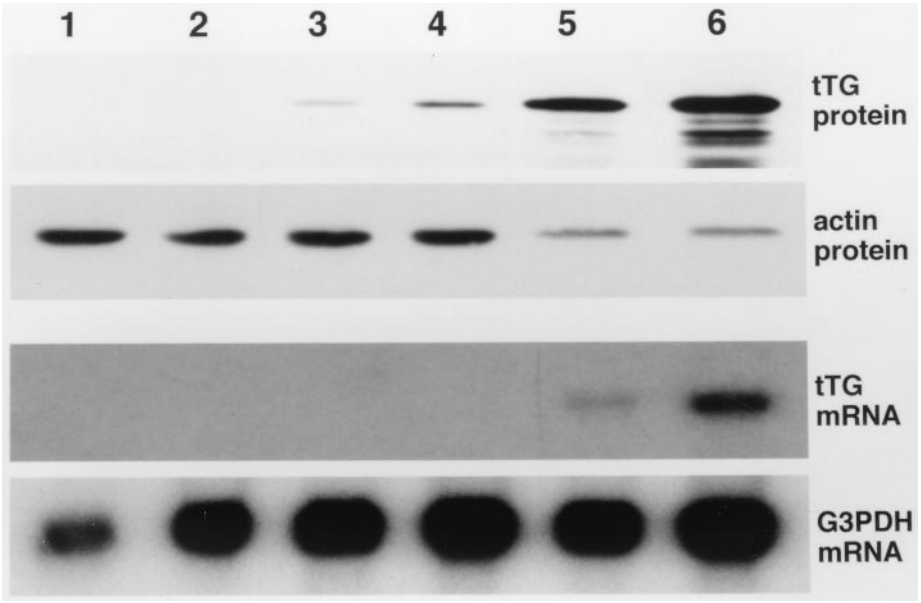

Figure 2 Constitutive levels of tissue transglutaminase (tTG) protein and mRNA. Total cell homogenate and total RNA were prepared from COLO201 (lane 1), COLO320DM (lane 2), CW-2 (lane 3), SW620 (lane 4), WiDr (lane 5), and SW480 (lane 6) cells. (*Upper panels*) Total cell extracts from 1×10^5 cells (lane 1–4) or 1×10^4 cells (lanes 5 and 6) were applied in each lane and subjected to 9% polyacrylamide slab gel electrophoresis followed by immunoblot analysis using monoclonal antibody TG100 to detect tTG. The blot was reprobed with anti-actin antibody to show the relative protein amount in each lane. (*Lower panels*) Twenty micrograms of total RNAs were separated by 1.2% agarose-formaldehyde gel electrophoresis followed by Northern blot hybridization using ^{32}P-labeled mouse tTG cDNA (pmTG700) as a probe. The blot was rehybridized with ^{32}P-labeled human glyceraldehyde 3 phosphate dehydrogenase (G3PDH) cDNA probe to demonstrate relative RNA amount applied in each lane.

study. Thus, SW480 and WiDr cells were suggested to have more differentiated and less aggressive phenotypes than COLO201, COLO320DM, CW-2, and SW620 cells based on morphology, cell adhesiveness, and growth activity.

Figure 2 demonstrates constitutive levels of tTG protein and mRNA. SW480 and WiDr cells had a high amount of cytosolic tTG protein. In this immunoblot analysis, 10 times more cell extracts were applied in each lane for COLO201, COLO320DM, CW-2, and SW620 cells compared with those of SW480 and WiDr cells. However, tTG protein was hardly detectable in COLO201, COLO320DM, and CW-2 cells, and weakly detectable in SW620 cells. Northern blot analysis showed similar results; tTG mRNA was detectable in SW480 and WiDr cells but hardly detectable in the other four cell lines.

Effects of SCFAs on the cytosolic tTG levels in colon cancer cell lines

Figure 3 shows the representative immunoblots demonstrating the cytosolic tTG levels in three colon cancer cell lines—COLO201, COLO320DM, and CW-2—24 hours after treatment with 4 mM acetate, propionate, butyrate, and isobutyrate. The basal levels of cytosolic tTG were kept low, and propionate and butyrate, but not acetate and isobutyrate, induced cytoplasmic accumulation of tTG protein in these colon cancer cell lines. Other differentiation-inducing factors such as TGF-β1, all-trans retinoic acid, 9-cis retinoic acid, dexamethasone, and dimethyl sulfoxide did not increase tTG level in these cell lines. Induction of tTG expression by propionate and n-butyrate was dose dependent and apparent at concentrations higher than 0.5 mM (*Figure 4*). Cytosolic TG protein was increased time-dependently and densitometric analysis showed that tTG amount reached a maximal level of more than 50-fold greater than that of the nontreated control cells 24 hours after 4 mM butyrate or propionate exposure in COLO201, COLO320DM, and CW-2 cells.

Effects of SCFAs on the growth rate and induction of cell death

The effects of SCFAs on cell proliferation and apoptosis were investigated in COLO201, COLO320DM, and CW-2 cells. Viable cell number was analyzed 72 hours after treatment with each SCFA. As shown in *Figure 5*, propionate and butyrate were shown to cause significant growth suppression in these cancer cells, whereas acetate and isobutyrate, which did not induce tTG, did not suppress the cell growth. The number of viable cells was markedly reduced by n-butyrate at concentrations of more than 4 mM. This suggests the induction of cell death. The rate of fragmented DNA was assessed to confirm cell death in these cells 24 and 48 hours after treatment with 4 mM n-butyrate. *Figure 6* shows that DNA fragmentation significantly increased after n-butyrate treatment. Characteristic morphologic features of apoptosis, such as chromatin condensation, nuclear fragmentation, and cell shrinkage, also were observed in cells treated with n-butyrate (data not shown).

Discussion

Although evidence is accumulating that there is a close correlation between tTG activity and cell proliferation and/or differentiation, the physiologic function of this enzyme is still in question.[12–14] According to the appealing theory of Birckbichler *et al*.,[15] tTG-mediated isopeptide crosslinks in proteins are maintained at low levels under proliferating conditions and tTG activity is increased as cells cease to proliferate and the cells are stabilized as the result of increased cross-linkage. Johnson *et al*.[16] reported that transfection of tTG into a highly malignant hamster fibrosarcoma led to a reduced incidence of primary tumor growth. Similarly, Melino *et al*.[21] demonstrated that the overexpression of tTG resulted in a drastic reduction in proliferative capacity of cells. These reports suggested that tTG is a negative regulator for the cell growth. The possible association of tTG with the process of cell death also has been reported in various cell types.[14,18,22] tTG induction and/or activation appear to have an advantageous effect on the process of cell death, because the probable consequence of tTG activation is an extensive cross-linking of cytoplasmic and membrane proteins providing a way to prevent the leakage of intracellular components. This caging effect by tTG is especially implicated in the process of apoptosis.[14,22,23] The process of malignant transformation involves

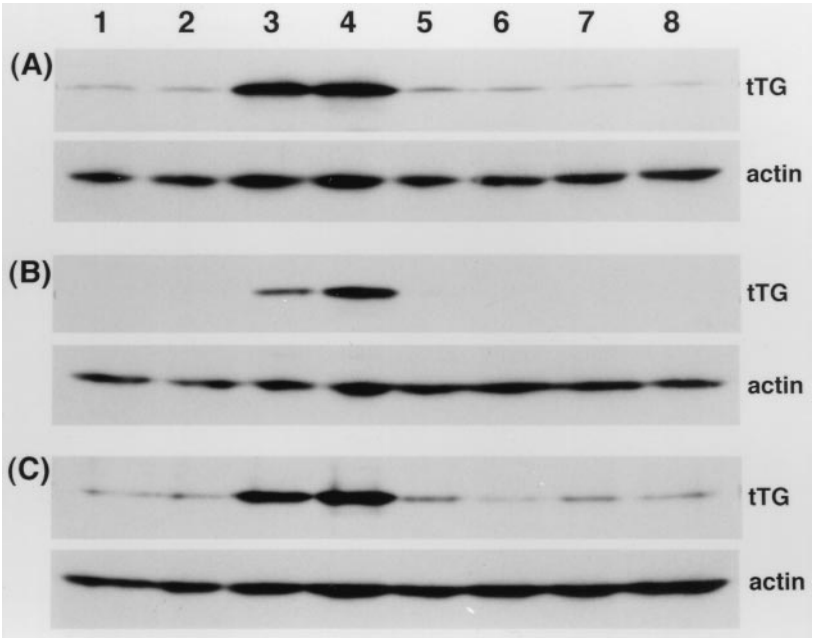

Figure 3 Representative immunoblots showing increase of cytosolic tissue transglutaminase (tTG) protein by treatment with propionate and n-butyrate in human colon cancer cell lines COLO201 (*A*), COLO320DM (*B*), and CW-2 (*C*). Cells were cultured for 24 hours in the presence of each short-chain fatty acid at 4 mM, transforming growth factor (TGF)-β1, all-trans retinoic acid, or 9-cis retinoic acid. Total cell extracts from 1×10^5 cells were applied in each lane and subjected to 9% polyacrylamide slab gel electrophoresis followed by immunoblotting using monoclonal antibody TG100. The blots, which were reprobed with anti-actin antibody, also are shown to evaluate the relative protein amount in each lane. Lanes: 1, control; 2, acetate (4 mM); 3, propionate (4 mM); 4, n-butyrate (4 mM); 5, isobutyrate (4 mM); 6, TGF-β (10 ng/mL); 7, all-trans retinoic acid (10 μM); 8, 9-cis retinoic acid (10 μM).

dysregulation of cell differentiation. The degree of dedifferentiation may be assumed depending on various markers such as morphology, cell adhesiveness, metastatic potential, expression of differentiation-associated proteins, and so forth. Although it is difficult to compare the differentiation state of each cell line, cells with increased cell-cell and cell-matrix interactions appear to have more differentiated and less aggressive phenotypes. If this notion is correct, the present findings are consistent with the prevailing theory that constitutive levels of tTG are higher in cells with well differentiated phenotypes than those with less differentiated phenotypes. This may indicate possible usefulness of tTG expression as a differentiation marker in colon cancer cells.

Colonic fermentation of dietary fiber produces n-butyrate and other SCFAs, acetate, and propionate.[1] Although n-butyrate is trophic to the intestinal mucosa with stimulating mucosal growth and cell differentiation, this SCFA has long been known as an inducer of cell differentiation and inhibitor of proliferation in numerous cell lines, probably acting via the inhibition of histone deacetylases and the resulting selective changes in gene expression.[24,25] The genes affected by this agent are those that are characteristic for the differentiated phenotype of the particular cell line and those coding for proteins involved in the regulation of cell cycle progression such as c-myc, cdc2, cyclin D1, or p21Waf-1.[26–29] Inhibition of phosphorylation of retinoblas-

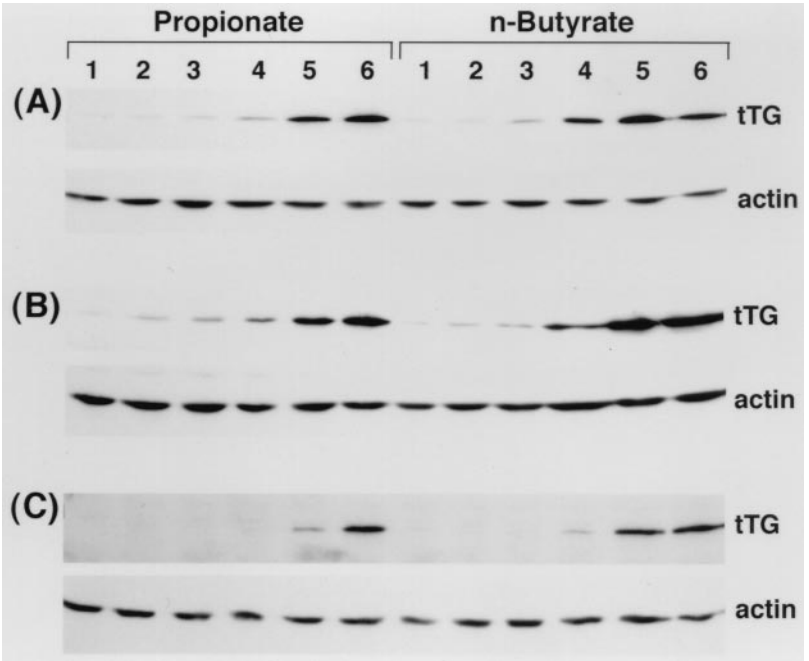

Figure 4 Dose-dependent effects of propionate and n-butyrate on cytosolic tissue transglutaminase (tTG) levels in COLO201 (*A*), COLO320DM (*B*), and CW-2 (*C*). Culture cells were treated with varying concentrations of propionate and n-butyrate for 24 hours. Total cell extracts from 1×10^5 cells were applied in each lane and subjected to 9% polyacrylamide slab gel electrophoresis followed by immunoblotting using monoclonal antibody TG100. The blots were reprobed with anti-actin antibody to demonstrate the relative protein amount in each lane. Lanes: 1, control; 2, 0.031 mM; 3, 0.125 mM; 4, 0.5 mM; 5, 2 mM; 6, 8 mM

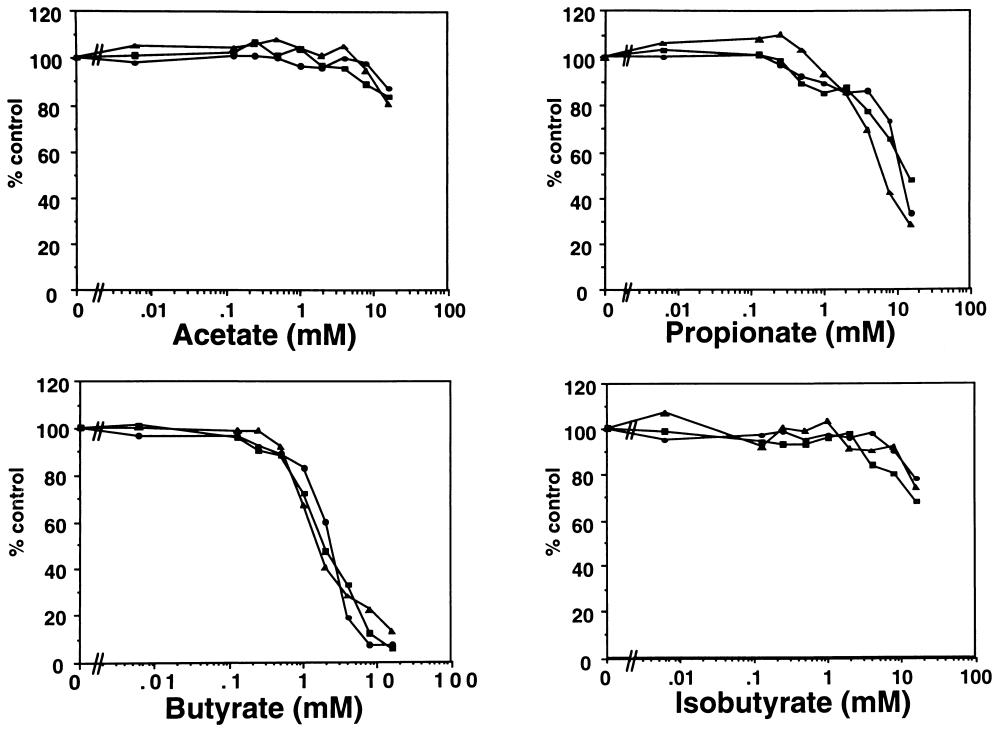

Figure 5 Effects of propionate and n-butyrate on the growth of colon cancer cell lines. Cells were seeded in 96 multi-well plates (1×10^4/well in 100 μL) and precultured for 24 hours before treatment. Each short-chain fatty acid was added in the medium at varying concentrations and relative viable cell number was determined after 72 hours of incubation by WST-1 assay. Data are expressed relative to control culture, which was set at 100%. Data shown are means of quadruplicate wells. ●, COLO201, ■, COLO320DM, ▲, CW-2 cells.

toma protein by n-butyrate is also likely to play a role in its antiproliferative activity.[30] Krupitza et al.[31] demonstrated that sodium butyrate blocks general mechanisms in signal transduction, such as the release of Ca^{2+} from intracellular stores, and modulates the activity of serine/threonine kinases. Therefore, the identification of the cellular targets of n-butyrate can be important for the understanding of the mechanisms of regulation of the proliferation and apoptosis by n-butyrate. The finding that n-butyrate and propionate can induce tTG expression appears to be related to their antineoplastic effects and seems to provide new insight into the molecular mechanisms of biological effects of SCFAs on colonic epithelium cells.

Because dedifferentiation and acquisition of resistance to apoptosis are hallmarks of malignant transformation,[32] induction of differentiation and apoptosis by SCFAs in colon cancer cells may be involved in the prevention of colon carcinogenesis.[33] Butyrate and propionate were shown to induce apoptosis in colon cancer cell lines, and close correlation between tTG induction and apoptosis was suggested in the present study. Basal levels of tTG protein in SW480 and WiDr are so high that these SCFAs could not increase the cytosolic tTG amount more than the basal level. However, measurement of intracellular tTG activity, as assessed by incorporation of biotin-labeled primary amines, demonstrated that intracellular tTG activity is low in living cells irrespective of high cellular tTG amount, but the activity increases during the process of apoptosis (unpublished observation). Although the regulation of transglutaminase activity remains poorly understood, several cofactors have been shown to affect transglutaminase function. tTG activity in the cytosol is regulated by Ca^{2+} concentration and levels of adenosine triphosphate and GTP.[34] It is reported that tTG activity is extremely low at physiologic levels of nucleoside diphosphate and triphosphate. Smethurst and Griffin[35] reported that tTG is unlikely to be activated in the cytosol without (1) decreases in both nucleoside triphosphate and diphosphate levels, (2) a major influx of extracellular Ca^{2+}, and (3) a posttranslational modification of tTG that removes the inhibitory activity of nucleotide and/or the need for Ca^{2+} binding in order for enzyme activation. Given these possibilities, tTG may be activated during apoptosis, when nucleoside triphosphate and diphosphate levels may be depleted and intracellular Ca^{2+} concentrations may increase. In the extracellular environment, in which Ca^{2+} level is high and nucleoside triphosphate and diphosphate levels are low compared with the intracellular environment, tTG is activated and may contribute to increased cell-cell and cell-matrix interaction. Therefore, it is suggested that the increase in the amount of cellular tTG is associated with cell adhesiveness and differentiated states, and enzymatic activation of intracellular tTG is associated with apoptosis.

In addition to the effects on differentiation and proliferation, tTG and SCFAs both are involved in the formation of extracellular matrix and wound repair processes.[4] It is reported that butyrate is an effective therapeutic tool in colitis,[36] and an increase in transglutaminase activity plays

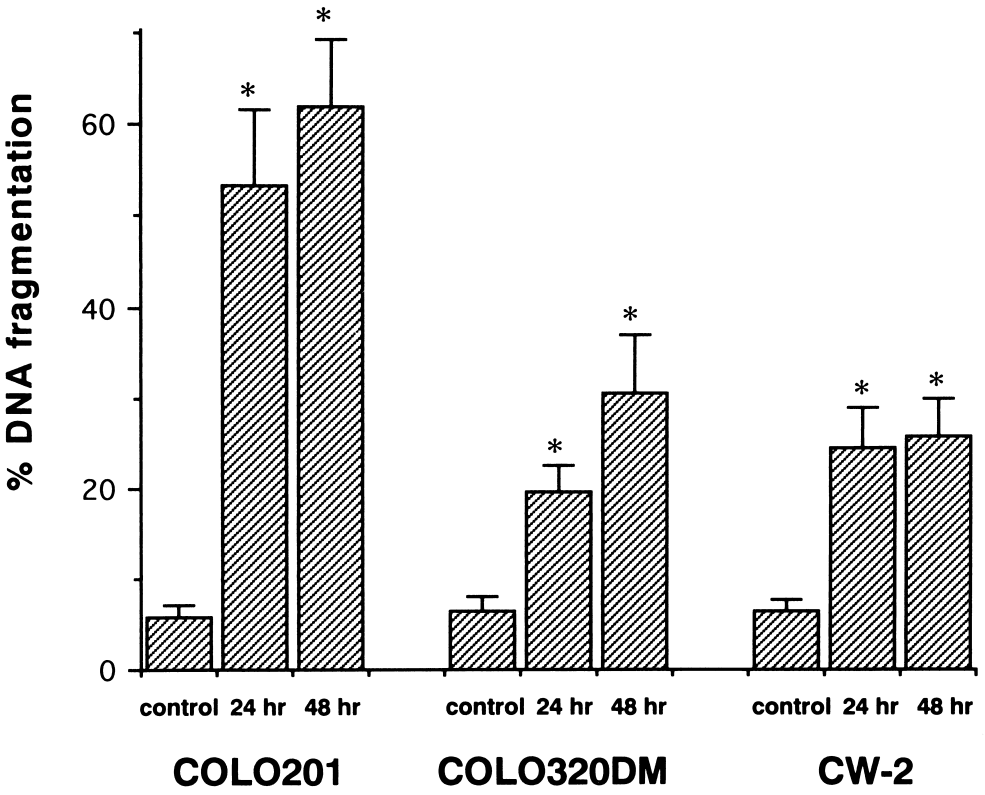

Figure 6 DNA fragmentation assay in COLO201, COLO320DM, and CW-2 cells treated with n-butyrate. The ratio of fragmented DNA was measured after incubation in the absence (control) or presence of 4 mM n-butyrate for 24 and 48 hours. Data shown are means ± SE of quadruplicate wells. *Significantly different from the nontreated control values at $P < 0.05$.

a role in ulcer healing by butyrate.[37] In ulcerative colitis and other premalignant conditions, hyperproliferation of the mucosa is considered a biomarker of increased cancer risk.[38,39] Rectal administration of butyrate reduces mucosal hyperproliferation in patients with ulcerative colitis.[40] These reports support the view that SCFAs may be regarded as protective agents in colorectal carcinogenesis. Thus, transglutaminase induction by SCFAs may be related to their effects on the maintenance of the integrity of colonic mucosa by promoting tissue repair and protecting colonic epithelial cells from malignant transformation. Considering that n-butyrate and propionate induce differentiation and apoptosis in colon cancer cells and that they are produced in large amounts by fermentation of dietary fiber in the colon, induction of tTG by these SCFAs may be closely involved in the mechanisms by which dietary fiber shows preventive effects against colon carcinogenesis.

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