[CANCER RESEARCH 54, 5992–6000, November 15, 1994]

Concomitant Increases in Galectin-1 and Its Glycoconjugate Ligands (Carcinoembryonic Antigen, Lamp-1, and Lamp-2) in Cultured Human Colon Carcinoma Cells by Sodium Butyrate[1]

David W. Ohannesian, Dafna Lotan, and Reuben Lotan[2]

Department of Tumor Biology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

ABSTRACT

Galactoside-binding lectins (galectins) with molecular masses of about 14.5 kilodaltons (galectin-1) and 31 kilodaltons (galectin-3) have been found in a variety of normal and malignant cells and have been implicated in the regulation of cell growth, cell adhesion, and metastasis. The KM12 human colon carcinoma cell line was found to express only galectin-3. Because the levels of both galectins are developmentally regulated and can be modulated during the differentiation of several cultured tumor cell lines, we studied the ability of 11 differentiation-inducing agents to induce galectin-1 expression in the KM12 cells. Treatment of these cells with sodium butyrate, an established differentiation-inducing agent for colon carcinoma cells, resulted in the induction of galectin-1, which was detected by immunoblotting as well as by affinity chromatography. This effect was not seen with any of the 10 other differentiating agents: hexamethylene bisacetamide, dimethyl sulfoxide, dimethyl formamide, herbimycin A, mycophenolic acid, retinoic acid, difluoromethyl ornithine, dibutyryl cAMP, 8-chloro cAMP, and transforming growth factor β1. Galectin-1 induction by butyrate was observed in seven other human colon carcinoma cell lines. Further studies with the KM12 cells revealed that butyrate caused cell flattening, suppressed cell proliferation and colony formation in agarose, and increased the level of carcinoembryonic antigen, a marker of human colon carcinoma cell differentiation, within 48 h of treatment. The increase in galectin-1 level was dependent linearly on butyrate concentration (range, 1–4 mM). Galectin-1 mRNA expression was detected by Northern blotting as early as 6 h, and the protein was detected after 24 h of treatment initiation. The level of the constitutively expressed galectin-3 was also increased by butyrate but to a lesser extent than the level of galectin-1. Butyrate-induced galectin-1 was detected on the cell surface by immunoprecipitation from radioiodinated cell surface proteins as well as by indirect immunofluorescence labeling. Affinity-purified human galectin-1 was found to bind to purified polylactosamine-containing glycoproteins and to detergent-solubilized cellular proteins electroblotted onto nitrocellulose membranes. Affinity chromatography of [^{3}H]glucosamine-labeled KM12 cell extracts on immobilized galectin-1 followed by immunoprecipitation from the lactose-eluted material demonstrated that lysosome-associated membrane glycoprotein-1, carcinoembryonic antigen, and nonspecific cross-reacting antigen are the major galectin-1-binding proteins in these cells. These results indicate that galectin-1 expression may be associated with the differentiation of KM12 cells and that several glycoproteins shown to be important in colon carcinoma adhesion and metastasis are capable of functioning as its endogenous ligands.

INTRODUCTION

Galactoside-binding proteins have been found in a variety of vertebrate tissues and cells in culture, and they constitute a family of molecules that share sequence homology in addition to carbohydrate-binding specificity (1–6). The two predominant classes of vertebrate galactoside-binding lectins have apparent molecular masses of about 14 and 31 kDa,[3] respectively (1, 4, 5). Recently, these lectins have been named galectins; the 14.5-kDa protein was named galectin-1, and the 31-kDa protein was named galectin-3 (6). Galectins have been localized in the cell nucleus, in the cytoplasm, on the cell surface, and in the extracellular milieu into which they are secreted by certain cell types (1–6). Various functions have been proposed for the galectins. These include a role in cell-cell interactions, cell adhesion to ECM, organization of the ECM, growth regulation, and tissue remodeling (1–5). Because they bind galactosides, it is plausible to assume that galectins function by binding complementary carbohydrate-containing glycoconjugates inside the cell, on the cell surface, or in the ECM (1–7). A relatively high affinity for polylactosamine-containing oligosaccharides compared to lactose has also been demonstrated for both galectin-1 and galectin-3 (8). It is noteworthy in this regard that the polylactosamine chains of glycoconjugates have been found to be developmentally regulated in some tissues and to be involved in cellular interactions (9, 10). Likewise, galectins have been found to be developmentally regulated in various tissues (1–6, 11–14). Furthermore, galectins and lactosamine-containing glycoconjugates appear to be expressed coordinately in some tissues (15).

Galectins have been found in a variety of tumors and tumor cell lines, where they have been proposed to play a role in the expression of the transformed phenotype and in metastasis (16–20). Most of the tumors and tumor cell lines analyzed contained primarily galectin-1, although several of these also expressed galectin-3 as well as other galactoside-binding lectins with higher molecular masses (16, 20, 21). Transformation enhanced the expression of galectins (17), and the levels of galectin-3 protein and mRNA were found to be higher in virally transformed fibroblasts than in their untransformed counterparts (17, 22, 23). Transfection of galectin-3 into immortalized cells enhanced their tumorigenic and metastatic phenotype (24). Although the level of the galectin-1 mRNA appeared to be unmodified after malignant transformation (23), it has been demonstrated recently that stable overexpression of galectin-1 in 3T3 fibroblasts confers on these cells a transformed phenotype and the ability to form tumors in nude mice (18).

Several reports described the expression of galactoside-binding lectins in normal and malignant human colon tissues (20, 21, 25–27). Most of these studies detected the presence of galectin-1 in primary colon tissue and tumor samples after affinity chromatography on immobilized lactose. Differences were detected in the lectin profiles of metastases to either lung or liver from similar primary colon carcinomas (25). Recently, we showed that 20 of 21 HCC cell lines constitutively expressed galectin-3, whereas only 7 of 21 expressed

Received 6/30/94; accepted 9/14/94.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported by Grant 2546 from the Council for Tobacco Research U. S. A., Inc.

[2] To whom requests for reprints should be addressed, at Department of Tumor Biology, Box 108, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030.

[3] The abbreviations used are: kDa, kilodaltons; ECM, extracellular matrix; HCC, human colon carcinoma; FBS, fetal bovine serum; DPBS, Dulbecco's phosphate-buffered saline; HMBA, hexamethylene bisacetamide; DFMO, difluoromethyl ornithine; DMSO, dimethyl sulfoxide; DMF, dimethyl formamide; TGF-β1, transforming growth factor β1; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; CEA, carcinoembryonic antigen; mAb, monoclonal antibody; poly(A)$^+$, polyadenylated; cDNA, complementary DNA; sulfo-SHPP, sulfosuccinimidyl (hydroxyphenyl) propionate; FITC, fluorescein isothiocyanate; PBS, phosphate-buffered saline; lamp, lysosome-associated membrane glycoprotein.

galectin-1.[4] Galectin-1 and -3 expression in a variety of murine and human tumor cell lines was modulated by differentiation-inducing agents (28). One intriguing result of that study was that only one tumor cell line, the KM12 HCC cell line, expressed galectin-3 but did not express galectin-1. Furthermore, treatment of the KM12 cells with sodium butyrate, a known differentiation-inducing agent for colon carcinoma cells (29–31), caused an induction of galectin-1 in these cells (28). The present study was undertaken to further explore the nature of butyrate-induced galectin-1 expression in the context of *in vitro* differentiation and to attempt to characterize potential galectin-1 ligands in the KM12 cells.

MATERIALS AND METHODS

Tissue Culture. The KM12 cell line and its various sublines were established from a Duke's B2 primary HCC (32). Cell line KM12-c was established by direct culture of an aliquot of enzymatically dissociated tumor specimen. Cell line KM12-SM was derived from a liver metastasis in an athymic mouse that had been injected into its cecum with cultured KM12-c cells (32). The cells were grown in a 1:1 (v/v) mixture of Dulbecco's modified Eagle's minimum essential medium and Ham's F-12 medium supplemented with 10% FBS (Hyclone Laboratories, Logan, UT), L-glutamine, nonessential amino acids, and 2-fold vitamin solution (GIBCO, Grand Island, NY) in plastic tissue culture dishes (Corning Glass, Corning, NY). LoVo, LS174T, SW403, HCT-15, and HT-29 HCC cell lines were obtained from the American Type Culture Collection (Rockville, MD) and grown in Dulbecco's modified Eagle's minimum essential medium/F-12 medium containing 10% FBS with (LS174T) or without (LoVo, SW403, HCT-15, and HT-29) 1% nonessential amino acids. The MIP-101 cell line (33) was maintained in RPMI 1640 with 10% FBS. Sodium butyrate (Aldrich, Milwaukee, WI) was added to the medium from a 200 mM stock solution diluted in DPBS. β-all-*trans*-Retinoic acid (1 μM) was a gift from Hoffmann-La Roche (Basel, Switzerland); HMBA (5 mM) and herbimycin A (0.1 μg/ml) were gifts from Dr. G. Gallick (University of Texas M. D. Anderson Cancer Center); DFMO (10 mM) was a gift from Dr. K. Nishioka (University of Texas M. D. Anderson Cancer Center); DMSO (1.2%), DMF (0.8%), dibutyryl cAMP (1 mM), and mycophenolic acid (3 μM) were purchased from Sigma Chemical Co. (St. Louis, MO); 8-chloro-cAMP (25 μM) was a gift from Dr. E. Tahara (Hiroshima University, Hiroshima, Japan); and TGF-β1 (2 ng/ml) was a gift from Dr. A. Rizzino (Eppley Cancer Center, Omaha, NE). Cells were maintained in the presence of these agents for 6 days with one refeeding with fresh medium on day 3. The anchorage-independent growth of the cells in 0.5% agarose-containing medium was assayed as described in detail elsewhere (34) to determine the effect of 2 mM butyrate.

Gel Electrophoresis. Electrophoresis in polyacrylamide slab gels was performed in the presence of SDS as described before (35). The same amounts of total protein were loaded in each lane (usually between 60 and 100 μg). Unlabeled proteins were detected by staining with Coomassie brilliant blue, whereas radiolabeled proteins were detected by fluorography or autoradiography. The relative amounts of specific bands were quantitated by densitometric scanning of the X-ray films using a Beckman DB scanning densitometer and comparing the integrated areas under the respective peaks.

Immunoblotting of Lectins with Anti-Lectin Antibodies. HCC cell extracts or purified lectins were immunoblotted using methods described elsewhere (28). The following anti-lectin anti-sera were used: a rat mAb M3/38 (36) raised against the murine macrophage antigen Mac-2 (mouse galectin-3; Boehringer-Mannheim, Indianapolis, IN); a polyclonal antiserum raised against a mixture of galectins-1 and -3 (37) purified from bovine lung (a gift from Dr. S. Barondes, University of California, San Francisco, CA); and an affinity-purified rabbit polyclonal antibody raised against purified human placenta galectin-1 (26). Samples were resolved by electrophoresis in 14% SDS-PAGE and transferred electrophoretically to nitrocellulose (0.1 μm; Micron Separation, Inc., Westboro, MA) at 150 V for 2 h. After blocking overnight in a solution containing 15% skim milk, 0.9% NaCl, and 0.02% sodium azide in a 10 mM Tris-HCl buffer (pH 7.4), the nitrocellulose membranes were incubated for 3 h with the appropriate antibodies in 10 ml of blocking buffer, washed to remove excess unbound antibodies, and further incubated for 2 h with ^{125}I-labeled goat anti-rabbit IgG (1.5×10^6 cpm; 12.7 μCi/μg; ICN Biomedicals, Irvine, CA) in 10 ml blocking buffer. The membranes were then washed twice with blocking solution with 0.1% Tween-20, dried, and placed against an X-ray film for autoradiography at −80°C.

Analysis of CEA. Cell extracts were subjected to electrophoresis and processed for immunoblotting as described above, except that the primary antibody used was a mouse anti-human CEA mAb 7F (38) (a generous gift from Dr. J. Chan, M. D. Anderson Cancer Center) and the second antibody was ^{125}I-labeled goat anti-mouse IgG (ICN).

Northern Blotting. Poly(A)$^+$ RNA was purified from the KM12-c cells using a Fast Track Kit (Invitrogen, San Diego, CA) according to the manufacturer's instructions. Samples containing 3 μg of poly(A)$^+$ RNA were subjected to electrophoresis on a 1.2% formaldehyde-agarose gel, transferred to a nylon membrane, and cross-linked by UV irradiation. The following DNA fragments were used for hybridization: 881 base pairs of human galectin-3 cDNA (a gift from Dr. D. Cooper, University of California San Francisco, San Francisco, CA); 549 base pairs of rat galectin-1 cDNA (also a gift of Dr. D. Cooper); and a 1.2-kilobase cDNA for chicken glyceraldehyde phosphate dehydrogenase.

Radioiodination of Cell Surface Proteins. Sulfo-SHPP (Pierce) was iodinated by incubating 40 nmol of a 4 mM stock solution prepared in 100 mM 3-(*N*-morpholino)propane sulfonic acid (pH 7.2) and 2 mCi Na ^{125}I (ICN) in an Iodogen-coated (Pierce) microcentrifuge tube with constant mixing for 2 min. Iodinated sulfo-SHPP was added to washed cell monolayers in 5 ml DPBS to a final concentration of 8 μM. This mixture was incubated for 30 min on ice with occasional swirling. The reaction was terminated by aspirating the iodination mixture and washing the cell monolayers three times, each with 10 ml of ice-cold DPBS containing 1 mg/ml lysine. After scraping, suspending, and pelleting the cells, cell extracts were prepared by solubilizing the cells in lysis buffer (0.25 M sucrose, 10 mM Tris-HCl, 0.05 mM $CaCl_2$, 100 μM phenylmethylsulfonyl fluoride, and 0.5% Nonidet-P40) for 15 min at room temperature. Lysates were clarified by pelleting the cell nuclei by centrifugation at 16,000 × *g* for 5 min. Protein content was determined using Coomassie Plus reagent (Pierce).

Immunoprecipitation of Cell Surface Radioiodinated Galectin-3. Aliquots of the ^{125}I-cell surface-labeled cell lysates containing 1 mg protein were precleared by an overnight incubation at 4°C with recProtein G-Sepharose 4B (Zymed, South San Francisco, CA). Samples were then incubated for 3 h with rat anti-galectin-3 mAb M3/38 or rabbit anti-human galectin-1 antibodies at 4°C and then with 75 μl recProtein G-Sepharose 4B for 30 min at 4°C with vigorous shaking. After five washes in a buffer containing 50 mM Tris (pH 8.0), 0.5 M NaCl, 0.5% deoxycholate, 0.5% Triton X-100, 0.1% SDS, 5 mM EDTA, and 0.02% sodium azide and one wash in lysis buffer, the Sepharose beads were boiled in an SDS-PAGE sample buffer [2% SDS, 62.5 mM Tris-PO_4 (pH 6.7), 144 mM β-mercaptoethanol, 0.5 mM EDTA, and 10% glycerol], and the soluble material was subjected to SDS-PAGE and analyzed by autoradiography at −70°C.

Localization of Lectins by Indirect Immunofluorescence. Cells were grown on glass coverslips (Corning Glass, Corning, NY), washed, and processed in one of the following ways: prefixation in 3.5% freshly dissolved paraformaldehyde in DPBS for 1 h; no fixation; or brief fixation in 3.5% paraformaldehyde for 10 min followed by permeabilization with 0.1% Triton X-100 for 15 min on ice. The prefixed cells were washed with DPBS, incubated with anti-galectin-3 mAb M3/38 (5 μg/ml) for 1 h on ice, washed, and incubated for an additional 1 h with FITC-conjugated rabbit anti-rat IgG (Boehringer Mannheim). The unfixed cells and the permeabilized cells were washed in DPBS and incubated for 1 h on ice with affinity-purified rabbit anti-human galectin-1 antibodies, washed, and incubated with FITC-conjugated goat anti-rabbit IgG (Sigma). All of the above labeled cells were then washed, fixed with 3.5% paraformaldehyde in PBS, and mounted in 90% glycerol on microscope slides. The cells were then observed and photographed using a Nikon fluorescence microscope.

Galectin-1 Interaction with Polylactosaminoglycan-containing Glycoproteins. CEA purified from a liver metastasis of a colon carcinoma (39) was a gift from Dr. P. Thomas (New England Deaconess Hospital, Boston, MA). Human lamp-1 and lamp-2 purified from chronic myelogenous leukemia cells

[4] D. W. Ohannesian, D. Lotan, P. Thomas, J. M. Jessup, M. Fukuda, H. J. Gabius, and R. Lotan. Expression of galectin-3 and complementary glycoproteins in human colon carcinoma cells, submitted for publication.

(40) were a gift from Dr. M. Fukuda (La Jolla Cancer Foundation, La Jolla, CA). Each glycoprotein (4 μg) was subjected to SDS-PAGE in 5% gels and electroblotted onto nitrocellulose membrane as described above. The nitrocellulose membranes were stained with 0.5% Ponceau S in 1% acetic acid to assess the loading and efficiency of transfer, and then subsequently blocked overnight in wash buffer containing 1% hemoglobin. The filters were then incubated in wash buffer containing 0.1% Tween-20 for 15 min prior to their incubation for 3 h at room temperature with a solution of galectin-1 purified from human placenta as described elsewhere (41) at a concentration of 40 μg/ml in blocking solution. A control membrane was incubated with galectin-1 in the presence of lactose (0.1 M) to block carbohydrate-mediated binding. The nitrocellulose membranes were then washed four times in wash buffer [0.9% NaCl-10 mM Tris-HCl (pH 7.4)] with 0.1% Tween-20. The membranes were incubated with affinity-purified rabbit anti-human galectin-1 at a concentration of 5 μg/ml in blocking solution for 2 h before washing four times as above. Finally, the membranes were incubated with ^{125}I-labeled goat anti-rat IgG (1.5×10^6 cpm; ICN) for 1.5 h and then washed six times in wash buffer with 0.1% Tween-20 before being dried and exposed to an X-ray film for autoradiography at −70°C.

Purification of Galectin-1 Complementary Glycoconjugates from KM12 Cells by Affinity Chromatography. KM12-c cells were labeled metabolically for 48 h with [^{3}H]glucosamine (0.5 mCi/ml). The cells were then harvested in calcium- and magnesium-free PBS containing 2 mM EDTA and collected by centrifugation at 500 × *g* for 5 min. The cells were resuspended in a lysis buffer [0.5% Nonidet P-40, 10 mM Tris-HCl (pH 8.0), 10 mM NaCl, 2 mM $MgCl_2$, 0.1 mM phenylmethylsulfonyl fluoride, 0.5 μg/ml leupeptin, and 60 KIU/ml aprotinin] and incubated for 30 min on ice. The lysates were then cleared by centrifugation for 5 min at 16,000 × *g*. The cell lysate from a single 10-cm dish was then applied to a galectin-1-Affi-gel 10 affinity column (coupled at a ratio of 250 μg galectin-1 per ml of gel) and allowed to bind for 15 min. The column was then washed extensively with lysis buffer and eluted with lysis buffer containing 0.1 M lactose. The peak fractions from the lactose-eluted material were subjected to SDS-PAGE in 6% gels and analyzed by fluorography. One of the lactose peak fractions was diluted to 1 ml with buffer A (0.1% SDS, 0.5% Nonidet P-40, 5 mM EDTA, and 0.1% bovine serum albumin in PBS) and immunoprecipitated with an anti-human lamp-1 mAb (a kind gift from Dr. M. Fukuda) or with a polyclonal anti-CEA antibody (Signet Laboratories, Dedham, MA) overnight at 4°C. The immune complexes collected on protein A-Sepharose beads were washed several times with buffer A and eluted by boiling in SDS sample buffer. The suspension was then centrifuged, and the supernatant fraction was subjected to SDS-PAGE on 6% gels and analyzed by fluorography.

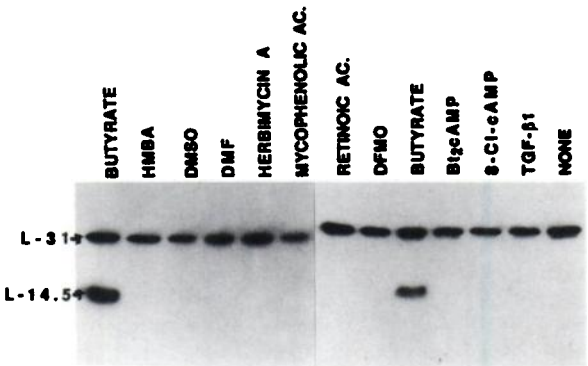

Fig. 1. Galectin expression in KM12-c cells after treatment with differentiation-inducing agents. Cells were treated with either butyrate (2 mM), HMBA (5 mM), DMSO (1.2%), DMF (0.8%), herbimycin A (0.1 μg/ml), mycophenolic acid (3 μM), retinoic acid (1 μM), DFMO (10 mM), dibutyryl cAMP (1 mM), 8-chloro-cAMP (25 μM), TGF-β1 (2 ng/ml), or normal growth medium (none) for 6 days. Cell lysates were then subjected to SDS-PAGE in 14% gels and transferred to nitrocellulose, followed by immunoblotting with anti-galectin-3 mAb M3/38 (*L-31*) and rabbit anti-human galectin-1 (*L-14.5*) antibody.

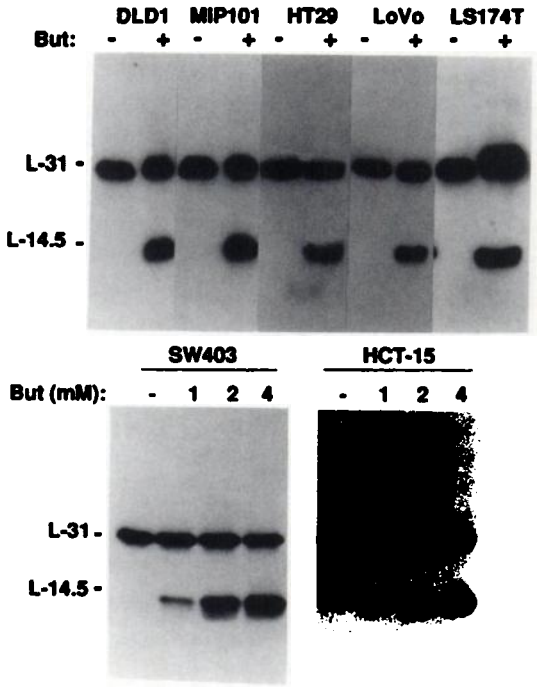

Fig. 2. Induction of galectin-1 expression in several HCC cell lines by sodium butyrate. *Top*, cells were grown with or without butyrate (2 mM) for 5 days, and cell extracts were then analyzed by immunoblotting with antibodies against galectin-3 (*L-31*) and anti-galectin-1 (*L-14.5*). *Bottom*, SW403 and HCT-15 HCC cells were grown in the presence of the indicated concentrations of sodium butyrate for 48 h. Cell extracts were then analyzed by immunoblotting as in Fig. 1.

RESULTS

Modulation of Galectin Expression in KM12-c Cells by Differentiation-inducing Agents. The KM12-c cells express only galectin-3 constitutively; however, after treatment with butyrate, these cells also express galectin-1 (Fig. 1) as we demonstrated previously (28). The induction of galectin-1 was specific for butyrate and was not shared by any of the other 10 compounds (HMBA, DMSO, DMF, herbimycin A, mycophenolic acid, retinoic acid, DFMO, dibutyryl cAMP, 8-chloro cAMP, and TGF-β1) selected because of their reported ability to induce the differentiation of various tumor cell types. The expression of galectin-3 was not modulated markedly by any of these agents.

Butyrate Induction of Galectin-1 in Other HCC Cell Lines. Recently, we found that, like KM12 cells, most HCC cell lines express galectin-3 but do not express galectin-1.[4] To determine whether galectin-1 could be induced by butyrate in other HCC cell lines, we analyzed the expression of galectins in seven cell lines. Fig. 2 shows that 2 mM butyrate induced galectin-1 in each of these cell lines. The expression of galectin-3 in these cells was only modestly affected by butyrate treatment, except for the LS174T cells in which its level more than doubled.

Effects of Butyrate on the Growth and Differentiation of KM12 HCC Cells. A 5-day treatment of KM12-SM cells with 2 mM butyrate resulted in increased flattening of these cells (Fig. 3, *a* and *b*). Both the proliferation of the cells in monolayer cultures and the ability of the cells to form colonies in semisolid agarose were dramatically inhibited by butyrate treatment in a dose-dependent fashion. Half-maximal growth inhibitory responses to butyrate were observed at 0.5–1.0 mM for growth in monolayer as well as in agarose (Fig. 3). The growth inhibitory effect of butyrate was reversible within 48 h after removal of the compound from the culture medium, which suggests that its effects are not the result of cytotoxicity in these cells (data not shown).

The KM12-SM cells constitutively expressed a low level of cell-associated CEA, a marker of differentiation in HCC cells.

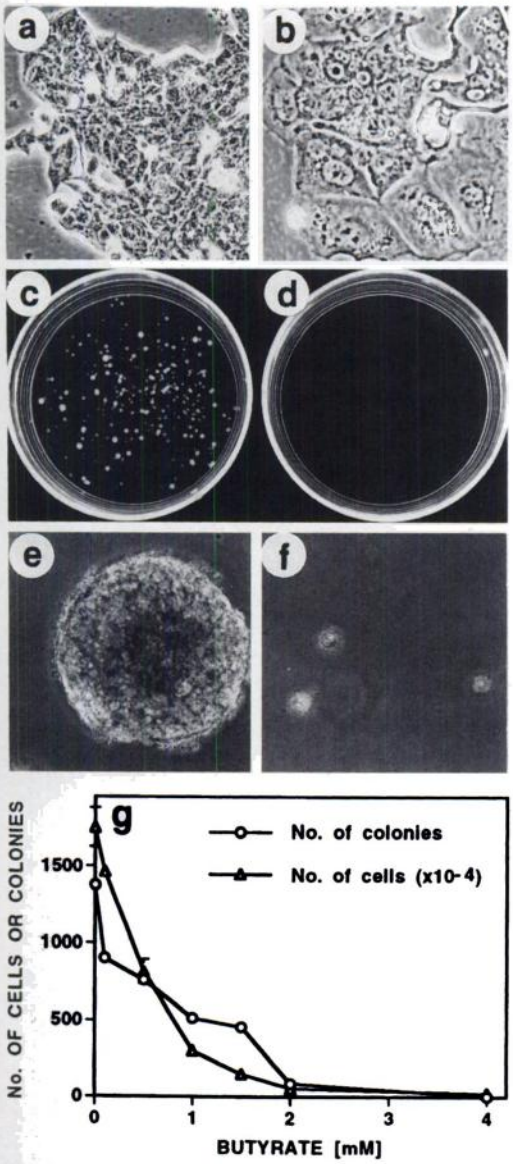

Fig. 3. Effect of butyrate on the morphology and growth of KM12-SM HCC cells. Cells were grown in monolayer culture for 5 days in the (*a*) absence or (*b*) presence of 2 mM butyrate. The anchorage-independent growth of these cells in 0.5% agarose was assessed after 14 days in the (*c*) absence or (*d*) presence of 2 mM butyrate. *e*, a representative colony from an untreated culture. *f*, single cells that failed to form a colony in a butyrate-treated culture. The butyrate dose-dependent decrease in cell proliferation and colony formation are depicted graphically in (*g*).

Butyrate (2 mM) increased CEA expression after 48 h of treatment, and CEA levels continued to increase through 4 days of treatment (Fig. 4). This increase in expression was nearly 8-fold after 4 days of butyrate exposure. Untreated cells showed little change in CEA expression during this time. Butyrate also increased the expression of CEA in some of the other HCC cell lines (*e.g.*, HT29 and LS174T; data not shown).

The Dose-Response and Time Course of Galectin-1 Induction in KM12 Cells by Butyrate. To further characterize the effects of butyrate on galectin-1 expression, we analyzed the dose dependence of its induction in both KM12-c and KM12-SM cells. Galectin-1 was initially detectable in cultures treated with 1 mM butyrate for 5 days, and its levels increased linearly with the increase in butyrate concentration up to 4 mM (Fig. 5, *A* and *B*). The increased expression of galectin-1 by butyrate in two other HCC cell lines (SW403 and HCT-15) was also dependent on butyrate concentration; however, 2 mM butyrate induced a near-maximal level of the lectin in these cells (Fig. 2). The level of galectin-3 also increased after butyrate treatment, especially in the KM12-SM cells (Fig. 5, *A* and *B*). Subsequent studies used 2 mM butyrate as a standard dose. At this concentration, butyrate induced a half-maximal increase in galectin-1 expression after 24 h in both the KM12-c and the KM12-SM cell lines (Fig. 5*C*). The maximal expression observed after 48 h of treatment remained relatively constant throughout 5 days of treatment (Fig. 5*C*). The level of galectin-3 also increased with butyrate treatment, albeit at a slower rate, reaching maximum levels in the KM12-c cells after 3–4 days (Fig. 5*C*). The increase in expression of both galectins is reversible upon removal of butyrate from the culture medium. In cells treated for 2 days with 2 mM butyrate, the galectin-1 level declined within 3 days after cessation of treatment, whereas in cells treated for 5 days, the decline in galectin-1 level lasted for 7 days after treatment was discontinued (data not shown).

The induction of galectins by butyrate was found also at the mRNA level by Northern analysis. Undetectable in untreated cells, galectin-1 mRNA increased within 6 h after addition of butyrate to the culture medium of the KM12-c cells, and its level continued to increase through 24 h (Fig. 6). Galectin-3 mRNA, consistent with the analysis of the proteins, was detected prior to butyrate exposure of the cells; however, its level increased 2–3 fold after butyrate treatment (Fig. 6).

Localization of Galectin-1 on the Cell Surface. To be able to interact with glycoconjugates at the cell surface or in the ECM, galectins must be present on the cell surface. We have demonstrated that galectins-1 and -3 are present on the surface of these cells using two different methods. First, KM12-SM cells were radioiodinated in monolayer culture by a membrane impermeant reagent (^{125}I-sulfo-SHPP), and galectin-1 was immunoprecipitated from cell extracts with affinity-purified polyclonal antibody (Fig. 7, *Lane B*). Galectin-1 could only be immunoprecipitated from butyrate-treated cells and not from untreated ones as expected (Fig. 7, *Lanes A* and *B*). Galectin-3

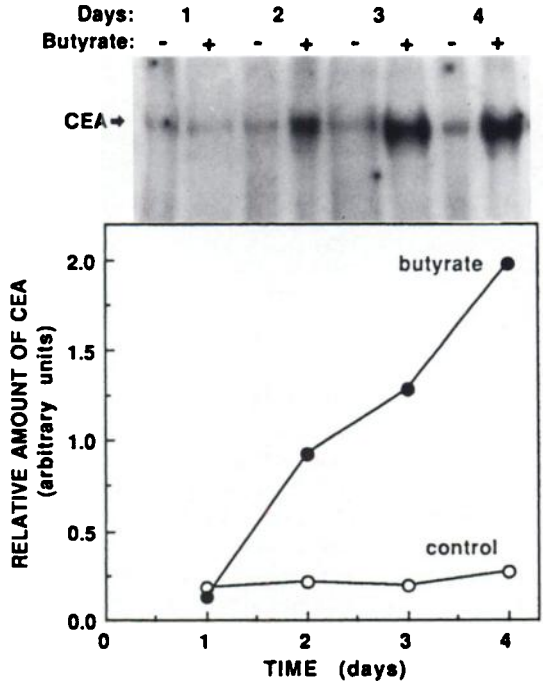

Fig. 4. Time course of CEA induction in KM12-SM cells by sodium butyrate. Cells were grown in the absence (−) or presence (+) of butyrate (2 mM) for 1 to 5 days. Cell lysates were prepared at the indicated times and subjected to SDS-PAGE in 5% gels, transferred to nitrocellulose, and immunoblotted with an anti-CEA mAb 7F. The quantitation of the immunoblotting results obtained after densitometric scanning of the autoradiogram is shown in the *lower panel*.

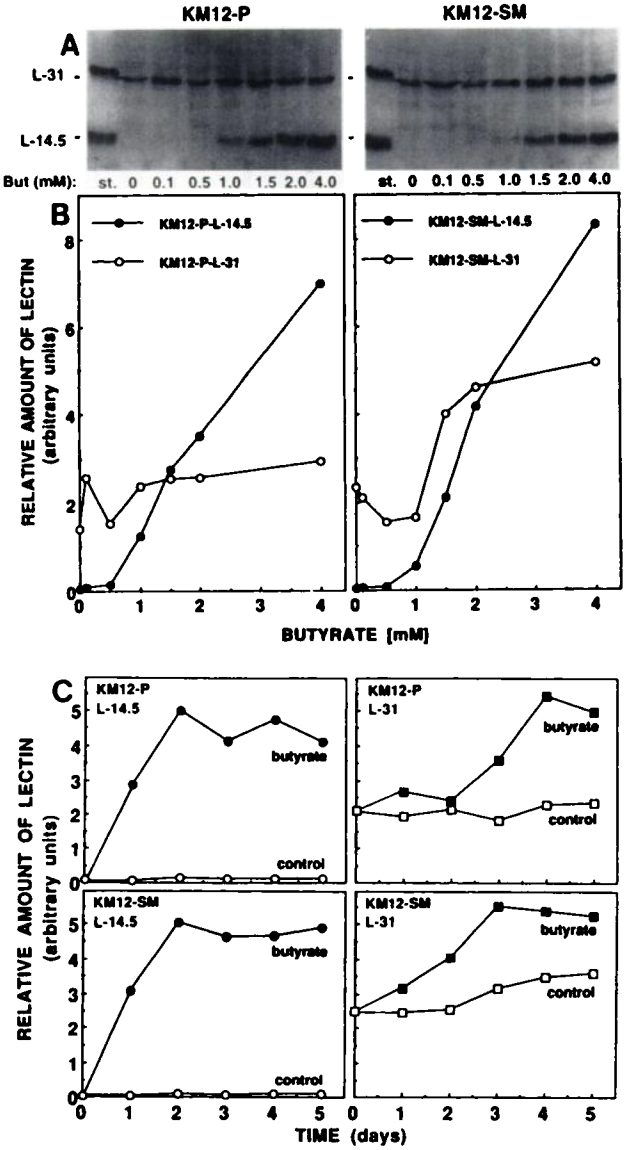

Fig. 5. Dose-dependent (*A* and *B*) and time-dependent (*C*) modulation of galectin-1 and galectin-3 in KM12-c and KM12-SM HCC cells by sodium butyrate. *A*, the cells were grown for 5 days with the indicated concentrations of sodium butyrate, and cell extracts were prepared and analyzed by immunoblotting using a rabbit anti-bovine antibody to galectin-1 and -3. The *leftmost lane* on each gel is a positive control extract of a mouse lymphosarcoma cell line, RAW117P. *B*, the results of a densitometric scan of each autoradiogram shown in (*A*). *C*, cells were grown for the indicated time in the presence of 2 mM butyrate. Cell extracts were analyzed by immunoblotting using a rabbit anti-bovine antibody to galectin-1 and -3. The relative amounts of galectin expression were determined after densitometric scanning of the autoradiograms.

was immunoprecipitated from both untreated and butyrate-treated cells (Fig. 7, *Lanes A'* and *B'*).

Indirect immunofluorescence analysis of KM12 cells demonstrated the presence of galectin-3 on the surface of untreated and butyrate-treated cells (Fig. 8, *a* and *b*); in contrast, galectin-1 was detected only on cells treated with butyrate for 48 h (Fig. 8*d*). Since prefixation apparently abolished the immunogenicity of galectin-1 on the cell surface, we had to stain the cells without fixation. A more intense staining was observed in permeabilized cells, suggesting that most of the induced galectin-1 may be intracellular (Fig. 8*f*). Butyrate treatment seemed to result in a slight increase in the cell surface expression of galectin-3 as well (Fig. 8*b*).

Identification of Potential Endogenous Ligands for Galectin-1 in KM12 Cells. Purified human galectin-1 was found to bind to several proteins produced by KM12-c cells, including prominent bands with molecular masses of ~18, 32, 70, 80, and 120 kDa (Fig. 9, *A* and *C*). Extracts from butyrate-treated cells displayed a similar pattern of reactivity with galectin-1 but with some notable differences. Butyrate diminished the reactivity of several proteins of 18–30 kDa (Fig. 9*C*) and increased binding to a 120-kDa protein and in particular to a 190-kDa protein (Fig. 9*A*). Incubation of galectin-1 with identical blots in the presence of 0.1 M lactose almost completely inhibited its binding to all of these proteins (Fig. 9, *B* and *D*). The prominent band

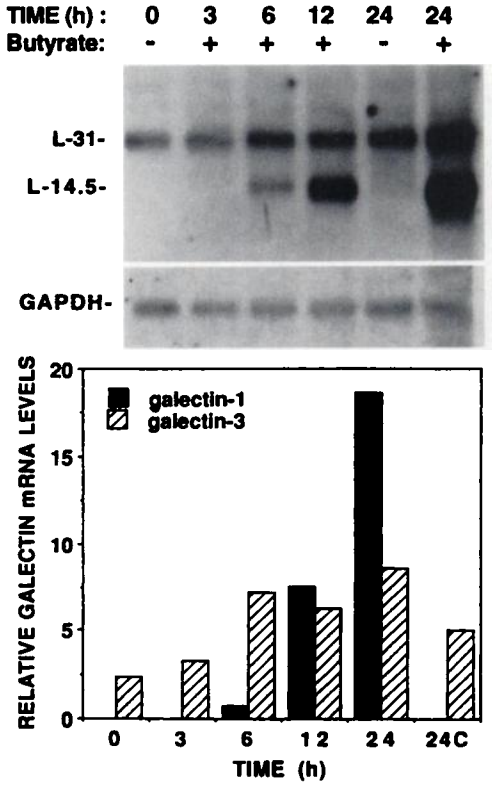

Fig. 6. Time-dependent modulation of the level of the mRNA of galectin-1 and galectin-3 in KM12 cells after butyrate treatment. Cells were grown in the presence of 2 mM butyrate (+) or in control medium (−) for the indicated times, and their RNA was extracted. Samples containing 3 μg of poly(A)$^+$ RNA were separated on a 1.2% agarose gel and transferred to a nylon membrane. The membrane was then hybridized sequentially with ^{32}P-labeled cDNA probes for human galectin-3 (*L-31*), rat galectin-1 (*L-14.5*), and chicken glyceraldehyde phosphate dehydrogenase (*GAPDH*). The results of a densitometric scan of the autoradiogram normalized according to the GAPDH level in each lane is shown in the *lower panel*. The 24-h time point (*24C*) was moved to the end of the graph for continuity.

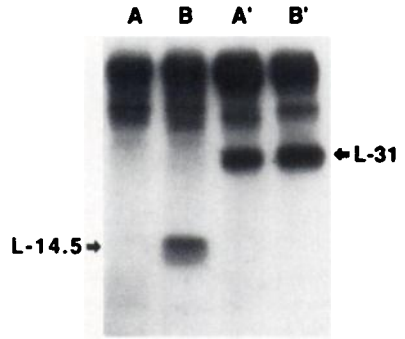

Fig. 7. Expression of galectins on the surface of KM12-SM HCC cells before and after butyrate treatment. KM12-SM cells were grown for 48 h either without (*A* and *A'*) or with (*B* and *B'*) with 2 mM butyrate, and then cell surface proteins were radioiodinated using the water-soluble, membrane-impermeable agent, ^{125}I-sulfo-SHPP. Aliquots of cell extracts (1 mg) were immunoprecipitated with either affinity-purified polyclonal anti-human galectin-1 antibodies (*Lanes A* and *B*) or anti-galectin-3 mAb M3/38 (*Lanes A'* and *B'*). The resulting immunoprecipitates were subjected to SDS-PAGE in 14% gels. Iodinated proteins were visualized in dried gels by autoradiography.

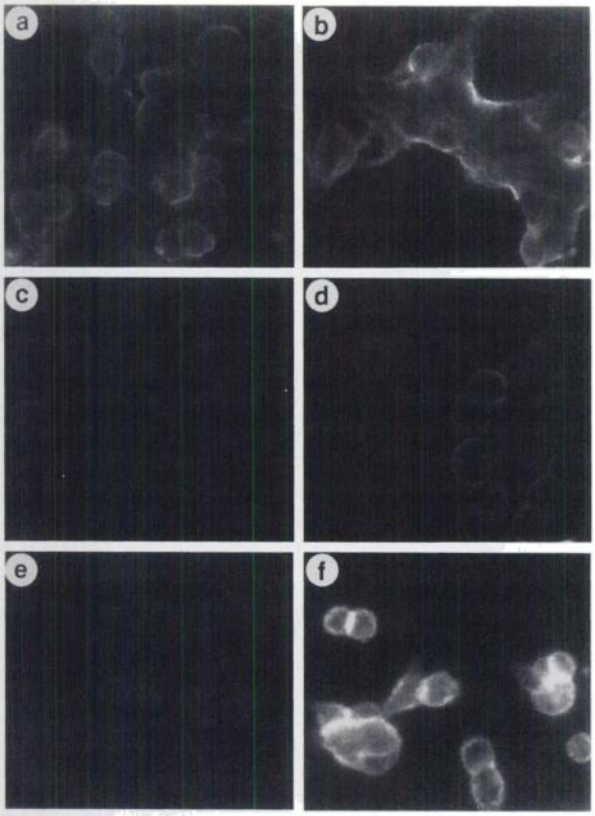

Fig. 8. Localization of galectin-1 and galectin-3 on KM12 cells by indirect immunofluorescence staining. Cells were grown on glass coverslips without (*a*, *c*, and *e*) or with (*b*, *d*, and *f*) 2 mM butyrate for 48 h. The cells were then prefixed with formaldehyde (*a* and *b*), left unfixed (*c* and *d*), or fixed and permeabilized with Triton X-100 (*e* and *f*). They were then incubated with either an anti-galectin-3 mAb M3/38 (*a* and *b*) or affinity purified anti-human galectin-1 polyclonal antibody (*c-f*), followed by FITC-labeled second antibodies as described in "Materials and Methods."

at 14.5 kDa on the 14% gels (Fig. 9, *C* and *D*) was the butyrate-induced endogenous galectin-1 itself. Its detection by the anti-galectin-1 antibody was unaffected by the presence of lactose as expected (Fig. 9*D*).

Galectin-1 Binding to CEA, Lamp-1, and Lamp-2. It seemed likely that the 190-kDa protein detected by lectin blotting might be CEA, whose expression increased in this cell line after butyrate treatment (Fig. 4). To test this hypothesis, we examined the interaction of purified galectin-1 with CEA purified from a colon carcinoma liver metastasis using an identical lectin blotting protocol. In addition, two other purified polylactosaminoglycan-containing glycoconjugates (human lamp-1 and lamp-2) with relevance to colon carcinoma adhesion events were analyzed alongside CEA to determine if they, too, could bind to galectin-1 in a carbohydrate-dependent manner. Fig. 10 shows that all these glycoproteins were bound by galectin-1. Galectin-1 binding to lamp-1 was greater than to lamp-2. Binding of galectin-1 to these glycoproteins was completely abrogated in the presence of 0.1 M lactose (Fig. 10*B*).

Identification of Endogenous KM12 Glycoproteins Bound by Immobilized Galectin-1. To further characterize the endogenous ligands bound by galectin-1, we isolated such molecules by affinity chromatography of [^{3}H]glucosamine-labeled KM12-c cell extracts using immobilized human galectin-1. Prominent bands of 116–120, 190, and >200 kDa were present in the material eluted with lactose from this column (Fig. 11*A*). Antibodies specific for human lamp-1 precipitated a 116-kDa glycoprotein (Fig. 11*B*), and anti-CEA antibodies precipitated glycoproteins of 120- and 190-kDa (Fig. 11*C*). These glycoproteins have the molecular weights expected for lamp-1 and CEA. Additional glycoprotein(s) immunoprecipitated with the anti-CEA antibodies may be other members of the CEA family (*e.g.*, nonspecific cross-reacting antigen or biliary glycoprotein). The identity of the higher molecular mass glycoprotein(s) in Fig. 11*A* remains unknown.

DISCUSSION

The regulated expression of cellular glycoconjugates and their carbohydrate side chains provides an abundant mutable source of information for cellular interactions involving recognition events such as cell-cell and cell-ECM adhesion, growth factor-receptor binding, and the transduction of signals responsible for the elaboration and

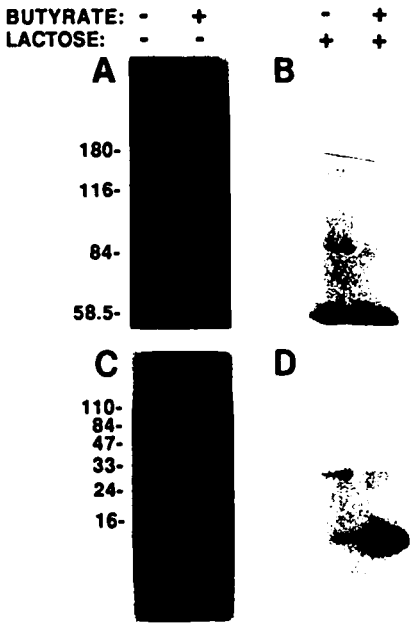

Fig. 9. Binding of purified galectin-1 to complementary molecules in extracts of untreated (−) and butyrate-treated (+) KM12-c cells. Samples of cell extracts containing 100 μg protein were subjected to SDS-PAGE on either 5% (*A* and *B*) or 14% (*C* and *D*) gels, transferred to nitrocellulose membranes, and incubated with a solution containing 40 μg/ml of human galectin-1 in 1% hemoglobin in the absence (*A* and *C*) or presence (*B* and *D*) of 0.1 M lactose. After extensive washing, the blots were incubated with affinity-purified polyclonal anti-galectin-1 antibody, followed by ^{125}I-labeled goat anti-rabbit antibody. Antibody binding was visualized by autoradiography. The 14.5-kDa band observed in butyrate-treated cell extracts (*C* and *D*) is the endogenous galectin-1, which is induced by butyrate and recognized by the anti-galectin-1 antibody used to localize the exogenously added purified galectin-1.

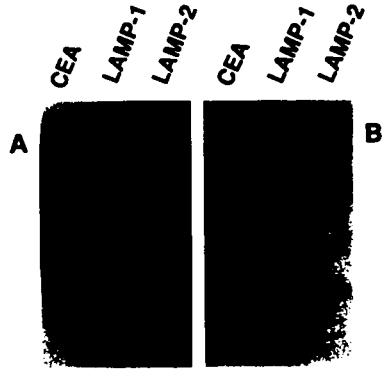

Fig. 10. Binding of purified galectin-1 to immobilized CEA, lamp-1, and lamp-2. Purified glycoproteins (4 μg each) were subjected to SDS-PAGE in 5% gels and transferred to a nitrocellulose membrane. Duplicate blots were incubated with a solution containing 40 μg/ml affinity-purified human galectin-1 in 1% hemoglobin in the absence (*A*) or presence (*B*) of 0.1 M lactose. After extensive washing, the blot was incubated with affinity-purified polyclonal anti-human galectin-1 antibody, followed by ^{125}I-labeled goat anti-rabbit antibody. Antibody binding was visualized by autoradiography.

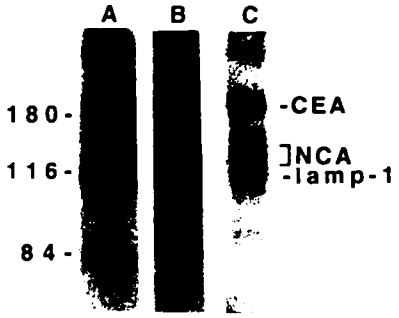

Fig. 11. Isolation of complementary glycoproteins from KM12-c HCC cells by affinity chromatography on immobilized human galectin-1. Cells were labeled for 2 days with [^{3}H]glucosamine (0.5 mCi/ml). Cell lysate was prepared and applied to a column of immobilized galectin-1. The column was washed extensively, first with buffer to remove unbound material, and then with 0.1 M lactose to elute specifically bound material. Samples of the lactose eluate were subjected to SDS-PAGE in a 6% gel, and the gel was processed for fluorography (*A*). Other samples of the lactose eluate were used for immunoprecipitation with anti-lamp-1 (*B*) or polyclonal anti-CEA antibodies (*C*), and the resulting immunoprecipitates were then subjected to SDS-PAGE in a 6% gel and processed for fluorography.

maintenance of genetic programs controlling growth and differentiation (7, 42). The process of mediating the recognition of carbohydrate-encoded information born by cellular glycoconjugates often relies on carbohydrate-binding proteins (1–7, 42). Whereas the regulation of glycoconjugate biosynthesis during development, differentiation, and transformation is well studied and documented (7, 9, 10, 43), there is comparatively little information on the regulation of lectin expression, especially in the context of cellular differentiation. In the present study, we characterized the induction of galectin-1 expression in human colon carcinoma cells in response to the differentiation-inducing agent sodium butyrate. In addition, we have identified endogenous glycoproteins which may represent *in vivo* recognition partners for this lectin in HCC cells.

The induction of galectin-1 expression was observed only in HCC cells treated with butyrate. None of the other 10 differentiation-inducing agents we examined was able to induce the lectin. The induction of galectin-1 by butyrate occurred not only in the KM12 cell line but also in seven other HCC cell lines. Two of these, SW403 and HCT-15, were reported previously to be insensitive to the butyrate-induced down-regulation of pp60$^{c\text{-}src}$ kinase activity that occurs in a number of other HCC cell lines that respond to this agent (44). These findings indicate that galectin-1 induction by butyrate may serve as a marker of differentiation of HCC cells. This suggestion is also supported by the observation that galectin-1 induction coincided with the increased expression of CEA, an established marker of differentiation in HCC cells (29–31). Previous studies have shown that galectin-1 is induced during epidermal differentiation *in vivo* and can be induced in day-13 embryonal chicken skin explants by hydrocortisone (13). Because we were not able to induce galectin-1 in HCC cells with steroids (data not shown), it appears that galectin-1 regulation may be distinct in different cell types.

Sodium butyrate is a potent differentiation-inducing agent in a variety of normal and tumor cell types (45, 46). We found that this fatty acid suppressed the growth of HCC KM12 cells in monolayer cultures and in semisolid medium. These results are similar to those in previous reports that have shown that butyrate treatment of HCC cells *in vitro* enhances the expression of the differentiated phenotype and suppresses their transformed phenotype as reflected by the ability to form colonies in semisolid medium (30, 31).

The dual effect of butyrate on galectin-1 expression and on cell growth raised the possibility that the two effects are related. However, we found that both butyrate and retinoic acid (data not shown) could suppress the ability of the KM12 HCC cells to form colonies in soft agarose, but only the butyrate-treated cells exhibited induction of galectin-1. This finding suggests that growth suppression of the KM12 HCC cells *per se* is not sufficient for galectin-1 induction. Retinoic acid was reported to suppress anchorage-independent growth of other HCC cell lines but to have only a marginal effect on differentiation (30, 31). Likewise, we did not observe CEA induction by retinoic acid in the KM12 cells. Thus, it appears that galectin-1 induction by butyrate may be related to differentiation and not to growth suppression. Furthermore, the ability of galectin-1 to suppress the growth of mouse embryo fibroblasts *in vitro* by acting as a negative autocrine factor (47) raised the possibility that the early increase in galectin-1 in butyrate-treated cells may be responsible for the subsequent growth inhibition of the KM12 cells. However, the establishment of a definitive role of galectin-1 in regulation of HCC cell growth would require more direct experimental evidence.

The analysis of butyrate-induced differentiation has particular physiological relevance in HCC cells because butyrate, a by-product of fiber fermentation by saccharolytic intestinal flora (48), is present in millimolar concentrations in the gut lumen and is readily absorbed from the colonic contents (49). Furthermore, a recent report described the ability of butyrate, delivered in liposomes to HCC tumors growing in athymic *nu/nu* mice, to suppress tumor growth and enhance differentiation (50). Butyrate derivatives are currently being developed as antineoplastic agents (51).

The exact mechanism by which butyrate induces the expression of galectin-1 is not clear. We found a rapid increase in mRNA level and additional studies not shown here indicate that butyrate treatment induces the expression of a reporter gene linked to the galectin-1 promoter. Butyrate has been shown to either enhance or suppress the expression of a variety of genes and induce or inhibit the differentiation of numerous cell types (45, 46). It has been demonstrated that butyrate treatment of cells in culture increases the level of histone acetylation by inhibiting histone deacetylase activity, and it was suggested that butyrate alters gene expression by inducing changes in chromatin conformation (52, 53). However, changes in histone acetylation alone do not readily explain the selective activation of specific genes by butyrate (54). In addition to histone hyperacetylation, butyrate has been shown to cause changes in DNA methylation (55) and histone phosphorylation (56). Butyrate modulates the expression of a number of genes that are involved in the control of cell growth. Differentiation induction in several HCC cell lines in response to butyrate was found to cause a decrease in the levels of pp60$^{c\text{-}src}$ and p56^{lck} (44) and a rapid decrease in the expression of c-*myc* (57). In the SW837 HCC cells, the decrease in c-*myc* expression has been found to result from an activity that blocks transcriptional elongation and is accompanied by a shift in the relative usage of the two major promoters of this gene (58). In light of these findings, it is of interest to determine whether the induction of galectin-1 is related to the subsequent growth inhibition in the KM12 cells.

The butyrate-induced galectin-1 was localized on the cell surface as well as in the cytoplasm. Because it is thought that the functions of galectins are mediated by binding complementary polylactosamine-containing glycoconjugates (1–7), it was of interest to identify the cognate ligands of galectin-1. Four glycoproteins of 160–200 kDa and a smaller component of 75 kDa were purified from extracts of rat lung on a column of immobilized galectin-1 (59). Whereas the identity of these molecules is not known, it was found that one of the major glycoproteins purified on immobilized porcine galectin-1 from F9 cell extracts is laminin (60), and the major galectin-1 binding protein isolated from CHO cells is lamp-1 (61). Recently, polymerized human galectin-1 has also been found to bind to lamp-1 and/or lamp-2 from human ovarian carcinoma A121 cells (62). In the study reported here,

we confirmed the ability of galectin-1 to bind purified lamp-1 and lamp-2 and characterized for the first time a few endogenous ligands for this galectin, including CEA. Purified human placenta galectin-1 was shown to interact in a carbohydrate-dependent manner with a number of endogenous proteins in KM12-P cell extracts, including a major protein of about 120 kDa in untreated cells. The major difference between the galectin-1 binding glycoconjugates of untreated and butyrate-treated cell extracts was seen in the expression of a protein of 190 kDa. It was plausible to suspect that this protein was CEA because butyrate increased the level of CEA in these cells, and CEA has been reported to contain polylactosaminoglycan oligosaccharide chains (63), which are recognized by galectin-1 (8). Indeed, human galectin-1 was found to bind to immobilized CEA purified from colon carcinoma tumor metastasis to liver, and both CEA and the related glycoprotein, non-cross-reacting antigen, were identified in material purified from KM12 cell extract by affinity chromatography on immobilized galectin-1.

This first demonstration that galectin-1 can bind CEA and that these two proteins are coordinately regulated by butyrate in the KM12 cells is important, not only because CEA is a marker of differentiation of HCC cells, but also because CEA has been demonstrated to function as a Ca^{2+}-independent cell-cell adhesion molecule (64, 65) and to be involved in cellular interactions relevant for metastasis (66). The presence of both galectin-1 and CEA on the KM12 cell surface suggests that the two complementary molecules may interact. In addition, cell surface galectin-1 may mediate interactions with ECM laminin. We have recently reported that galectin-3 from the KM12 cells also recognizes the lamps as well as CEA.[4] Thus, both lectins may contribute to the cellular interactions of butyrate-treated KM12 cells.

In conclusion, we propose that the induction of galectin-1 in the KM12 cells by butyrate represents one aspect of their acquisition of a more normal phenotype, associated with a more differentiated state, and that both galectins may mediate carbohydrate-dependent cellular interactions with CEA.

ACKNOWLEDGMENTS

We thank Drs. S. Barondes and J. Wang for their gifts of anti-galectin antibodies, Dr. D. Cooper for providing the galectin cDNAs, and Dr. J. Chan for the anti-CEA mAb. We would also like to thank Jack Pienazek for excellent technical assistance.

REFERENCES

1. Lotan, R. β-Galactoside-binding vertebrate lectins: synthesis, molecular biology, function. *In:* H. Allen and E. Kisailus (eds.), Glycoconjugates: Composition, Structure, and Function, pp. 635–671. New York, Marcel Dekker, Inc., 1992.
2. Zhou, Q., and Cummings, R. D. Animal lectins: a distinct group of carbohydrate-binding proteins involved in cell adhesion, molecular recognition, and development. *In:* M. Fukuda (ed.), Cell Surface Carbohydrates and Cell Development, pp. 99–126. Boca Raton, FL: CRC Press, 1992.
3. Drickamer, K., and Taylor, M. E. Biology of animal lectins. Annu. Rev. Cell Biol., *9:* 237–264, 1993.
4. Harrison, F. L. Soluble vertebrate lectins: ubiquitous but inscrutable proteins. J. Cell Sci., *100:* 9–14, 1991.
5. Hughes, R. C. Mac-2: a versatile galactose-binding protein of mammalian tissues. Glycobiology, *4:* 5–12, 1994.
6. Barondes, S. H., Castronovo, V., Cooper, D. N. W., *et al.* Galectins: a family of animal β-galactoside-binding lectins. Cell, *76:* 597–598, 1994.
7. Feizi, T. Carbohydrate differentiation antigens: probable ligands for cell adhesion molecules. Trends Biochem. Sci., *16:* 84–86, 1991.
8. Sparrow, C. P., Leffler, H., and Barondes, S. H. Multiple soluble β-galactoside-binding lectins from human lung. J. Biol. Chem., *262:* 7383–7390, 1987.
9. Thorpe, S. J., Bellairs, R., and Feizi, T. Developmental patterning of carbohydrate antigens during early embryogenesis of the chick: expression of antigens of the poly-*N*-acetyllactosamine series. Development (Camb.), *102:* 193–210, 1988.
10. Kimber, S. J. Glycoconjugates and cell surface interactions in pre- and peri-implantation mammalian embryonic development. Int. Rev. Cytol., *120:* 53–167, 1990.
11. Clerch, L. B., Whitney, P., and Massaro, D. Rat lung lectin gene expression is regulated developmentally and by dexamethasone. Am. J. Physiol., *256:* C501–C505, 1989.
12. Hynes, M. A., Gitt, M., Barondes, S. H., Jessell, T. M., and Buck. L. B. Selective expression of an endogenous lactose-binding lectin gene in subsets of central and peripheral neurons. J. Neurosci., *10:* 1004–1013, 1990.
13. Akimoto, Y., Kawakami, H., Oda, Y., Obinata, A., Endo, H., Kasai, K-I., and Hirano, H. Changes in expression of the endogenous β-galactoside-binding 14-kDa lectin of chick embryonic skin during epidermal differentiation. Exp. Cell Res., *199:* 297–304, 1992.
14. Poirier, F., Timmons, P. M., Chan, C-T. J., Guenet, J-L., and Rigby, P. W. J. Expression of the L-14 lectin during mouse embryogenesis suggests multiple roles during pre- and post-implantation development. Development (Camb.), *115:* 143–155, 1992.
15. Regan, L., Dodd, J., Barondes, S. H., and Jessell, J. M. Selective expression of endogenous lactose-binding lectins and lactoseries glycoconjugates in subsets of rat sensory neurons. Proc. Natl. Acad. Sci. USA, *83:* 2248–2252, 1986.
16. Carding, S. R., Thorpe, S. J., Thorpe, R., and Feizi, T. Transformation and growth related changes in levels of nuclear and cytoplasmic proteins antigenically related to mammalian galactoside-binding lectin. Biochem. Biophys. Res. Commun., *127:* 680–686, 1985.
17. Raz, A., Meromsky, L., Zwibel, I., and Lotan, R. Transformation related changes in the expression of endogenous cell lectins. Int. J. Cancer, *39:* 353–360, 1987.
18. Yamaoka, K., Ohno, S., Kawasaki, H., and Suzuki, K. Overexpression of a β-galactoside binding protein causes transformation of 3T3 fibroblast cells. Biochem. Biophy. Res. Commun., *179:* 272–279, 1991.
19. Raz, A., and Lotan, R. Endogenous galactoside-binding lectins: a new class of functional tumor cell surface molecules related to metastasis. Cancer Metastasis Rev., *6:* 433–452, 1987.
20. Allen, H. J., Karakousis C., Piver, M. S., Gamarra, M., Nava, H., Forsyth, B., Matecki, B., Jazayeri, A., Sucato, D., Kisailus, E., and DiCicoccio, R. Galactoside-binding lectin in human tissues. Tumor Biol., *8:* 218–229, 1987.
21. Gabius, H-J., Engelhardt, R., Hellmann, T., Midoux, P., Monsigny, M., Nagel, G. A., and Vehmeyer, K. Characterization of membrane lectins in human colon carcinoma cells by flow cytometry, drug targeting and affinity chromatography. Anticancer Res., *7:* 109–112, 1987.
22. Agrwal, N., Wang, J. L., and Voss, P. G. Carbohydrate binding protein 35: levels of transcription and mRNA accumulation in quiescent and proliferating cells. J. Biol. Chem., *264:* 17236–17242, 1989.
23. Raz, A., Avivi, A., Pazerini, G., and Carmi, P. Cloning and expression of cDNA for two endogenous UV-2237 fibrosarcoma lectin genes. Exp. Cell Res., *173:* 109–116, 1987.
24. Raz, A., Zhu, D., Hogan, V., Shah, N., Raz, T., Karkash, R., Pazerini, G., and Carmi, P. Evidence for the role of 34-kDa galactoside-binding lectin in transformation and metastasis. Int. J. Cancer, *46:* 871–877, 1990.
25. Gabius, H-J., Ciesiolka, T., Kunze, E., and Vehmeyer, K. Detection of metastasis-associated differences for receptors of glycoconjugates (lectins) in histomorphologically unchanged xenotransplants from primary and metastatic lesions of human colon adenocarcinomas. Clin. Exp. Metastasis, *7:* 571–584, 1989.
26. Lotan, R., Matsushita, Y., Ohannesian, D., Carralero, D., Ota, D. M., Cleary, K. R., Nicolson, G. L., and Irimura, T. Lactose-binding lectin expression in human colorectal carcinomas. Relation to tumor progression. Carbohydr. Res., *213:* 47–57, 1991.
27. Rosenberg, I., Cherayil, B. J., Isselbacher, K. J., and Pillai, S. Mac-2 binding glycoproteins: putative ligands for a cytosolic β-galactoside lectin. J. Biol. Chem., *266:* 18731–18736, 1991.
28. Lotan, R., Lotan, D., and Carralero, D. M. Modulation of galactoside-binding lectins in tumor cells by differentiation-inducing agents. Cancer Lett., *48:* 115–122, 1989.
29. Tsao, D., Shi, Z-R., Wong, A., and Kim, Y. S. Effect of sodium butyrate on carcinoembryonic antigen production by human colonic adenocarcinoma cells in culture. Cancer Res., *43:* 1217–1222, 1983.
30. Gum, J. R., Kam, W. K., Byrd, J. C., Hicks, J. W., Sleisenger, M. H., and Kim, Y. S. Effects of sodium butyrate on human colonic adenocarcinoma cells. J. Biol. Chem., *262:* 1092–1097, 1987.
31. Niles, R. M., Wilhelm, S. A., Thomas, P., and Zamchek, N. The effect of sodium butyrate and retinoic acid on growth and CEA production in a series of human colorectal tumor cell lines representing different states of differentiation. Cancer Invest., *6:* 39–45, 1988.
32. Morikawa, K., Walker, S. M., Jessup, J. M., and Fidler, I. J. *In vivo* selection of highly metastatic cells from surgical specimens of different primary human colon carcinoma implanted into nude mice. Cancer Res., *48:* 1943–1948, 1988.
33. Niles, R. M., Wilhelm, S. A., Steele, G. D., Jr., Bohdana, B., Christensen, T., Dexter, D., O'Brien, M. J., Thomas, P., and Zamchek, N. Isolation and characterization of an undifferentiated human colon carcinoma cell line (MIP-101). Cancer Invest., *5:* 545–552, 1987.
34. Lotan, R., Lotan, D., and Raz, A. Inhibition of tumor cell colony formation in culture by a monoclonal antibody to endogenous lectins. Cancer Res., *45:* 4349–4353, 1985.
35. Lotan, R. Kramer, R. H., Neumann, G., Lotan, D., and Nicolson, G. L. Retinoic acid-induced modifications in the growth and cell surface components of a human carcinoma (HeLa) cell line. Exp. Cell Res., *130:* 401–414, 1980.
36. Ho, M-K., and Springer, T. A. Mac-2, a novel 32,000 M_r mouse macrophage subpopulation-specific antigen defined by monoclonal antibodies. J. Immunol., *128:* 1221–1228, 1982.
37. Cerra, R. F., Haywood-Reid, P. L., and Barondes S. H. Endogenous mammalian lectin localized extracellularly in lung elastic fibers. J. Cell Biol., *98:* 1580–1589, 1984.
38. Zhang, H-Z, Ordonez, N. G., Batsakis, J. G., and Chan, J. C. Monoclonal antibody recognizing a carcinoembryonic antigen epitope differentially expressed in human colonic carcinoma *versus* adult colon tissues. Cancer Res., *49:* 5766–5773, 1989.
39. Krupey, J., Wilson, T., Freedman, S. O., and Gold, P. The preparation of purified carcinoembryonic antigen of the human digestive system from large quantities of tumor tissue. Immunochemistry, *9:* 617–622, 1972.

40. Carlsson, S. R., Roth, J., Piller, F., and Fukuda, M. Isolation and characterization of human lysosomal membrane glycoproteins, h-lamp-1 and h-lamp-2: major sialoglycoproteins carrying polylactosaminoglycan. J. Biol. Chem., *263:* 18911–18919, 1988.
41. Couraud, P-O., Casentini-Borocz, D., Bringman, T. S., Griffith, J., McGrogan, M., and Nedwin, G. E. Molecular cloning, characterization, and expression of a human 14-kDa lectin. J. Biol. Chem., *264:* 1310–1316, 1989.
42. Sharon, N., and Lis, H. Lectins as cell recognition molecules. Science (Washington DC), *246:* 227–234, 1989.
43. Hakomori, S. Aberrant glycosylation in tumors and tumor-associated carbohydrate antigens. Adv. Cancer Res., *52:* 257–331, 1989.
44. Foss, F. M., Veillette, A., Sartor, O., Rosen, N., and Bolen, J. B. Alterations in the expression of $pp60^{c\text{-}src}$ and $p56^{lck}$ associated with butyrate-induced differentiation of human colon carcinoma cells. Oncogene Res., *5:* 13–23, 1989.
45. Kruh, J. Effects of sodium butyrate, a new pharmacological agent, on cells in culture. Mol. Cell. Biol., *42:* 65–82, 1982.
46. Kruh, J., Defer, N., and Tichonicky, L. Action moleculaire et cellulaire du butyrate. C. R. Soc. Biol., *186:* 12–25, 1991.
47. Wells, V., and Mallucci, L. Identification of an autocrine negative growth factor: mouse β-galactoside-binding protein is a cytostatic factor and cell growth regulator. Cell, *64:* 91–97, 1991.
48. Cummings, J. H., and Englyst, H. N. Fermentation in the human large intestine and the available substrates. Am. J. Clin. Nutr., *45:* 1243–1255, 1987.
49. McNeil, N. I., Cummings, J. H., and James, W. P. T. Short chain fatty acid absorption by the human large intestine. Gut, *19:* 819–822, 1978.
50. Otaka, M., Singhal, A., and Hakomori, S. Antibody-mediated targeting of differentiation inducers to tumor cells: inhibition of colonic cancer cell growth *in vitro* and *in vivo*: a preliminary note. Biochem. Biophys. Res. Commun., *158:* 202–208, 1989.
51. Raphaeli, A., Rabizadeh, E., Aviram, A., Shaklai, M., Ruse, M., and Nudelman, A. Derivatives of butyric acid as potential anti-neoplastic agents. Int. J. Cancer, *49:* 66–72, 1991.
52. Candido, E. P. M., Reeves, R., and Davie, J. R. Sodium butyrate inhibits histone deacetylation in cultured cells. Cell, *14:* 105–113, 1978.
53. Davie, J. R., and Candido, E. P. M. Acetylated histone H4 is preferentially associated with template-active chromatin. Proc. Natl. Acad. Sci. USA, *75:* 3574–3577, 1978.
54. Birren, B. W., Taplitz, S. J., and Herschman H. R. Butyrate-induced changes in nuclease sensitivity of chromatin cannot be correlated with transcriptional activation. Mol. Cell. Biol., *7:* 3863–3870, 1987.
55. Cosgrove, D. E., and Cox, G. S. Effects of sodium butyrate and 5-azacytidine on DNA methylation in human tumor cell lines: variable response to drug treatment and withdrawal. Biochim. Biophys. Acta, *1087:* 80–86, 1990.
56. Boffa, L. C., Gruss, R. J., and Allfrey, V. G. Manifold effects of sodium butyrate on nuclear function. J. Biol. Chem., *256:* 9612–9621, 1981.
57. Barnard, J. A., and Warwick, G. Butyrate rapidly induces growth inhibition and differentiation in HT-29 cells. Cell Growth & Differ., *4:* 495–501, 1993.
58. Heruth, D. P., Zirnstein, G. W., Bradley, J. F., and Rothberg, P. G. Sodium butyrate causes an increase in the block to transcriptional elongation in the c-*myc* gene in SW837. J. Biol. Chem., *268:* 20466–20472, 1993.
59. Powell, J. T., and Whitney, P. L. Endogenous ligands of rat lung β-galactoside-binding protein (galaptin) isolated by affinity chromatography on carboxyamidomethylated-galaptin-Sepharose. Biochem. J., *223:* 769–774, 1984.
60. Zhou, Q., and Cummings, R. D. L-14 lectin recognition of laminin and its promotion of *in vitro* cell adhesion. Arch. Biochem. Biophys., *300:* 6–17, 1993.
61. Do, K-Y., Smith, D. F., and Cummings, R. D. LAMP-1 in CHO cells is a primary carrier of poly-*N*-acetyllactosamine chains and is bound preferentially by a mammalian S-type lectin. Biochem. Biophys. Res. Commun., *173:* 1123–1128, 1990.
62. Skrincosky, D. M., Allen, H. J., and Bernacki, R. J. Galaptin-mediated adhesion of human ovarian carcinoma A121 cells and detection of cellular galaptin-binding glycoproteins. Cancer Res., *53:* 2667–2675, 1993.
63. Yamashita, K., Totani, K., Kuroki, M., Matsuoka, Y., Ueda, I., and Kobata, A. Structural studies of the carbohydrate moieties of carcinoembryonic antigens. Cancer Res., *47:* 3451–3459, 1987.
64. Benchimol, S., Fuks, A., Jothy, S., Beauchemin, N., Shirota, K., and Stanners, C. P. Carcinoembryonic antigen, a human tumor marker, functions as an intercellular adhesion molecule. Cell, *57:* 327–334, 1989.
65. Stanners, C. P., Rojas, M., Zhou, H., Fuks, A., and Beauchemin, N. The CEA family: a system in transitional evolution? Int. J. Biol. Markers, *7:* 137–142, 1992.
66. Jessup, J. M., and Thomas, P. Carcinoembryonic antigen: function in metastasis by human colorectal carcinoma. Cancer Metastasis. Rev., *8:* 263–280. 1989.