Cytotechnology **31:** 255–263, 1999.

Differential patterns of expression of glycosylphosphatidylinositol-anchored carcinoembryonic antigen and alkaline phosphatase in various cancer cell lines

Kaoru Yoshinari[1,*], Kunio Matsumoto[2] & Hideo Misaki[1]
[1] *Diagnostics R&D Department, Asahi Chemical Industry Co., Ltd., Ohito-cho, Shizuoka 410-2321, Japan*
(Author for correspondence, e-mail: a8111626@ut.asahi-kasei.co.jp)*
[2] *Department of Applied Chemistry, Kanagawa Institute of Technology, Shimoogino, Atsugi-shi, Kanagawa 243-0292, Japan*

Received 16 July 1998; accepted 15 December 1999

Key words: alkaline phophatase, cancer cell line, carcinoembryonic antigen, glycosylphosphatidylinositol, sodium butyrate, tumor-associated antigen

Abstract

The expression of glycosylphosphatidylinositol (GPI-anchored) carcinoembryonic antigen (CEA) and alkaline phosphatase (ALP) on the cell surface of various cancer cell lines and a lung diploid cell line (WI38) was investigated, with exposure of the cell lines to a cell differentiation agent (sodium butyrate) to induce cell differentiation and expression of the two tumor-associated antigens. In three colon (SW1222, SW1116, and HT-29) and stomach (MKN-45) cancer cell lines, all of which are double producers of CEA and ALP, the maximum expression of GPI-anchored CEA occurred with butyrate at a lower concentration than did that of GPI-anchored ALP. GPI-anchored ALP derived from colon (SW1222 and SW1116) and stomach (MKN-45 and MKN-1) cancer cell lines was heat-stable with and without exposure to butyrate, but GPI-anchored ALP derived from lung cancer cell lines (PC-6, PC13, PC-14, WI26VA4, and WI38VA13) showed a variety of heat stabilities, depending on cell line, butyrate exposure, and SV40 transformation.

Introduction

Carcinoembryonic antigen (CEA) (Gold and Freedman, 1965a, b) and alkaline phosphatase (ALP) (Fishman et al., 1968; Nakayama et al., 1970; Fishman et al., 1983), are both used as tumor-associated and oncodevelopmental markers and have been identified as members of the group of glycoproteins which are anchored by glycosyl-phosphatidylinositol (GPI) (reviewed in references: Low and Saltiel, 1988; Ferguson and Williams, 1988; Low, 1989).

CEA is one of the most thoroughly characterized tumor-associated antigens, in both its biochemical and its clinical aspects. Its molecular weight of 180 000–200 000 includes a carbohydrate content of approximately 50–60%, as demonstrated by cDNA cloning (Oikawa et al., 1987). It is anchored to the cell surface membrane by a GPI moiety (Takami et al., 1988), and functions as an intercellular adhesion molecule by the mediating homotypic aggregation of cultured human colon cancer cells (Pignatelli et al., 1990).

ALP comprises a group of four isozymes found in liver, kidney, intestine, placenta, bone and most other tissues (reviewed in reference McComb et al., 1979). ALP derived from various sources such as human placenta, pig kidney microsome, and rat liver plasma membrane has been shown to be anchored to the cell surface membrane by a GPI moiety (Low and Zilversmit, 1980; Kominami et al., 1985; Miki et al., 1985; Malik and Low, 1986; Howard et al., 1987). Placental ALP (Reagan) (Fishman et al., 1968) and germ cell ALP (Nagao) (Nakayama et al., 1970) isozymes, which are detectable in sera and cancerous tissue of patients with genitourinary and other cancers,

are stable to heat inactivation at 56 °C. The other two isozymes (liver/bone/kidney ALP and intestinal ALP), in contrast, are readily inactivated by heat (Fishman, 1983). Each ALP isozyme is expressed in association with distinct cancers or cancerous stages (Fishman et al., 1968; Nakayama et al., 1970; Fishman, 1983).

The present study is part of an investigation ultimately directed toward the development of immunological tools for cancer therapy and imaging. Our investigation is focused on the development of antibodies against tumor-associated, GPI-anchored antigens such as CEA and ALP, since cell-surface antigens may presumably be more efficiently targeted than cytoplasmic antigens. The use of two or more antibodies to such antigens in combination, moreover, may improve the detection and discrimination of cancer cells expressing these antigens. The present study was therefore performed as an initial investigation of the patterns of GPI-anchored CEA and ALP expression in various cancer cell lines at various stages of differentiation.

Butyrate is known to stimulate differentiation in a variety of cultured cells, and was therefore used for this purpose in the present study. Some studies have shown that butyrate induces morphological and biochemical changes in certain colon and breast cancer cell lines, including an increased expression of CEA consistent with a more differentiated state (Tsao et al., 1983; Abe and Kufe, 1984; Niles et al., 1988; Toribara et al., 1989; Saini et al., 1990). Other researchers have shown that exposure to butyrate increases expression of ALP in certain colon cancer cell lines (Chung et al., 1985; Gum et al., 1987). These studies have generally examined the expression of either CEA or ALP, in separate experiments.

In the present study, we investigate and compare the patterns of expression of both CEA and ALP, and the four isozymes of ALP, in various cancer cell lines under exposure to sodium butyrate as a differentiation-inducing agent.

Materials and methods

Materials

Phosphatidylinositol-specific phospholipase C (PI-PLC) from *Bacillus thuringiensis* was purchased from Funakoshi Co. (Tokyo, Japan). A CEA ELISA kit was obtained from Denka-Seiken (Tokyo, Japan).

Table 1. Cell lines used in this study

Cell line	Origin	Histology
SW1222	Colon	Well differentiated carcinoma
SW1116	Colon	Well differentiated carcinoma
HT-29	Colon	Well differentiated carcinoma
PC-6	Lung	Small cell carcinoma
PC-13	Lung	Large cell carcinoma
PC-14	Lung	Poorly differentiated adenocarcinoma
WI26VA4	Lung	SV40-transformed cancer cell
WI38VA13	Lung	SV40-transformed cancer cell
WI-38	Lung	Lung diploid fibroblast cell
MKN-45	Stomach	Poorly differentiated adenocarcinoma
MKN-1	Stomach	Adenosquamous cell carcinoma
NUGC-4	Stomach	Poorly differentiated adenocarcinoma

Cell lines

All cell lines were grown at 37 °C with 5% CO_2 in Minimum Essential Medium (MEM) (Flow Laboratories, McLean, VA) with 10% fetal calf serum (FCS) (Gibco-BRL, Gaithersburg, MD), and supplemented with penicillin and streptomycin (100 units ml^{-1} and 100 μg ml^{-1}, respectively). The colon cancer cell line SW1222 was originally established by Leibovitz et al. (1976). Stomach tumor cell lines MKN-1 and MKN-45 and lung tumor cell lines PC-6, PC-13, and PC-14 were purchased from IBL Co. (Gunma, Japan). The colon tumor cell line SW1116, lung tumor cell lines WI26VA4 and WI38VA13, and lung diploid cell line WI38 were purchased from Dai-Nippon Pharmaceutical Co. Ltd. (Osaka, Japan). The colon tumor cell HT-29 was obtained from American Type Culture Collection (Rockville, MD). The gastric tumor cell line NUGC-4 (Akiyama et al., 1988) was supplied by Japanese Cancer Research Resources Bank (Tokyo, Japan). All cell lines used in this study are summarized in Table 1.

Exposure to butyrate and PI-PLC

Subconfluent monolayer cells in three plastic tissue culture dishes (10 cm in diameter, Falcon #3003) were incubated for 4 days in FCS-supplemented MEM with sodium butyrate at various concentrations. The medium was renewed at the end of day 2 by replacement with fresh medium. After incubation, the adherent cells were washed once with 5 ml of phosphate-buffered saline (PBS), and 1 ml of serum-free RPMI-1640 medium (Flow Laboratories) was added to each

dish. The adhering cells were then detached from the dishes by scraping with a rubber policeman. The cells were collected by pooling the contents of the three dishes and centrifuging the combined contents (total medium volume, 3 ml) at 1000 rpm for 10 min. The collected cells were resuspended in 1 ml of serum-free RPMI-1640, and incubated at 37 °C for the indicated times with occasional gentle shaking in the presence or absence of 0.2 μ ml^{-1} of PI-PLC. Supernatant was then obtained by low speed (1000 rpm) centrifugation for 10 min followed by high speed (10 000 rpm, 12 000 g) centrifugation for 10 min, and its CEA content and ALP activity were measured as described below. Cell numbers were counted with a hemocytometer.

CEA content and ALP activity

CEA content was determined using a commercially available CEA ELISA kit (Denka-Seiken Co., Ltd., Tokyo, Japan). ALP activity was determined as follows: A reaction mixture containing 3.0 ml of 1 M diethanolamine/0.5 mM $MgCl_2$ buffer (pH 9.8), 0.05 ml of 0.67 M *p*-nitrophenylphosphate solution, and 50 μl of the test sample was incubated at 37 °C for 15 min. Reaction was terminated by addition of 1 ml of 1 N NaOH and results were measured by absorbency at 405 nm.

Results

Optimum conditions for CEA and ALP release by PI-PLC

A colorectal tumor cell line, SW1222, was used to determine the optimum concentration of PI-PLC for release of the GPI-anchored CEA and ALP from the cells. A concentration of 0.2 μ ml^{-1} was sufficient for maximal release, and even 0.01 μ ml^{-1} showed effective activity (Figure 1). With 0.2 μ ml^{-1} of PI-PLC, the optimum incubation period was 2 h, as cell disruption and release of cytoplasmic CEA and ALP were low throughout this period but increased rapidly with longer incubation times (Figure 2).

Expression of GPI-anchored CEA and ALP by tumor cell lines

Expression of GPI-anchored CEA and ALP on the representative tumor cell surfaces was examined, with sodium butyrate induction. Table 2 summarizes the number of adhering (i.e., viable) cells under exposure to various concentrations of butyrate. Figure 3 shows the patterns of the butyrate-induced CEA and ALP expression by the twelve cell lines used in this study (Table 1). In the two colon cancer cell lines SW1222 (Figure 3A) and SW1116 (Figure 3B), the expression of GPI-anchored CEA generally decreased with increasing butyrate concentration, and that of GPI-anchored ALP was maximum around 3 mM butyrate. In a third highly differentiated colon tumor cell line, HT-29, the expression of both CEA and ALP peaked in a similar manner, at 2 and 4 mM of butyrate, respectively (Figure 3C).

Figures 3D–3F show the patterns of GPI-anchored CEA and ALP expression in the three stomach cell lines MKN-45, MKN-1, and NUGC-4. In MKN-45, expression of GPI-anchored CEA reached a plateau at 4 mM butyrate while that of GPI-anchored ALP increased exponentially over the range of butyrate concentrations tested (Figure 3D). In MKN-1 (Figure 3E), no expression of GPI-anchored CEA was observed, but GPI-anchored ALP expression peaked at 1 and 2 mM of butyrate. In NUGC-4 (Figure 3F), no detectable GPI-anchored CEA or ALP was observed with or without exposure to butyrate medium during the 4-day period of incubation.

Figures 3G–3L show the expression of ALP by various lung cancer cell lines and a diploid lung fibroblast cell line, WI-38, and the absence of any detectable CEA expression in any of these six lung cell lines. Expression of GPI-anchored ALP in the small cell lung carcinoma cell line, PC-6, decreased gradually with increasing butyrate concentration throughout the tested range of 0–10 mM (Figure 3G). The rate of GPI-anchored ALP expression by the diploid lung fibroblast cell line, WI-38, was consistently high over the 0–10 mM range of butyrate concentrations (Figure 3L), although approximately 70% of the cells were damaged by exposure to 10 mM butyrate (Table 2). Butyrate induction of GPI-anchored ALP expression in both the lung large cell carcinoma cell line PC-13 and the lung adenocarcinoma cell line PC-14 was observed at low butyrate concentrations (2 or 4 mM, respectively; Figures 3H and 3I), but exposure to higher butyrate concentrations resulted in pervasive cell death (Table 2).

The results shown in Figure 3 suggest that double producers of both GPI-anchored CEA and ALP (SW-1222, SW-1116, HT-29, and MKN-45) express maximum CEA at lower concentration of butyrate than that for ALP.

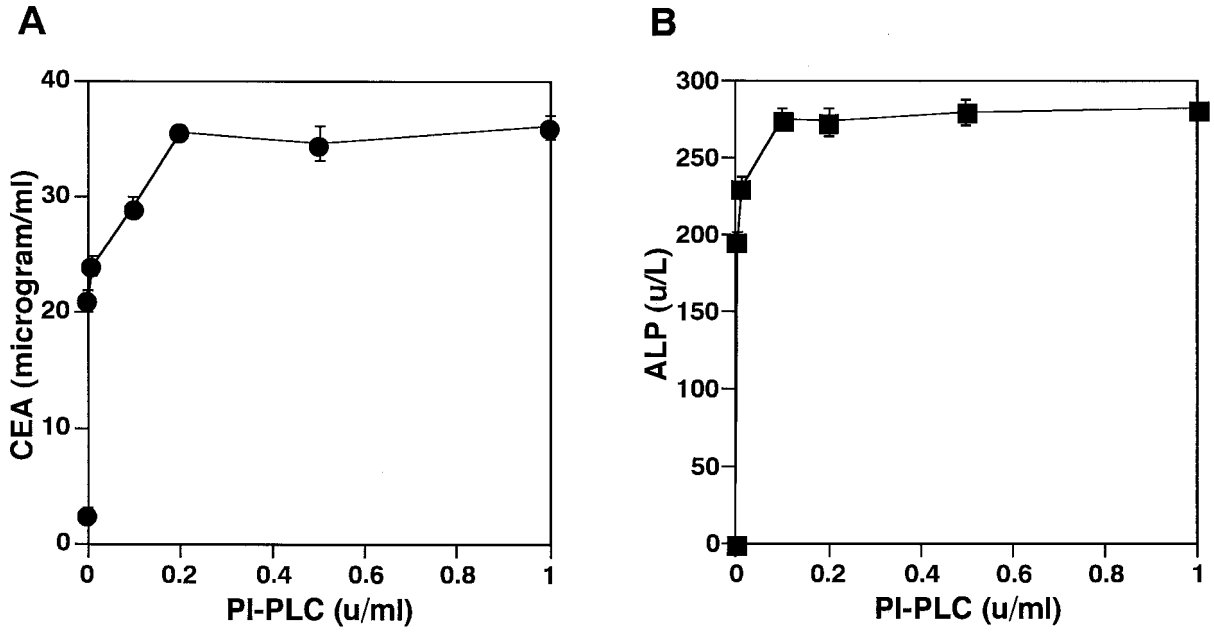

Figure 1. Determination of optimal PI-PLC. A colon cancer cell line, SW1222, was used for determination of the optimal conditions for PI-PLC digestion. Mechanically scraped and collected cells were incubated at 37 °C at the indicated concentrations of PI-PLC for 2 h. Released CEA (Figure 1A) and ALP (Figure 1B) were measured as described in Materials and Methods. All data points are the average of two determinations.

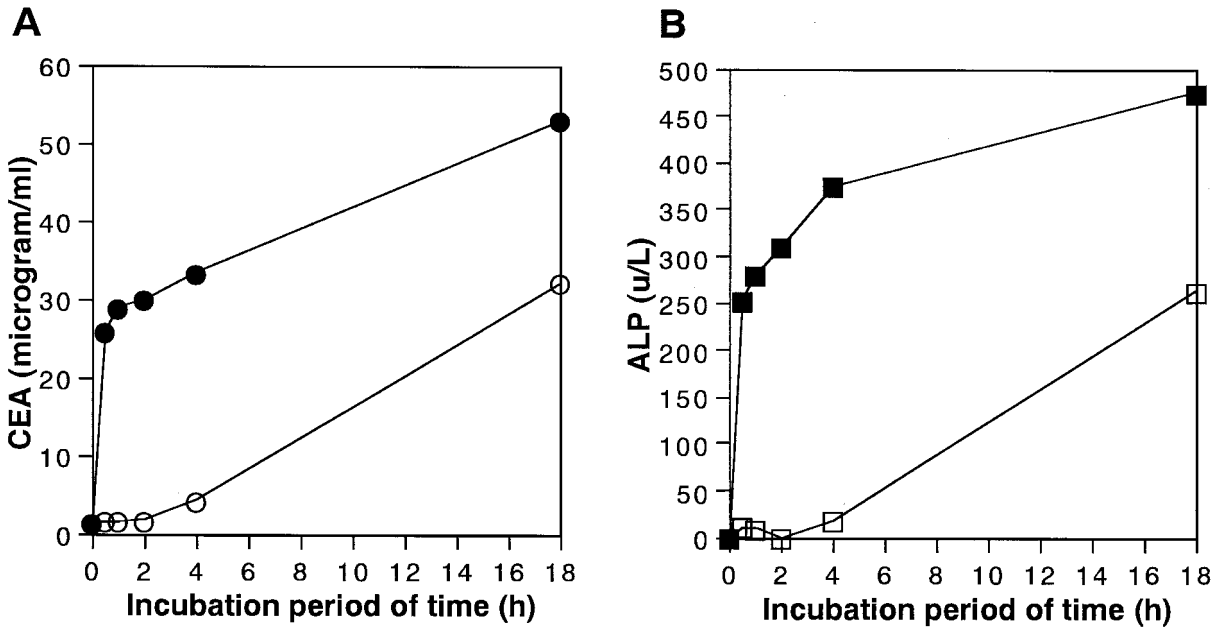

Figure 2. Determination of optimal incubation time for PI-PLC treatment. A colon cancer cell line, SW1222, was used for determination of the optimal time for PI-PLC digestion. Collected cells were incubated at 37 °C in the presence (closed symbols) or absence (open symbols) of 0.2 μ ml^{-1} of PI-PLC for the indicated periods. Representations are as described in Figure 1. All data points are the average of two determinations. Standard deviations are within 15%.

Table 2. Number of adhered cells after exposure to butyrate. Subconfluent cell monolayer was exposed for 4 days to sodium butyrate at the indicated concentration (renewal of medium at day 2). Data represent the average number and standard deviation of the total adhered cells from three plastic petri dishes, each being calculated from two determinations

Cell line	Sodium butyrate (mM)				
	0	1	2	4	10
SW1222	41.5±3.5	38.5±3.5	42.0±2.8	44.0±4.2	36.0±2.8
SW1116	60.0±2.8	61.0±4.2	58.5±3.5	59.5±3.5	51.5±3.5
HT-29	99.0±7.1	N.D.	54.0±4.2	45.0±2.8	28.5±2.1
PC-6	72.0±5.7	22.0±2.8	20.0±2.8	6.6±0.42	1.5±0.14
PC-13	30.0±2.8	13.0±1.4	5.7±0.35	0.18±0.02	0.046±0.007
PC-14	8.1±0.42	7.8±0.35	8.0±0.42	3.3±0.42	0.81±0.04
WI26VA4	66.0±4.2	N.D.	9.0±0.42	3.0±0.28	0.60±0.04
WI38VA13	48.5±3.5	9.9±0.28	8.4±0.49	3.0±0.21	0.90±0.03
WI-38	21.5±2.1	N.D.	16.1±0.99	16.0±1.4	6.60±0.35
MKN-45	86.5±5.0	42.5±3.5	26.5±2.1	21.5±2.1	27.0±2.8
MKN-1	8.4±0.42	7.8±0.42	8.2±0.64	3.9±0.42	0.64±0.05
NUGC-4	34.0±2.8	21.5±2.1	12.8±1.1	3.9±0.85	0.96±0.06

Numbers in $\times 10^6$ cells. N.D. = not determined.

Heat stability of GPI-anchored ALP induced by butyrate

ALP is known to consist of four isozymes. The placental ALP (Reagan) and germ cell ALP (Nagao) isozymes are stable at 56 °C while the liver/bone/kidney and intestinal isozymes are readily heat inactivated (Fishman, 1983).

Figure 4 shows the results of heat treatment (56 °C for 10 min) for the GPI-anchored ALP obtained from various cell lines with and without butyrate. The ALP activity before and after the heat treatment is shown in Figure 4A, and the ratio of post- to pre-exposure ALP activity, as calculated from these values, is shown in Figure 4B. In the two colon cancer cell lines, SW1222 and SW1116, and two stomach cancer cell lines, MKN-45 and MKN-1, the ALP activity was stable under heat treatment irrespective of prior exposure to butyrate (Figure 4B). The lung cancer cell lines, in contrast, showed three different patterns of heat sensitivity. In the PC-13 and PC-6 lines, the percentage of heat-stable ALP was lower in cells that had been exposed to butyrate than in those that had not. In WI26VA4 and WI38VA13, the percentage of heat-stable ALP was the same with or without prior butyrate exposure. In WI38, finally, the percentage of heat-stable ALP was increased by butyrate exposure.

These results show that the ALPs of the colon cancer and stomach cancer cell lines were different from those in the lung cancer cell lines, and that in their profiles of ALP expression by butyrate induction, colon and stomach cancer cell lines clearly differ from the lung cancer cell lines other than SV40-transformed WI26VA4 and WI38VA13.

Discussion

Butyrate has been shown to slow proliferation and elicit differentiation in a variety of cell lines (Tsao et al., 1983; Abe and Kufe, 1984; Chung et al., 1985; Gum et al., 1987; Niles et al., 1988; Toribara et al., 1989; Saini et al., 1990; Fleming et al., 1995; Velcich et al., 1995; Hodin et al., 1996). Particularly in some colon or breast cancer cell lines, butyrate has been shown to increase the levels of CEA or ALP and cause alterations in cellular differentiation (Tsao et al., 1983; Abe and Kufe, 1984; Chung et al., 1985; Gum et al., 1987; Niles et al., 1988; Toribara et al., 1989; Saini et al., 1990). Those reports, however, only describe the expression of CEA or ALP singly. Therefore, as a fundamental step toward the ultimate goal of developing a means of immunological therapeutics or in vivo diagnostics for cancer using a combination of antibodies against two separate tumor-associated, GPI-anchored antigens, we attempted to elucidate the relationship between expression of GPI-anchored CEA and ALP as a model system, in 12 cell lines, under exposure to butyrate. Expression of both GPI-anchored CEA and ALP was observed in four (SW1222, SW1116, HT-

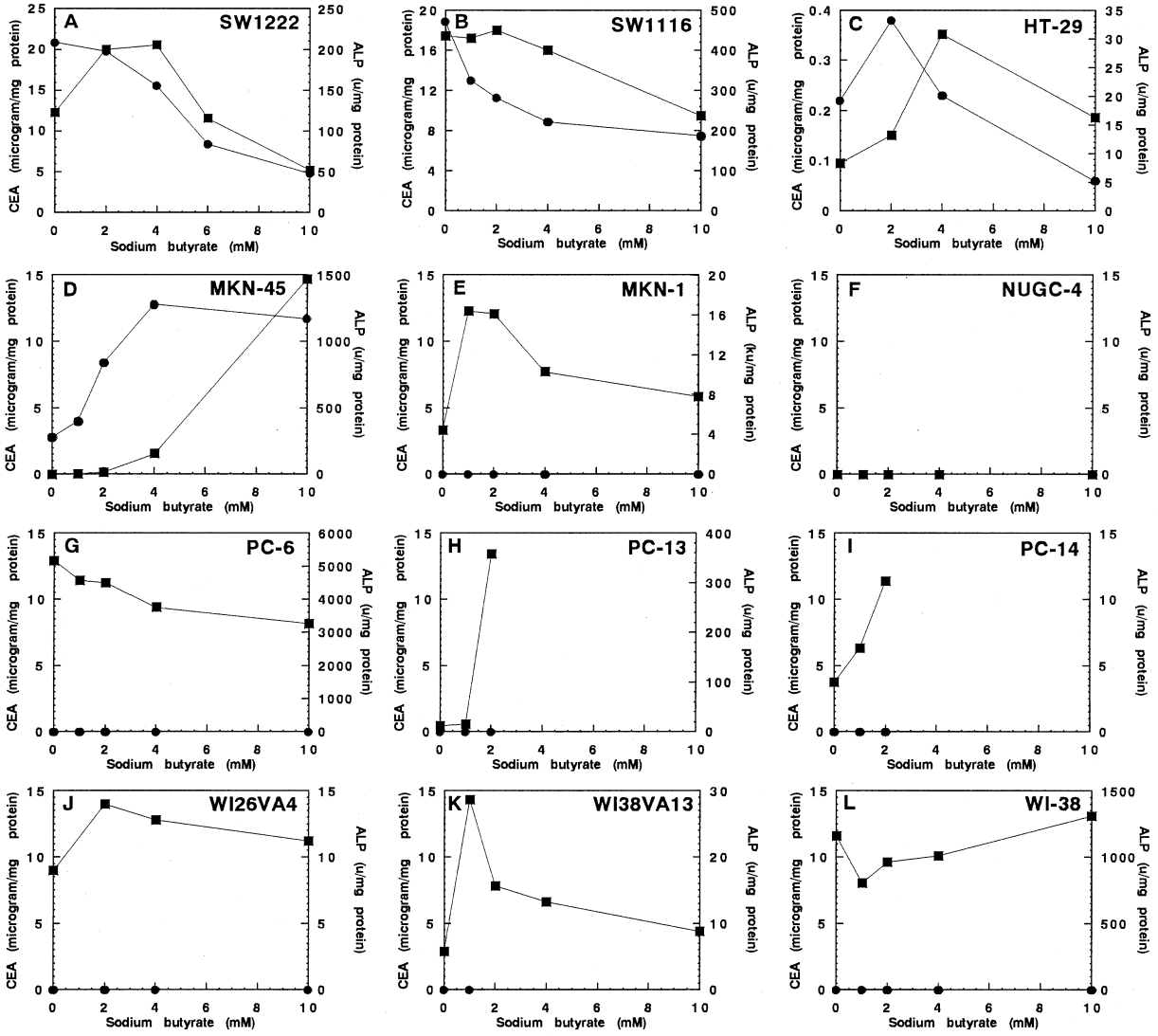

Figure 3. Patterns of GPI-anchored CEA and ALP co-expression in various cell lines. Expression patterns of GPI-anchored CEA and ALP were investigated with exposure to sodium butyrate, using three colon cancer cell lines (SW1222, SW1116, and HT-29), three stomach cancer cell lines (MKN-45, MKN-1, and NUGC-4), five lung cancer cell lines (PC-6, PC-13, PC-14, WI26VA4, and WI38VA13) and a dipolid lung fibroblast cell line (WI-38). Release of GPI-anchored CEA and ALP from the cell surface was performed at 37 °C with 0.2 μ ml^{-1} of PI-PLC for 2 h. CEA content (closed circles) and ALP activity (closed squares) are shown per mg protein. All data points are the average of two determinations. Standard deviations are within 15%.

29, and WKN-45) of the twelve cell lines. Maximum expression of CEA in these cell lines was observed at lower concentrations of butyrate than that those resulting in maximum ALP expression. On the other hand, the lung cancer cell lines used in this study expressed ALP only, in varying combinations of heat-stable and heat-labile ALP isozymes.

Some of the results observed here are inconsistent with those by other investigators. Tsao et al. (1983) have reported that SW1116 cells are relatively insensitive to butyrate treatment and that sodium butyrate at a concentration of 5 mM or higher is cytotoxic. In the present study, however, it was found that CEA expression on SW1116 cells decreased with butyrate treatment and the chemical exhibited little or no toxicity, as 80% of the CEA cells were viable after 4 h treatment with 10 mM sodium butyrate.

Velcich et al. (1995) have reported that in the colon cancer cell line HT-29, accumulation of ALP mRNA was first detected at 24 h after 5 mM sodium butyr-

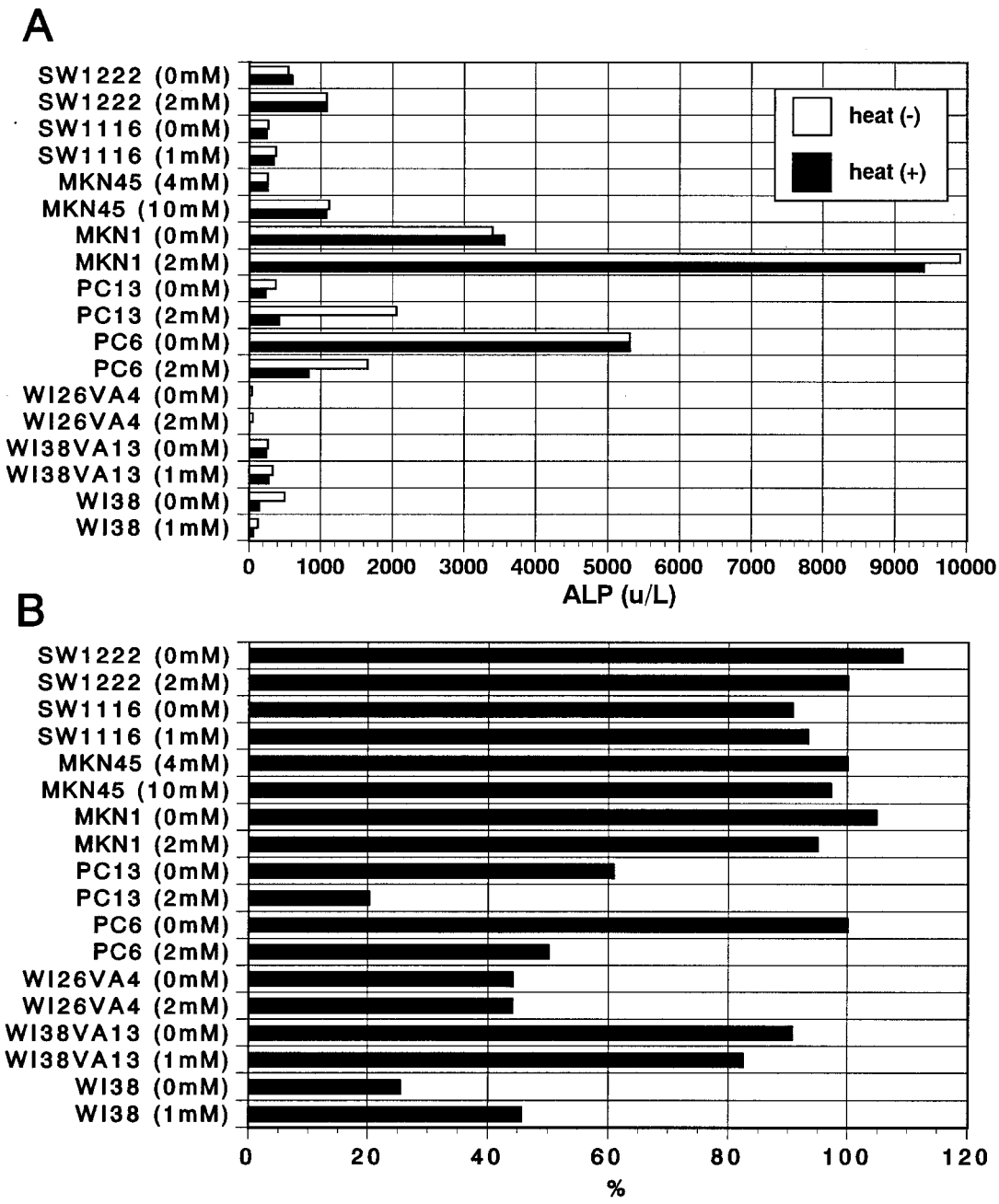

Figure 4. Effects of heat treatment on ALP derived from various cell lines with and without butyrate. Representative samples with ALP activity were tested for heat inactivation at 56 °C for 15 min. In Figure 4A, the open bars show the ALP activity without heat inactivation and the closed bars show the residual ALP activity after heat inactivation. Figure 4B shows the residual ALP after heat exposure as a percentage of that without heat exposure, calculated from the values shown in Figure 6A. The concentration of sodium butyrate used in the ALP induction is shown in parentheses for each cell line.

ate treatment, and the mRNA increased continuously up to 72 h, whereas the detectable CEA mRNA accumulation is observable around 48 h treatment. Our findings, with a different experimental protocol and focused on cell membrane surface levels rather than mRNA levels, were almost the opposite of those by Velcich et al. (1995). Our results clearly showed the effect of butyrate on expression of GPI-anchored

CEA and ALP. Since the maximum expression of GPI-anchored CEA peaked at a lower concentration of sodium butyrate than that of GPI-anchored ALP in the four double producers, the expression of CEA and ALP may be sequentially regulated in oncodevelopmental stages. The mechanism of the action of butyrate on CEA and ALP gene expression in cancer cells is unknown, but it has been proposed that butyrate activates some genes in a transcriptional level via hypermethylation of histones by inhibiting histone deacetylase (Riggs et al., 1977; Sealy and Chalkley, 1978; Vidali et al., 1978). Therefore, given a uniform action of butyrate to genes, it may be that CEA and ALP genes, as oncodevelopmental genes, are sequentially regulated, i.e. CEA genes may be expressed earlier than ALP genes in cancer cells.

The heat lability of the GPI-anchored ALP is also of interest, as it may provide a basis for relatively simple discrimination among the several ALP isozymes. The five lung cancer cell lines showed three distinct patterns of thermal stability in their GPI-anchored ALP, in contrast to the uniformly heat-stable ALP from the two colon cancer cell lines SW1222 and SW1116 and the stomach cancer cell lines MKN-45 and MKN-1. In the small cell lung cancer cell line PC-6, the ALP expressed in the absence of sodium butyrate was virtually all heat-stable, but heat-labile ALP was induced by 2 mM sodium butyrate. Sodium butyrate (2 mM) increased heat-labile ALP several fold in large lung cancer cell line PC-13. This result coincides with the observation with human endometrial cancer cells by Fleming et al. (1995).

In the SV40-transformed cell lines WI26VA4 and WI38VA13, it is of note that the percentages of heat-stable ALP with and without butyrate induction were almost the same. With human diploid lung fibroblast cell lines WI-26 and WI38, Knaup et al. clearly observed an all-or-nothing shift from a liver-like type ALP to a placental-like variant after the transformation by the DNA-tumor virus SV40 (i.e., WI26SV40 and WI38SV40) (1978). In the present study, however, heat-stable ALP comprised approximately 25% of the GPI-anchored ALP in WI-38 cells and approximately 90% of that in a subline of the SV40-transformed WI-38 cells (WI38VA13). With butyrate exposure (1 mM), heat-stable ALP increased to 46% (from 25% without butyrate exposure) in WI-38 cells and decreased sightly, to 83% (from 91%), in WI38VA13 cells.

In conclusion, the present study suggests that the expression of GPI-anchored CEA and ALP is sequentially regulated in oncodevelopmental stages, as the maximum expression of GPI-anchored CEA peaked at a lower concentration of sodium butyrate than that of GPI-anchored ALP did in the four double producers tested. In addition, the expression patterns of ALP isozymes by butyrate induction in colon and stomach cancer cell lines, showing generally heat-stable ALP, were different from those of lung cancer cell lines, which comprised varying combinations of heat-stable and heat-labile ALP.

Acknowledgement

We thank Tomomi Akiyama-Usami for technical assistance, and O.M. Stever for critical reading of the manuscript.

References

Abe M and Kufe DW (1984) Effect of sodium butyrate on human breast carcinoma (MCF-7) cellular proliferation, morphology, and CEA production. Breast Cancer Res Treat 4: 269–274.

Akiyama S, Amo H, Watanabe T, Matuyama M, Sakamoto J, Imaizumi H, Kondo T and Takagi H (1988) Characteristics of three human gastric cancer cell lines, NU-GC–2, NU-GC-3 and NU-GC-4. Jpn J Surg 18: 438–446.

Chung YS, Song IS, Erickson RH, Sleisenger MH and Kim YS (1985) Effect of growth and sodium butyrate on brush border membrane-associated hydrolases in human colorectal cancer cell lines. Cancer Res 45: 2976–2982.

Ferguson MAJ and Williams AF (1988) Cell-surface anchoring of proteins via glycosyl-phosphatidylinositol structures. Annu Rev Biochem 57: 285–320.

Fishman WH (1983) Oncodevelopmental markers. In: Oncodevelopmental markers; Biologic, Diagnostic, and Monitoring Aspects. Academic Press, Inc., New York, pp. 3–19.

Fishman WH, Inglis NR, Green S, Anstiss CL, Ghosh NK, Reif AE, Rustigan R, Krant MJ and Stolbach LL (1968) Immunology and biochemistry of Regan isoenzyme of alkaline phosphatase in human cancer. Nature (Lond.) 219: 697–699.

Fleming H, Begley M, Campi T, Condon R, Dobyns K, McDonagh J and Wallace S (1995) Induction of heat labile alkaline phosphatase by butyrate in differentiating endometrial cells. J Cell Biochem 58: 509–516.

Gold P and Freedman SO (1965a) Demonstration of tumor-specific antigens in human colonic carcinoma by immunological tolerance and absorption techniques. J Exp Med 121: 439–462.

Gold P and Freedman SO (1965b) Specific carcinoembryonicantigens of the human digestive system. J Exp Med 122: 467–481.

Gum JR, Kam WK, Byrd JC, Hicks JW, Sleisenger MH and Kim YS (1987) Effects of sodium butyrate on human colonic adenocarcinoma cells. J Biol Chem 262: 1092–1097.

Hodin RA, Meng S, Archer S and Tang R (1996) Cellular growth state differentially regulates enterocyte gene expression in butyrate-treated HT-29 cells. Cell Growth Differ 7: 647–653.

Howard AD, Berger J, Gerber L, Familletti P and Udenfriend S (1987) Characterization of the phophatidylinositol-glycan membrane anchor of human alkaline phophatase. Proc Natl Acad Sci USA 84: 6055–6059.

Knaup G, Pfleiderer G and Bayreuther K (1978) Human diploid lung fibroblast cell lines WI-26 and WI38 exhibit isozyme shift of alkaline phosphatase after viral transformation. Clin Chim Acta 88: 375–383.

Kominami T, Miki A and Ikehara Y (1985) Electrophoretic characterization of hepatic alkaline phosphates released by phosphatidylinositol-specific phospholipase C. Biochem J 227: 183–189.

Leibovitz A, Stinson JC, McCombs WB III, McCoy CE, Mazur KC and Marby ND (1976) Classification of human colorectal adenocarcinoma cell lines. Cancer Res 36: 4562–4569.

Low MG (1989) The glycosyl-phosphatidylinositol anchor of membrane proteins. Biochim Biophys Acta 988: 427–454.

Low MG and Saltiel AR (1988) Structural and functional roles of glycosyl-phosphatidylinositol in membranes. Science 239: 268–274.

Low MG and Zilversmit DB (1980) Role of phosphatidylinositol in attachment of alkaline phosphates to membranes. Biochemistry 19: 3913–3918.

Malik AS and Low MG (1986) Conversion of human placental alkaline phophatase from a high Mr form to a low Mr form during butanol extraction. Biochem J 240: 519–527.

McComb RB, Bowers Jr GN and Posen S (1979) Alkaline phosphatase, Plenum Publishing Corp., New York.

Miki A, Kominami T and Ikehara Y (1985) pH-dependent conversion of liver-membranous alkaline phosphatase to a serum-soluble from by n-butanol extraction. Biochem Biophys Res Commun 126: 89–95.

Nakayama T, Yoshida M and Kitamura M (1970) L-Leucine sensitive, heat-stable alkaline phosphates isoenzyme detected in a patient with pleuritis caricinomatosa. Clin Chim Acta 30: 546–548.

Niles RM, Wilhelm SA, Thomas P and Zamcheck N (1988) 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.

Oikawa S, Nakazato H and Kosaki G (1987) Primary structure of human carcino-embryonic antigen (CEA) deduced from cDNA sequence. Biochem Biophys Res Commun 142: 511–518.

Pignatelli M, Durbin H and Bodmer WF (1990) Carcinoembryonic antigen functions as an accessory adhesion molecule mediating colon epithelial cell-collagen interactions. Proc Natl Acad Sci USA 87: 1541–1545.

Riggs MG, Whittaker RG, Neumann JR and Ingram VM (1977) n-butyrate causes histone modification in HeLa and Friend erythroleukemia cells. Nature 268: 462–464.

Saini K, Steele G and Thomas P (1990) Induction of carcinoembryonic-antigen-gene expression in human colorectal carcinoma by sodium butyrate. Biochem J 272: 541–544.

Sealy L and Chalkley R (1978) The effect of sodium butyrate on histone modification. Cell 14: 115–121.

Takami N, Misumi Y, Kuroki M, Matsuoka Y and Ikehara Y (1988) Evidence for carboxyl-terminal processing and glycolipid-anchoring of human carcinoembryonic antigen. J Biol Chem 263: 12716–12720.

Toribara NW, Sack TL, Gum JR, Ho SB, Shively JE, Willson JKV and Kim YS (1989) Heterogeneity in the induction and expression of carcinoembryonic antigen-related antigens in human colon cancer cell lines. Cancer Res 49: 3321–3327.

Tsao D, Shi Z, Wong A and Kim YS (1983) Effect of sodium butyrate on carcinoembryonic antigen production by human colonic adenocarcinoma cells in culture. Cancer Res 43: 1217–1222.

Velcich A, Palumbo L, Jarry A, Laboisse C, Racevskis J and Augenlicht L (1995) Patterns of expression of lineage-specific markers during *in vitro* induced differentiation of HT29 colon carcinoma cells. Cell Growth Differ 6: 749–757.

Vidali G, Boffa LC, Bradbury EM and Allfrey VB (1978) Butyrate suppression of histone deacetylation leads to accumulation of multiacetylated forms of histones H3 and H4 and increased DNase I sensitivity of the associated DNA. Proc Natl Acad Sci USA 75: 2239–2243.