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Tumor-promoting Phorbol Ester Induces Alterations of Sialidase and Sialyltransferase Activities of JB6 Cells

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Sialidase and sialyltransferase activities were studied in JB6 mouse epidermal cells before and after exposure to phorbol ester, 12-O-tetradecanoyl phorbol-13-acetate (TPA), which irreversibly induces anchorage-independent growth and tumorigenicity. JB6 cells exhibited sialidase activities toward 4-methylumbelliferyl-α-D-N-acetylneuraminic acid (4MU-NeuAc) and gangliosides at pH 4.5 in the particulate fraction but apparently not in the cytosol at pH 4.5 or 6.0. In JB6 cells exposed to TPA and in the anchorage-independent transformants, the sialidase activity toward 4MU-NeuAc was decreased and the activity toward gangliosides was increased compared with those in untreated JB6 cells. Immunological analysis with antisera against membrane-associated sialidases I and II revealed that plasma membrane-associated sialidase I was increased and lysosomal membrane-associated sialidase II was decreased under these conditions. TPA treatment also affected the sialyltransferase activities of JB6 cells: an elevation of the transfer activities toward asialo-orosomucoid and asialo-porcine submaxillary mucin but a reduction of GM_3 and GD_3 synthase activities were observed on exposure to TPA and in cells transformed by TPA to retain anchorage-independency. These results suggest that an increase in sialic acid bound to glycoproteins and a decrease in that bound to glycolipids may occur in JB6 cells exposed to TPA and in the anchorage-independent transformants.

Key words: Sialidase — JB6 cell — Phorbol ester — Anchorage independence — Ganglioside

Alterations in cell surface carbohydrates are known to be closely associated with malignant transformation.[1,2] In particular, alterations in terminal sugar units such as sialic acid may play a role in the expression of decreased adhesiveness[3,4] and of metastatic potential.[5–7] To answer the question of how the sialylation state of cell surface glycoconjugates is involved in malignant transformation, we have been studying neoplastic alterations of sialidase and sialyltransferase.

We previously demonstrated that hepatocarcinogenesis of rats was associated with changes in the activities of four types[8–12] of sialidase differing in their subcellular localization: hepatoma showed increased activity of intralysosomal sialidase,[13] and decreased activities of cytosolic[13] and membrane-associated[14] sialidases. Recent immunological studies on the membrane-asociated sialidases revealed that lysosomal membrane sialidase II was actually decreased but plasma membrane sialidase I remained unchanged in hepatoma.[15]

We also found that hepatoma possesses only a sialylated and activated form of Galβ1→4GlcNAcα2→6 sialyltransferase, while a considerable portion of the liver enzyme remained unsialylated and less active.[16] The finding fits well with the report that hepatoma was more active in α2→6 sialyl transfer toward asialo-orosomucoid than liver.[17]

JB6 cells derived from epidermal cells of newborn BALB/c mice were shown by Colburn and coworkers[18,19] to respond to TPA by irreversible induction of anchorage-independent growth, and this was well correlated with tumorigenicity. Srinivas *et al.*[20] reported that TPA[7] treatment reduces the synthesis of gangliosides, in particular of trisialo-ganglioside, in JB6 cells. In the present study, we examined the levels of sialidases and sialyltransferases of JB6 cells before and after TPA treatment.

MATERIALS AND METHODS

Materials TPA and phorbol were purchased from Calbiochem (La Jolla, CA) and Funakoshi Pharm. (Kyoto), respectively. CMP-[^{14}C]NeuAc was obtained from New England Nuclear (Boston, MA) and diluted with cold CMP-NeuAc from Sigma (St. Louis, MO) to give a final specific radioactivity of 2.5 Ci/mol.

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[7] Abbreviations used: TPA, 12-O-tetradecanoyl phorbol-13-acetate; 4MU-NeuAc, 4-methylumbelliferyl-α-D-N-acetylneuraminic acid.

Orosomucoid and lactosylceramide were purchased from Sigma. Bovine and porcine submaxillary mucin were prepared by the methods of Tsuiki *et al.*[21] and de Solegui and Plonska,[22] respectively. Orosomucoid and bovine submaxillary mucin were desialylated by mild acid hydrolysis (0.1 *N* H_2SO_4, 80°C, 1 h), and porcine submaxillary mucin was treated with 1 *N* H_2SO_4 at 70°C for 3 h to remove sialic acid and fucose. GM_3 was isolated from dog erythrocytes and GM_1 and GD_{1a} were from bovine brain. 4MU-NeuAc was purchased from Nakarai (Kyoto). Antisera against membrane sialidases I (anti-I) and II (anti-II) were prepared by immunizing rabbits with rat brain enzymes as described previously.[11]

Cells Parental JB6 cells were kindly supplied by Colburn *et al.* and grown in monolayer culture at 37°C under a humidified atmosphere of 5% CO_2; Eagle's MEM supplemented with 10% heat-inactivated fetal calf serum was used. The cells were harvested prior to confluency. To examine the effect of TPA, the cells were exposed to 10 ng/ml of TPA for 24 h then harvested. Anchorage-independent clones were isolated by plating on the surface of 0.5% agar plates containing 10 ng/ml of TPA by the method described by Hosoi *et al.*[23] Colonies were removed and grown in monolayer culture for 10 days in the absence of TPA. Clones that were found to retain anchorage-independent growth by re-examining the colony-forming capacity without TPA were used as clones A-D (Table I).

Preparation of particulate fraction Cultures containing approximately 5.0×10^6 cells were washed 3 times with phosphate-buffered saline (PBS), then the cells were collected using a rubber policeman and centrifuged at 600*g* for 5 min. The packed cells were washed with PBS and sonicated in 9 vol of 0.25 *M* sucrose/1 m*M* EDTA 3 times each for 20 min at maximum speed. The mixture was then centrifuged at 600*g* for 10 min and the supernatant at 105,000*g* for 60 min. The resultant supernatant was used as the cytosolic fraction, and the pellet suspended in 9 vol of 0.25 *M* sucrose/1 m*M* EDTA was used as the particulate fraction. Protein was determined by using a phenol reagent.[24]

Sialidase assay 4MU-NeuAc and GM_3 were routinely used as substrates, since based on the previous results[8-12] 4MU-NeuAc is an appropriate substrate for intralysosomal and cytosolic sialidases and GM_3 for membrane-associated sialidases. When 4MU-NeuAc was the substrate, the assay mixture contained 60 nmol of the substrate, 5 μmol of sodium acetate buffer (pH 4.5), 100 μg of bovine serum albumin and particulate fraction (40–100 μg protein) in a final volume of 0.1 ml. For the assay of cytosolic sialidase, sodium acetate buffer was replaced by sodium cacodylate buffer (pH 6.0). The incubation was conducted at 37°C for 1–2 h and terminated by adding 2.5 ml of 0.25 *M* glycine-NaOH (pH 10.4). 4MU released was measured fluorometrically as described previously.[9] GM_3 ^{14}C-labeled in the sialic acid moiety (1000 cpm/nmol) was prepared as described previously.[10] With this ^{14}C-labeled GM_3 as a substrate, the assay mixture contained 5 nmol of ^{14}C-GM_3, 5 μmol of sodium acetate (pH 4.5), 50 μg of bovine serum albumin, 50 μg of sodium deoxycholate and enzyme in 0.1 ml. After incubation at 37°C for 1–2 h, 1 ml of 5% trichloroacetic acid/1% phosphotungstic acid was added and the radioactivity in the trichloroacetic acid-soluble fraction was determined in an emulsion scintillator. When other substrates were employed, sialic acid released was determined by the thiobarbituric acid method[25] after AG1X-2 column chromatography.[14] One unit of sialidase was defined as the amount of enzyme which catalyzed the release of 1 nmol of sialic acid/h.

Sialyltransferase assay With desialylated glycoprotein acceptors, the assay mixture contained 0.04 μmol of CMP-[^{14}C]NeuAc, 50–100 μmol (expressed in terms of acceptor site) of acceptor, 5 μmol of sodium cacodylate (pH 6.3), 100 μg of Triton X-100 and enzyme (40–100 μg) in 0.1 ml. After incubation at 37°C for 30–60 min, protein-bound radioactivity was determined as described previously.[16] When glycolipids were acceptors, the assay mixture contained 0.02 μmol of CMP-[^{14}C]NeuAc, 20–50 nmol of acceptor, 2.5 μmol of sodium cacodylate (pH 6.3), 50 μg of Triton X-100 and enzyme in 0.05 ml. The reaction mixture was incubated for 1–2 h at 37°C and the radioactivity incorporated was determined by paper chromatography with 1% sodium tetraborate followed by development with chloroform/methanol/water (60:30:6) as described by Chien *et al.*[26] Gangliosides formed were identified as described previously.[17] One unit of enzyme was defined as the amount which catalyzed the transfer of 1 nmol of sialic acid/h.

Immunoprecipitation studies Deoxycholate was added to the particulate fraction to give a final concentration of 0.5% (w/v), and the mixture was homogenized in a small glass/Teflon homogenizer and centrifuged at 105,000*g* for 1 h. The resultant supernatant contained over 80% of the sialidase activity originally present in the particulate fraction. The solubilized membrane sialidase (2–0.3 units) was preincubated at 37°C for 30 min with antiserum (10–50 μl) that had been bound to Protein A bacterial adsorbent (100 μl, Seikagaku Kogyo) by previous incubation at 37°C for 30 min. After centrifugation at 10,000*g* for 10 min, sialidase remaining in the supernatant was assayed with ^{14}C-GM_3 as the substrate. In a certain case, ^{14}C-GM_3 was replaced by 4MU-NeuAc.

RESULTS

Table I shows colony formation on the surface of 0.5% agar plates of JB6 cells and the anchorage-independent

transformants in the presence and absence of 10 ng/ml TPA. When parental JB6 cells (10^3) were plated on the agar, about 4.6% of the cells formed colonies in the presence of 10 ng/ml of TPA while in the absence of TPA only a few (0.1%) colonies were observed. Anchorage-independent transformants A, B, C and D had acquired TPA-independency for colony formation on the agar plate.

These JB6 cell lines, both parental and transformed, exhibited sialidase activities toward both 4MU-NeuAc and gangliosides in the particulate fraction at pH 4.5 (Table II), but the cytosolic fraction did not attack any of these substrates at either pH 4.5 or 6.0.

The sialidase activities were slightly increased as the cell population density was increased; the activities at confluency were approximately 1.2 to 1.5 times those at the exponential phase. The experiments described below were conducted with cells just before confluency.

The effect of TPA treatment on the sialidase activities of JB6 cells is shown in Table II. Exposure of parental JB6 cells to TPA for 24 h brought about a substantial decrease in 4MU-NeuAc sialidase activity and a slight increase in GM_3 sialidase activity. Anchorage-independent transformants A-D also exhibited changes similar to those of JB6 cells exposed to TPA, as compared with the untreated parental cells, although the deviations occurred to a much greater extent than those following TPA exposure of the parental cells: the 4MU-NeuAc sialidase activity was decreased by approximately 50–80% and the ganglioside sialidase was increased by 30–130%. When the transformants were exposed to TPA, they tended to have somewhat lower and higher activities

Table I. Induction by TPA of Anchorage-independent Growth in JB6 Cells

Cell line	TPA in agar	Colony-forming efficiency (%)
Parental JB6	−	0.1±0.1
	+	4.6±1.3
Anchorage-independent transformants		
A	−	5.3±0.3
	+	7.3±0.5
B	−	1.7±0.4
	+	4.0±0.7
C	−	4.1±0.6
	+	5.2±1.2
D	−	3.3±0.5
	+	8.7±2.5

JB6 cells were plated at 1,000 cells/dish on 0.5% agar with or without TPA as described under "Materials and Methods."

Table II. Sialidase Activities in the Particulate Fractions of JB6 Cell Lines

Cell line	TPA[a]	Substrate	
		4MU-NeuAc (units/mg protein)[b]	[^{14}C]GM_3 (units/mg protein)[b]
Parental JB6	−	26.7±4.4	3.1±0.5
	+	17.5±2.9	4.0±0.4
Anchorage-independent transformants			
A	−	6.4±0.4	6.8±1.3
B	−	9.6±1.9	4.9±0.5
C	−	13.6±2.4	4.0±0.2
D	−	8.4±0.4	5.6±0.8

a) TPA exposure was carried out for 24 h at a concentration of 10 ng/ml.
b) The values are means±SD of 3–6 experiments.

Table III. Immunoprecipitation of Ganglioside-sialidase Activity by Antisera against Membrane Sialidases I and II

Cell line	TPA	Total activity (units/mg protein)	Activity[a] precipitated by (units/mg protein)	
			Anti-I	Anti-II
Parental JB6	−	2.9	1.7	1.3
	+	3.6	2.2	1.0
Anchorage-independent transformants				
A	−	6.3	4.9	0.3
B	−	4.2	3.4	0.0
D	−	5.5	4.9	0.7

a) The values are computed from total activity and % activity precipitated by anti-I or anti-II.

of the former and the latter, respectively, than those of the cells without TPA (data not shown).

To elucidate the nature of ganglioside sialidase, the particulate fraction from these cells was treated with sodium deoxycholate, and the solubilized enzyme was examined for immunoprecipitation with antisera against rat brain membrane sialidases I and II. The two antibodies did not cross-react with each other.[11] The results obtained are shown in Table III. The data for parental JB6 cells indicate that sialidases I and II are the major ganglioside sialidases in these cells.

Table III also shows that plasma membrane-associated sialidase I is increased after exposure of JB6 cells to TPA whereas lysosomal membrane-associated sialidase II is reduced. Alterations in the same direction have been observed for ganglioside sialidases of anchorage-independent transformants when compared with those of parental JB6 cells: all of clones A-D possessed higher and lower levels of membrane-sialidases I and II, respectively, than those in the parental cells (Table III).

Although membrane sialidase II hydrolyzes 4MU-NeuAc in addition to gengliosides,[11] the much higher activity toward 4MU-NeuAc compared with that toward gangliosides (Table II) suggests that the majority of the 4MU-NeuAc sialidase activity of JB6 cells comes from a third form of sialidase, most probably intralysosomal sialidase. This is supported by the two observations that (i) only a trace of 4MU-NeuAc sialidase activity of JB6 cells was immunoprecipitated with anti-II (data not shown) and (ii) the particulate fraction of JB6 cells hydrolyzed fetuin glycopeptides as well as 4MU-NeuAc but had little effect on native fetuin (data not shown). The latter finding is in agreement with previous results[8] on the substrate specificity of rat liver intralysosomal sialidase, which was proved to hydrolyze only low-molecular substrates such as 4MU-NeuAc and glycopeptides and is supposed to play a major role in glycoprotein degradation by collaborating with lysosomal proteases. JB6 cells thus were found to contain at least three types of sialidase that are altered in different manners by TPA treatment.

To elucidate further the significance of sialidase alterations, the levels of sialyltransferases were determined. Table IV shows that the activities toward asialo-orosomucoid and asialo-porcine submaxillary mucins of JB6 cells are increased by TPA treatment; the activity

Table IV. Glycoprotein-sialyltransferase Activities in JB6 Cell Lines

Cell line	TPA	Acceptor		
		Asialo-orosomucoid (units/mg protein)[a]	Asialo-porcine submaxillary mucin (units/mg protein)[a]	Asialo-bovine submaxillary mucin (units/mg protein)[a]
Parental JB6	−	0.24±0.05	0.86±0.23	0.79±0.23
	+	0.42±0.10	1.33±0.12	0.82±0.20
Anchorage-independent transformants				
A	−	0.75±0.15	1.91±0.48	1.21±0.17
D	−	0.47±0.11	3.09±0.69	1.19±0.10

a) The values are means±SD of 3 experiments.

Table V. Ganglioside-sialyltransferase Activities in JB6 Cell Lines

Cell line	TPA	Acceptor			
		Lac-cer	GM_3	GM_1	GD_{1a}
		(units/mg protein)[a]			
Parental JB6	−	0.31±0.05	0.091±0.011	0.35±0.04	0.12±0.03
	+	0.10±0.01	0.070±0.008	0.30±0.01	0.10±0.01
Anchorage-independent transformants					
A	−	0.18±0.04	0.033±0.010	0.32±0.01	0.09±0.02
D	−	0.11±0.02	0.042±0.007	0.30±0.05	0.14±0.03

a) The values are means±SD of 3 experiments.

toward asialo-bovine submaxillary mucin, on the other hand, was largely unaffected. When the transformants were examined, all the transferase activities, including the activity toward asialo-bovine submaxillary mucin, were found to be much higher than those of the parental JB6 cells (Table IV).

We have also examined sialyl transfer to glycolipids using lactosylceramide, GM_3, GM_1 and GD_{1a} as acceptors. Table V shows that, in contrast to glycoprotein sialylation, anchorage-independent transformants showed substantial reduction of GM_3 and GD_3 formations or at least little change in GD_{1a} and GT formations. None of the changes observed above were induced by a non-promoter, phorbol (data not shown).

DISCUSSION

The present study demonstrated that JB6 cells possess three different sialidases, i.e., intralysosomal, and membrane-associated I and II, and sialyltransferases capable of sialylating a variety of glycoproteins and glycolipids. In response to TPA, the cells undergo characteristic alterations in the levels of these sialidases and sialyltransferases. The alterations observed upon TPA exposure occur much more markedly in anchorage-independent transformants, which were transformed to retain anchorage-independent growth in the absence of TPA, compared with the parental JB6 cells. This enhancement in the transformants suggests that the alterations in these enzyme activities are due not only to the direct effects of TPA but also to induction of anchorage-independent growth.

Overall, the present results on sialidase and sialyltransferase favor the general view that sialic acid bound to glycoproteins tends to be increased while that bound to glycolipids is decreased in TPA-treated and TPA-transformed JB6 cells: elevation in glycoprotein sialyltransferase and reduction in 4MU-NeuAc (glycoprotein) sialidase may lead to the former, but elevation in total ganglioside sialidase and reduction of ganglioside sialyltransferase may lead to the latter in association with anchorage-independent growth.

Colburn and co-workers[20] have reported that treatment of JB6 with TPA reduces the synthesis of total gangliosides, in particular of trisialogangliosides. Their report is not contradictory to ours since we found that exposure of JB6 cells to TPA elevates the ganglioside sialidase activity, though only slightly, and reduces the GM_3 and GD_3 synthase activities. It remains, however, unclear how a selective decrease in trisialoganglioside is developed.

Alterations in the level of ganglioside sialidase by malignant transformation have also been described by others using different systems: Yogeeswaran and Hakomori[27] reported the loss of density-dependent suppression of ganglioside sialidase in 3T3 transformed cells. In our system, however, cell density-dependent changes in sialidase activities were not affected by TPA exposure or by anchorage-independency. The observation by Schengrund *et al.*[28] that the activity of ganglioside sialidase appeared in BHK transformed cells is consistent with the present study, although no information is presently available for identification of their sialidase as our sialidase I.

We have reported elsewhere changes of the sialidases[13–15] and sialyltransferases[16, 17] during hepatocarcinogenesis of the rat. In hepatoma, membrane sialidase II but not membrane sialidase I was strongly reduced as compared with normal liver.[15] Thus a decrease in the ratio of membrane sialidase II to I may be an alteration occurring independently of cell origin and in the pathway of tumor induction. We also found that the activation of Galβ1→4GlcNAcα2→6 sialyltransferase is accelerated in hepatoma.[16] Since TPA also elevates the level of asialo-orosomucoid sialyltransferase in JB6 cells, the acceleration of the sialylation of N-glycosylated proteins may be considered as a common alteration in many types of neoplastic transformation. This conclusion is consistent with the reports made by others[1, 29–32] that enrichment in sialic acid-containing, highly branched N-glycan structure has been observed in transformed cells: an increase of highly branching structure could be accompanied by the increased substitution of sialic acid, due to the elevation of the sialyltransferase.

Compared to glycoprotein sialyltransferase, alterations in ganglioside sialyltransferases appear to be more diverse among cell lines.[2] Although the reduction in GM_3 and GD_3 formation in TPA-treated JB6 cells and the transformants has also been seen in many types of transformed cells,[2] hepatoma was found to exhibit a higher level of GM_3 synthesis than liver.[17] Momoi *et al.*[33] reported that TPA activates GM_3 synthesis in HL-60 cells in association with differentiation.

Extensive studies are required to clarify the mode of TPA action on sialidases and sialyltransferases.

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REFERENCES

1) Warren, L., Buck, C. A. and Tuszynski, G. P. Glycopeptide changes and malignant transformation, a possible role for carbohydrate in malignant behaviour. *Biochim. Biophys. Acta,* **516,** 97–127 (1978).
2) Hakomori, S. Aberrant glycosylation in tumor and tumor-associated carbohydrate antigens. *Adv. Cancer Res.,* **52,** 257–331 (1989).
3) Reutter, W., Kottgen, E., Bauer, C. and Gerok, W. Biological significance of sialic acid. *In* "Sialic Acid: Chemistry, Metabolism and Functions," ed. R. Schauer, pp. 263–305 (1982). Springer-Verlag, Vienna, New York.
4) Symington, B. E., Symington, F. W. and Rohrschneider, L. R. Phorbol ester induces increased expression, altered glycosylation, and reduced adhesion of K562 erythroleukemia cell fibronectin receptors. *J. Biol. Chem.,* **264,** 13258–13266 (1989).
5) Yogeeswaran, G. and Salk, P. Metastatic potential is positively correlated with cell surface sialylation of cultured murine tumor cell lines. *Science,* **212,** 1514–1516 (1981).
6) Fogel, M., Altevogt, P. and Schirrmacher, V. Metastatic potential severely altered by changes in tumor cell adhesiveness and cell-surface sialylation. *J. Exp. Med.,* **157,** 371–376 (1983).
7) Collard, J. G., Schijven, J. F., Bikker, A., La Riviere, G., Bolscher, J. G. M. and Roos, E. Cell surface sialic acid and the invasive and metastatic potential of T-cell hybridomas. *Cancer Res.,* **46,** 3521–3527 (1986).
8) Miyagi, T. and Tsuiki, S. Rat-liver lysosomal sialidase. Solubilization, substrate specificity and comparison with the cytosolic sialidase. *Eur. J. Biochem.,* **141,** 75–81 (1984).
9) Miyagi, T. and Tsuiki, S. Purification and characterization of cytosolic sialidase from rat liver. *J. Biol. Chem.,* **260,** 6710–6716 (1985).
10) Miyagi, T. and Tsuiki, S. Evidence for sialidase hydrolyzing gangliosides GM_2 and GM_1 in rat liver plasma membrane. *FEBS Lett.,* **206,** 223–228 (1986).
11) Miyagi, T., Sagawa, J., Konno, K., Handa, S. and Tsuiki, S. Biochemical and immunological studies on two distinct ganglioside-hydrolyzing sialidases from the particulate fraction of rat brain. *J. Biochem.,* **107,** 787–793 (1990).
12) Miyagi, T., Sagawa, J., Konno, K. and Tsuiki, S. Immunological discrimination of intralysosomal, cytosolic, and two membrane sialidases present in rat tissues. *J. Biochem.,* **107,** 794–798 (1990).
13) Miyagi, T., Goto, T. and Tsuiki, S. Sialidase of rat hepatomas: qualitative and quantitative comparison with rat liver sialidase. *Gann,* **75,** 1076–1082 (1984).
14) Sagawa, J., Miyagi, T. and Tsuiki, S. Membrane-associated sialidase of rat liver and its decrease in hepatomas. *Jpn. J. Cancer Res.,* **79,** 69–73 (1988).
15) Miyagi, T., Konno, K., Sagawa, J. and Tsuiki, S. Neoplastic alteration of a membrane-associated sialidase of rat liver. *Jpn. J. Cancer Res.,* **81,** 915–919 (1990).
16) Miyagi, T. and Tsuiki, S. Purification and characterization of β-galactoside ($\alpha 2 \rightarrow 6$) sialyltransferase from rat liver and hepatomas. *Eur. J. Biochem.,* **126,** 253–261 (1982).
17) Miyagi, T., Koseki, M. and Tsuiki, S. Comparative study of the levels of sialyltransferases responsible for the formation of sugar chains in glycoproteins and gangliosides in rat liver and hepatomas. *Jpn. J. Cancer Res.,* **79,** 742–749 (1988).
18) Colburn, N. H., Bruegge, W. F. V., Bates, J. R., Gray, R. H., Rossen, J. D., Kelsey, W. H. and Shimada, T. Correlation of anchorage-independent growth with tumorigenicity of chemically transformed mouse epidermal cells. *Cancer Res.,* **38,** 624–634 (1978).
19) Colburn, N. H., Former, B. F., Nelson, K. A. and Yuspa, S. H. Tumour promoter induces anchorage independence irreversibly. *Nature,* **281,** 589–591 (1979).
20) Srinivas, L., Gindhart, T. D. and Colburn, N. H. Tumor-promoter-resistant cells lack trisialoganglioside response. *Proc. Natl. Acad. Sci. USA,* **79,** 4988–4991 (1982).
21) Tsuiki, S., Hashimoto, Y. and Pigman, W. Comparison of procedures for the isolation of bovine submaxillary mucin. *J. Biol. Chem.,* **236,** 2172–2178 (1961).
22) de Salegui, M. and Plonska, H. Preparation and properties of porcine submaxillary mucins. *Arch. Biochem. Biophys.,* **129,** 49–56 (1969).
23) Hosoi, J., Abe, E., Suda, T., Colburn, N. H. and Kuroki, T. Induction of anchorage-independent growth of JB6 mouse epidermal cells by 12,25-dihydroxyvitamin D_3. *Cancer Res.,* **46,** 5582–5586 (1986).
24) Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.,* **193,** 265–275 (1951).
25) Warren, L. The thiobarbituric acid assay of sialic acids. *J. Biol. Chem.,* **234,** 1971–1975 (1959).
26) Chien, J-L., Williams, T. and Basu, S. Biosynthesis of a globoside-type glycosphingolipid by a β-N-acetylgalactosaminyltransferase from embryonic chicken brain. *J. Biol. Chem.,* **248,** 1778–1785 (1973).
27) Yogeeswaran, G. and Hakomori, S. Cell contact-dependent ganglioside changes in mouse 3T3 fibroblasts and a suppressed sialidase activity on cell contact. *Biochemistry,* **14,** 2151–2156 (1975).
28) Schengrund, C. L., Lausch, R. N. and Rosenberg, A. Sialidase activity in transformed cells. *J. Biol. Chem.,* **248,** 4424–4428 (1973).
29) Yamashita, K., Ohkura, T., Tachibana, Y., Takasaki, S. and Kobata, A. Comparative study of the oligosaccharides released from baby hamster kidney cells and their polyoma transformant by hydrazinolysis. *J. Biol. Chem.,* **259,** 10834–10840 (1984).
30) Collard, J. G., Van Beek, W. P., Janssen, J. W. G. and Shijven, J. F. Transfection by human oncogenes: concom-

itant induction of tumorigenicity and tumor-associated membrane alterations. *Int. J. Cancer,* **35,** 207–214 (1985).
31) Pierce, M. and Arango, J. Rous sarcoma virus-transformed baby hamster kidney cells express higher levels of asparagine-linked tri- and tetraantennary glycopeptides containing [GlcNAc-β(1,6)Man-α(1,6)] and poly-N-acetyllactosamine sequences than baby hamster kidney cells. *J. Biol. Chem.,* **261,** 10772–10777 (1986).
32) Santer, U. V., De Santis, R., Hard, K. J., van Kuik, J. A., Vliegenthart, J. F. G., Won, B. and Glick, M. C. N-Linked oligosaccharide changes with oncogenic transformation require sialylation of multiantennae. *Eur. J. Biochem.,* **181,** 249–260 (1989).
33) Momoi, T., Shinmoto, M., Kasuya, J., Senoo, H. and Suzuki, Y. Activation of CMP-N-acetylneuraminic acid: lactosylceramide sialyltransferase during the differentiation of HL-60 cells induced by 12-O-tetradecanoylphorbol-13-acetate. *J. Biol. Chem.,* **261,** 16270–16273 (1986).