Correlation of Retinoic Acid-enhanced Sialyltransferase Activity and Glycosylation of Specific Cell Surface Sialoglycoproteins with Growth Inhibition in a Murine Melanoma Cell System

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ABSTRACT

Retinoic acid inhibits the proliferation of the murine melanoma clone S91-C-2 cells, enhances the glycosylation of specific cell surface sialoglycoproteins, and stimulates sialyltransferase activity. Mutant clones, selected from the S91-C-2 cells for resistance to the growth-inhibitory effect of retinoic acid, were used to explore whether cell surface modulation by retinoic acid is related to growth inhibition. Glycoprotein synthesis was assessed by analysis of [3H]glucosamine incorporation into glycoconjugates, and cell surface sialo- and galactoglycoproteins were analyzed after radiolabeling by the NaI04:NaB3H4 and the neuraminidase plus galactose oxidase:NaB3H4 methods, respectively. The cells were solubilized and the labeled molecules were separated by polyacrylamide gel electrophoresis and identified by fluorography. Sialyltransferase activity was measured in detergent-solubilized cells, using cytidine 5'-monophosphate-[14C]sialic acid as a sugar donor and asialofetuin as an exogenous acceptor. The results demonstrated that retinoic acid enhanced [3H]glucosamine incorporation into a M, 160,000 glycoprotein in the S91-C-2 cells but not in any of the resistant mutant clones, while the pattern of [35S]methionine-labeled proteins was not modified in either the sensitive or the resistant clones. Radiolabeling of a M, 160,000 sialoglycoprotein on the surface of S91-C-2 and of several retinoic acid-sensitive subclones of S91-C-2 was augmented by retinoic acid. A considerably smaller effect was observed on the labeling of M, 160,000 sialoglycoprotein on one of the resistant clones, and no significant effect could be detected on the other resistant mutant clones. Sialyltransferase activity was increased 2- to 3-fold by retinoic acid in the S91-C-2 cells and in several sensitive subclones, but not in any of the resistant mutant clones. Tetradecanoylphorbol acetate, which inhibits the proliferation of both retinoic acid-sensitive and retinoic acid-resistant cells, failed to increase sialyltransferase activity or cell surface labeling of sialoglycoproteins. These findings suggest that the ability of retinoic acid to stimulate sialyltransferase activity and glycosylation of cell surface glycoproteins is related to the growth-inhibitory effect of this compound.

INTRODUCTION

Numerous recent reports on the ability of retinoids (vitamin A analogues) to modulate the growth and differentiation of certain normal, transformed, and tumor cells have focused interest on these compounds (4, 5, 7, 11, 16, 26, 29, 39-41, 46). Of particular importance is their ability to inhibit the growth of various tumor cells in culture, to enhance the differentiation of malignant cells, and to suppress the expression of the transformed phenotype (16, 26, 29, 40). Because of their unique influence on fundamental cellular processes, the elucidation of the mechanism of retinoid action is a most challenging and interesting endeavor.

During the last few years we have characterized the effects of retinoids, and in particular RA, on cultured murine melanoma clone S91-C-2 cells (17, 22). These cells proved to be an exquisite system for exploring the mechanism of the antiproliferative activity of retinoids, since we found that RA decreases their growth rate (17), induces a tight coupling between cell shape and DNA synthesis (25), inhibits colony formation in semisolid medium known as anchorage-independent growth (19, 22, 24), stimulates melanogenesis and morphological differentiation (18), and suppresses tumor growth in vivo (22). The presence of both a cellular retinol-binding protein and a cellular RA-binding protein in S91-C-2 cells has been demonstrated (21, 23, 24). Analyses of biochemical changes induced by RA in S91-C-2 cells revealed an enhancement of sialyltransferase activity (8), and an increased glycosylation of gp160, which is a constituent of the cell surface membrane (20).

Recently, we selected and isolated from the S91-C-2 cells several mutant clones which are resistant to the growth-inhibitory effects of RA and found that resistance is not the result of a reduced RA uptake, nor can it be correlated with changes in cellular RA-binding protein levels (24).

In the present investigation we used the RA-resistant mutant clones to determine whether the changes that RA induces in the surface glycoconjugates on S91-C-2 cells are related to growth inhibition.

MATERIALS AND METHODS

Cell Culture. The S91-C-2 cloned mouse melanoma cell line (17), and the RA-resistant mutant clones derived from it (24), were grown on plastic tissue culture dishes or in 0.5% agarose and propagated in various media containing 10% fetal calf serum with or without 10-8M retinoic acid. The RA-resistant clone was derived from such colonies in plastic tissue culture dishes.

Treatment with RA and Assays of Growth Inhibition. Treatment of cells with RA (BASF Aktiengesellschaft, Ludwigshafen, Federal Republic of Germany) and assays for inhibition of growth as a result of such a treatment were performed exactly as described elsewhere (24).

1The abbreviations used are: RA, 5-all-trans-retinoic acid; gp160, M, 160,000 glycoprotein; gp135, M, 135,000 glycoprotein; SDS-PAGE, polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate; TPA, 12-O-tetradecanoylphorbol-13-acetate.

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Metabolic Radiolabelling of Glycoproteins and Proteins. Cellular glycoproteins were labeled by incubating cells with L-[6-3H]glucosamine (31 Ci/mmol; New England Nuclear, Boston, MA), and proteins were labeled by incubating cells with L-[35S]methionine (1390 Ci/mmol; Amer sham International, Ltd., Buckinghamshire, England), as described in more detail previously (20, 28).

Radiolabelling of Cell Surface Sialo- and Galactoglycoproteins. Sialic acid residues exposed on the cell surface were labeled by oxidation of intact adherent cells in monolayer cultures with dilute NaOCl, followed by reduction with NaBH₄, and exposed galactose (and/or galactosamine) residues were labeled, after removal of sialic acid residues, with neuraminidase, by oxidation with galactose oxidase, followed by reduction with NaBH₄, exactly as described elsewhere (28).

Analysis of Radiolabeled Macromolecules. After metabolic labeling the media were removed by aspiration, centrifuged at 1000 rpm for 5 min to pellet a few floating cells, and the supernatants were used for analysis of macromolecules released (shed or secreted) into the medium. Cells labeled metabolically or by cell surface-specific methods were washed, treated with detergents, and the solubilized macromolecules were analyzed. The analysis was based on subjecting the radiolabeled molecules, found in the medium or in the cell extracts, to SDS-PAGE, and identifying them by autoradiography or fluorography. The details of the procedures for cell solubilization, SDS-PAGE, and autoradiography were described previously (20, 28).

Sialyltransferase Assay. Sialyltransferase activity was assayed by measuring the transfer of [14C]sialic acid from CMP-[14C]sialic acid to an exogenous acceptor (asialofetuin), as catalyzed by cell extracts. The procedure was based on the method described by Liu et al. (15). Cells were solubilized by suspending them at 2.5 × 10⁶ cells/ml in a solution containing 0.4% Triton X-100 and 0.15 M NaCl. After centrifugation at 10,000 × g for 30 sec in a Beckman microfuge, the supernatant was removed and used as a crude enzyme preparation. Asialofetuin was prepared by removal of sialic acid residues from fetuin (Spiro method; Grand Island Biological Co., Grand Island, NY) by acid hydrolysis (0.1 n H₂SO₄, 80°, 60 min). The assay consisted of mixing 40 nM crude enzyme (about 100 µg protein); 10 µl asialofetuin (30 mg/ml in distilled water); 10 µl 0.25 M sodium phosphate buffer, pH 7.0, containing 0.05 M MgCl₂; 15 µl CMP-[4,5,6,7,8,9-14C]sialic acid (2 µCi/ml 247 mCi/mmol; New England Nuclear); and distilled water to adjust the final volume to 100 µl. Incubation was for 2 h at 37°. Transfer of [14C]sialic acid to endogenous acceptors was determined in reaction mixtures from which asialofetuin had been omitted. The endogenous activity was subtracted from the exogenous activity. The reaction was terminated by the addition of 10 µl cold 100% trichloroacetic acid, and the precipitates that formed during 18 hr at 4° were collected on GF/C glass fiber filters. The filters were washed successively with 5% cold trichloroacetic acid and 95% ethanol, and dried. The radioactivity remaining on the filters was then counted, using a Packard Tri-Carb scintillation counter.

RESULTS

Effect of RA on Cell Growth and Morphology. S91-C-2 cells exposed to RA for 3 days became more elongated and extended longer cellular processes than did untreated cells (Fig. 1, A and D). Cultures of the untreated S91-C-2 cells became confluent by the end of a 5-day incubation, whereas the growth of the cells in the presence of RA was inhibited (Fig. 1, B and E). In contrast, neither the morphology of the RA-resistant mutant clone S91-C-154 nor their proliferation were affected significantly by RA (Fig. 1, G, J, H, and K). Likewise, the morphology of the other RA-resistant clones was not altered by RA treatment (data not shown). Furthermore, the formation of multicellular colonies by the multiplication of single cells of S91-C-2 clones suspended in semisolid medium (0.5% agarose) was inhibited by RA (Fig. 1, C and F), whereas colony formation by the RA-resistant S91-C-154 cells was not suppressed by RA (Fig. 1, I and L).

Effect of RA on Incorporation of Radioactive Precursors into Cellular Glycoproteins and Proteins. The incorporation of [3H]glucosamine and [35S]methionine into trichloroacetic acid-insoluble material in the S91-C-2 cells and in the RA-resistant clones is presented in Table 1. A few of the mutant clones incorporated less [3H]glucosamine than the sensitive S91-C-2 clone, and in all except clone S91-C-163, treatment with RA reduced [3H]glucosamine incorporation by 10 to 30%. Incorporation of [35S]methionine decreased by 20% in S91-C-2 cells and was either not significantly affected or somewhat stimulated in the RA-resistant clones.

Identification of the glycoproteins and proteins that were labeled by the incorporation of the above precursors was achieved by fluorography of polyacrylamide slab gels, in which solubilized cell components had been separated by electrophoresis. As found previously (20), RA enhances [3H]glucosamine incorporation into gp160 in the RA-sensitive S91-C-2 cells (Fig. 2). In contrast, no similar increase could be detected in the RA-resistant clones (Fig. 2). However, in clone S91-C-83, RA caused an increased [3H]glucosamine incorporation into a glycoprotein which migrates somewhat faster than does gp160 (Fig. 2). The patterns of cellular proteins radiolabeled with [35S]methionine and separated by SDS-PAGE were similar in S91-C-2 cells and in the RA-resistant cells, whether the cells had been grown in the absence or presence of RA (Fig. 2).

Table 1

<table>
<thead>
<tr>
<th>Clone</th>
<th>Growth inhibition by RA</th>
<th>Radioactivity in trichloroacetic acid-insoluble material (dpm/10⁵ cells)⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[IC₅₀] (µM)</td>
<td>[3H]Glucosamine</td>
</tr>
<tr>
<td>S91-C-2</td>
<td>0.02</td>
<td>16,400 ± 190</td>
</tr>
<tr>
<td>S91-C-83</td>
<td>3.4</td>
<td>5,330 ± 110</td>
</tr>
<tr>
<td>S91-C-110</td>
<td>10</td>
<td>8,380 ± 730</td>
</tr>
<tr>
<td>S91-C-154</td>
<td>&gt;50</td>
<td>7,970 ± 420</td>
</tr>
<tr>
<td>S91-C-163</td>
<td>5.5</td>
<td>12,820 ± 180</td>
</tr>
</tbody>
</table>

⁵ IC₅₀ concentration of RA required for 50% inhibition of cell proliferation on plastic tissue culture dishes (24).

⁶ Cells were cultured in 10-cm-diameter dishes in the absence or presence of 10 µM RA for 5 days, of which the last 48 hr were in medium containing [3H]glucosamine at 10 µCi/ml. Other cultures were labeled for the last 5 hr with [35S]methionine at 50 µCi/ml in serum-free and methionine-free medium after a 30-min incubation in a similar medium without radiolabeled methionine. Qualification of label incorporation into trichloroacetic acid-insoluble material was performed as described previously (29). Similar results were obtained in 2 experiments.

Mean ± S.E. of triplicate samples.

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Effect of RA on Radiolabeling of Cell Surface Sialo- and Galactoglycoproteins. Treatment of S91-C-2 cells with RA increased the labeling of gp160 and gp135 on the surface of intact cells by the sialic acid-directed NaI2O4:NaB3H4 method (Fig. 3). The labeling of galactose (and/or galactosamine) residues on the desialylated derivatives of gp160 and gp135 by the galactose oxidase:NaB3H4 method was also enhanced by RA (Fig. 4). A similar effect of RA on gp160 labeling was found in several RA-sensitive subclones of the S91-C-2 cells (Fig. 3). A considerably smaller increase in gp160 labeling by the above methods was observed following treatment of the S91-C-83 clone with RA (Figs. 3 and 4). Furthermore, no significant increase in the labeling of gp160 or gp135, or of their desialylated derivatives, could be detected on RA-treated clones S91-C-110, S91-C-154, and S91-C-163 (Figs. 3 and 4).

Effect of RA on Release of Glycoproteins into Growth Medium. To determine whether the differences observed in the labeling of gp160 on the RA-treated S91-C-2 cells and on the RA-treated resistant clones are the result of differential release of these surface molecules into the medium, we incubated untreated and RA-treated cells with [3H]glucosamine, and analyzed the release of labeled macromolecules into the medium by fluorography after SDS-PAGE. Fig. 5 shows that all of the clones released several [3H]glucosamine-labeled glycoconjugates into the medium, including glycoproteins which comigrated with gp160 and gp135. The amounts of gp160 released by RA-treated S91-C-2 cells, as well as by the RA-treated resistant clones, seemed lower than those released by the untreated counterparts. The difference in gp160 release between treated and untreated cells was particularly pronounced in the S91-C-83 cells (Fig. 5). These findings suggest that the increased cell surface labeling of gp160 on RA-treated S91-C-2 and S91-C-83 cells may be, at least in part, the result of increased retention of gp160 on the cell surface, as indicated by the decreased release into the medium. However, since the lower release of gp160 by RA-treated S91-C-110, S91-C-154, and S91-C-163 cells was not accompanied by an enhanced cell surface labeling of gp160 on these cells, we explored other possible explanations for the differential effect of RA on cell surface labeling of S91-C-2 cells and/or on these resistant clones.

Effect of RA on Sialyltransferase Activity. RA treatment of S91-C-2 cells and of several RA-sensitive subclones derived from these cells caused a 2- to 3-fold increase in sialyltransferase activity (Table 2). In contrast, RA treatment of all of the RA-resistant clones failed to stimulate sialyltransferase activity to any appreciable extent.

Comparison of the Effects of RA and TPA on Cell Proliferation, Cell Surface Sialoglycoproteins, and Sialyltransferase Activity. Treatment of either S91-C-2 or S91-C-154 cells with TPA resulted in growth inhibition, whereas RA treatment inhibited only the S91-C-2 cells (Table 3). Although the growth of the S91-C-2 cells was inhibited similarly by TPA and RA, under the experimental conditions used here, only the latter compound caused an increase in sialyltransferase activity (Table 3). Furthermore, whereas RA treatment of S91-C-2 cells resulted in an enhanced labeling of gp160 on the surface of the cells by the NaI2O4:NaB3H4 method, TPA treatment exerted no such effect (Fig. 6). These results indicated that growth inhibition per se is not sufficient to cause either an enhancement of sialyltransferase or an increased labeling of gp160 on the cell surface.

DISCUSSION

The investigation reported here was designed to explore whether cell surface modulation by RA is related to growth inhibition. For this purpose we used RA-resistant mutant clones, which we produced as a model for studying retinoid mechanism of antiproliferative action (24). We compared the effects of RA on these cells and on RA-sensitive cells from which the mutants had been derived. The principal finding of this study is that RA exerts differential effects on sialyltransferase activity and on the structure of cell surface sialoglycoproteins of RA-sensitive and RA-resistant cells. RA treatment of S91-C-2 cells, which are sensitive to the growth-inhibitory effect of RA, resulted in an increase in sialyltransferase activity. In contrast, similar treatment of RA-resistant mutant clones failed to augment the activity of this enzyme. Cell surface labeling of sialic acid and galactose (and/or galactosamine) residues of gp160 on RA-sensitive cells increased after RA treatment. In contrast, except for a small increase in gp160 labeling on S91-C-83 cells, RA failed to augment labeling of cell surface sialo- and galactoglycoproteins on the...
RA-resistant clones. Since clonal drifts in cell surface components have been found in melanoma cells (30), we derived subclones of the S91-C-2 cells after recloning in agarose. All these subclones were found to be as sensitive to RA growth-inhibitory effects, as the S91-C-2 parental clone and their treatment with RA also resulted in an increase in sialyltransferase activity and increased labeling of cell surface gp160. These results suggest that the differences between the responses of RA-resistant mutant clones and those of the parental RA-sensitive clone S91-C-2 are not due to clonal drifts.

Because RA-induced changes in sialyltransferase activity and in cell surface glycoproteins were observed mainly in cells that were also growth inhibited by RA, the question arose as to whether these biochemical alterations are secondary to growth inhibition. In an attempt to resolve this question, we used the phorbol ester tumor promoter TPA, which we had previously found to be capable of inhibiting the proliferation of the S91-C-2 melanoma cells (18); others have shown that it can enhance sialyltransferase activity (31) and modify cell surface glycoconjugates (1, 2, 45). Having found that growth inhibition of either the RA-sensitive S91-C-2 cells or the RA-resistant S91-C-154 cells by TPA failed to augment either sialyltransferase activity or labeling of cell surface sialoglycoproteins, we concluded that inhibition of cell proliferation in this system is not sufficient, in itself, to cause the effects that RA exerts on cell surface glycoprotein synthesis in RA-sensitive cells.

The increase in cell surface labeling of sialoglycoproteins on RA-sensitive cells following RA treatment can be accounted for by several mechanisms, including de novo synthesis of the entire molecule (polypeptide and covalently-bound carbohydrates), increased glycosylation of preexisting glycoprotein molecules, decreased rate of turnover of the polypeptide and/or the terminal sugar residues (sialic acid and galactose), or decreased rate of shedding of the sialoglycoproteins.

In a previous study we have demonstrated that gp160 is one of the endogenous acceptors of sialyltransferase in extracts of S91-C-2 cells, and suggested that the increased glycosylation of preexisting gp160 is the result of RA-stimulated enzyme activity (8). The present investigation, showing an excellent correlation between RA-induced increase in sialyltransferase and increased labeling of cell surface sialoglycoproteins, lends further support to the above proposal.

A crucial question is, of course, whether and how these results relate to the mechanism of retinoid action. Current thoughts on the mechanisms of action of retinoids are centered on effects on nuclear and extranuclear events (5, 7, 16, 26, 35, 40, 46). Notable among the latter effects are the increase in glycoprotein synthesis and the alterations in cell membrane glycoconjugates, some of which may occur even in enucleated cells (2, 7, 16, 20, 46). Indeed, the glycosylation of proteins is a postranslational, non-template process; it could therefore be modulated by epigenetic mechanisms (6). One such mechanism is the direct involvement of retinol, and possibly also RA, as an intermediate in glycosyl transfer reactions (7). In addition, recent studies have demonstrated that RA can increase the activity of certain glycosyltransferases (8, 31). The levels of glycosyltransferases, which are membrane-bound enzymes responsible for the sequential and concerted addition of sugar residues during the extension of covalently bound oligosaccharide side chains of glycoproteins, are controlled genetically (6). Ultimately then, the structure of glycoconjugates is also defined genetically. At present it is not clear how RA augments sialyltransferase activity in RA-sensitive S91 cells. The most likely possibility is by increased de novo enzyme synthesis. However, a decreased rate of enzyme turnover or changes in enzyme compartmentalization are also compatible with our findings.

Glycoconjugates associated with the cell surface membrane have been implicated in growth and differentiation control (10, 33, 34, 42, 44). They may be mediators of specific interactions with soluble regulatory effector molecules (e.g., hormones and growth factors), with insoluble components of the extracellular matrix, or with constituents of the surface of adjacent homotypic or heterotypic cells (9, 10, 33, 34, 37, 38, 42, 44). Sialic acids are important constituents of cell surface glycoconjugates, where they occupy almost exclusively terminal positions. Various biological functions have been attributed to these sugar residues (37). They endow cell surface components with negative charge and, thereby, may alter the conformation and mobility of sialoglycoconjugates within the membrane, and abrogate their function as receptors or antigens. Sialylation of galactosylglycoconjugates may "mask" receptors and prevent recognition and adhesion between cells, on the one hand, or "create" receptor sites for sialic acid-specific recognition, on the other hand. Hence, increased sialylation of cell surface glycoproteins on RA-treated S91-C-2 cells and subclones may mask receptors and thereby alter their function, so that they no longer bind essential growth factors, leading to the inevitable inhibition of growth.

Apart from their effect on cell growth, surface components of tumor cells may be important for the expression of the transformed and malignant phenotype (3, 33, 34, 44). Interestingly, changes in the glycosylation of specific cell surface glycoproteins have been correlated with transformation and malignancy (3, 13, 27). Thus, a M, 100,000 cell surface glycoprotein found on various tumor cells showed a lower affinity for wheat germ agglutinin, a lectin which specifically binds sialic acid and N-acetylglucosamine, than for concanavalin A, a mannose-specific lectin. In contrast, the counterpart of this molecule, isolated from nontumorigenic cells, exhibited an opposite pattern of affinities for these lectins. These findings suggest that the glycoprotein of less tumorigenic cells is more glycosylated and contains a higher proportion of "complex structure" than of "high mannose" (3, 13). Recent reports suggest that this glycoprotein may be the transferrin receptor (43). More direct demonstrations of increased sialylation of cell surface glycoproteins on less malignant cells have been reported. A higher degree of labeling of cell surface sialic acid and galactose (and/or galactosamine) residues was found on a M, 150,000 glycoprotein of low-tumorigenic, low-invasive variant cell lines of a rat sarcoma than on the same glycoprotein of the highly tumorigenic clone (13). Recently, a comparison of cell surface components of different neuraminidase-treated human colon carcinoma cell lines revealed an inverse relationship between the labeling of glycoproteins of M, 85,000 to 200,000 by galactose oxidase:NaB3H4 and the aggressiveness of the cell lines (27). Thus, the increased glycosylation of specific sialoglycoproteins on S91 melanoma cells following treatment with RA may reflect the suppression of the transformed phenotype of these cells (22).

Several reports have described that cell surface components, both membrane bound and solubilized (or shed into the conditioned medium), can modulate growth and differentiation (14, 32, 53, 54, 55). The present investigation, showing an excellent correlation between RA-induced increase in sialyltransferase and increased labeling of cell surface sialoglycoproteins, lends further support to the above proposal.
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36). Most of these components were found in normal cells and were implicated in regulation of density-dependent growth control of untransformed cells. However, a gp160 cell surface glycoprotein isolated from conditioned medium of hamster melanocytes has been found to restore density-dependent growth control to malignant melanoma cells (14). More directly related to our findings is perhaps the recent report on the inhibition of cell proliferation by galactosylation of certain cell surface glycoproteins (12).

The understanding of the mode of action of retinoids is germane to the comprehension of the neoplastic process, because retinoids influence cell proliferation, cell differentiation, and cellular interactions. Our current findings highlight the possibility that changes induced by RA in cell surface sialoglycoproteins may be the cause for the anti-proliferative action of retinoids.

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REFERENCES


Fig. 1. Photomicrographs of S91-C-2 cells (A to F) and S91-C-154 cells (G to L), grown for 3 days (A, D, G, J), 5 days (B, E, H, K), or 10 days (C, F, I, L) on plastic dishes (A, B, D, E, G, H, J, K), or in 0.5% agarose (C, F, I, L) in the absence (A to C, G to I), or presence of 10 μM RA (D to F, J to L). Phase contrast, x 120.
Fig. 2. Fluorographs of glycoproteins (10 lanes on left) and proteins (10 lanes on right) of the indicated clones grown for 5 days in the absence (−) or presence (+) of 10 μM RA, respectively. The cells were labeled with [3H]glucosamine (left lanes) or [35S]methionine (right lanes), as described in Table 1, and the labeled molecules were solubilized, subjected to SDS-PAGE in 8% slab gels, and analyzed by fluorography, as described in "Materials and Methods." Each lane represents glycoproteins extracted from 1 x 10⁶ cells (left lanes) or proteins extracted from 1 x 10⁶ cells (right lanes). The ordinate between the 2 groups of lanes represents the electrophoretic migration of standard-molecular-weight protein markers, including myosin (M, 210,000), β-galactosidase (M, 130,000), phosphorylase a (M, 94,000), and bovine serum albumin (M, 68,000); gp160, position of the glycoprotein with an apparent molecular weight of 160,000.

Fig. 3. Fluorographs of sialoglycoproteins exposed on the surface of the different sensitive and resistant clones grown for 5 days in the absence (−) or presence (+) of 10 μM RA. The sialoglycoproteins were labeled by the NaIO₄-NaBH₄ method. The cells were then solubilized and the extracted molecules were processed and analyzed by SDS-PAGE in 8% gels. Each lane contains proteins extracted from 1 x 10⁶ cells.
Fig. 4. Fluorographs of galactoglycoproteins exposed on the surface of neuraminidase-treated S91-C-2 cells and RA-resistant clones. The cells were grown for 5 days in the absence (−) or presence (+) of 10 μM RA. The cells were then treated with neuraminidase and galactose oxidase, and then labeled with NaB3H4. After cell solubilization and SDS-PAGE in 6% gels, the labeled galactoproteins were identified by fluorography.

Fig. 5. Fluorographs of glycoproteins released into the growth medium of cells labeled metabolically with [3H]glucosamine. Cells were grown for 5 days in the absence (−) or presence (+) of 10 μM RA and labeled with [3H]glucosamine for the last 48 hr. Thirty-μl aliquots of the medium containing 3 ± 0.5 × 10^4 trichloroacetic acid-precipitable dpm were mixed with 15 μl of 3 times concentrated SDS-PAGE sample buffer and were analyzed by SDS-PAGE in slab gels. "gp160," glycoprotein comigrating with gp160.

Fig. 6. Fluorographs of cell surface sialoglycoproteins on S91-C-2 and S91-C-154 cells grown in the absence (−) or presence (+) of 10 μM RA or 0.5 μM TPA for 5 days before labeling by the NaClO4/NaB3H4 method.
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