Effect of Retinoic Acid on the Synthesis of Glycoproteins of Mouse Skin Tumors during Progression from Promoted Skin through Papillomas to Carcinomas

Larissa V. Levin, Jeffrey N. Clark, Helen R. Quill, Paul M. Newberne, and George Wolf

Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

MATERIALS AND METHODS

ABSTRACT

Papillomas and carcinomas were induced on the skin of mice by initiation with dimethylbenzanthracene, followed by promotion with 12-O-tetradecanoylphorbol-13-acetate. Retinoic acid was applied topically, either chronically, throughout the promotion period, or acutely, to the papillomas or carcinomas. All tumor types were verified histologically. Tumor tissue was incubated with 12-O-tetradecanoylphorbol-13-acetate. Retinoic acid was applied topically, either chronically, throughout the promotion period, or acutely, to the papillomas or carcinomas. All tumor types were verified histologically. Tumor tissue was incubated with labeled glucosamine and labeled glycoproteins released into media were fractionated on DEAE-Sephadex. For papillomas, one peak (eluted with 0.17 m NaCl) appeared and another (0.40 m) all but disappeared as a result of retinoic acid treatment. Carcinomas also showed the 0.40 m peak released by papillomas, which was also suppressed by retinoic acid. Carcinomas released a 0.26 m peak instead of the 0.17 m peak in response to the retinoid. All three peaks yielded single, symmetrical peaks on gel filtration columns. They were all resistant to mild alkaline hydrolysis. Labeling experiments revealed the presence also of mannose, galactose, and traces of fucose in all three glycoproteins. The 0.17 and 0.26 m peaks were bound by concanavalin A-Sepharose columns, the 0.40 m peak was not. Molecular weights, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, were approximately 80,000 and 105,000 (0.17 m peak), 67,000 (0.26 m peak), 70,000 and 80,000 (0.4 m peak).

INTRODUCTION

Changes in the cell surface glycoproteins have been known to occur in the transformation from the normal to the tumor cell (8), especially with respect to the "large, external, transformation-sensitive" glycoprotein ("LETS" protein) or fibronectin (8). Less is known about the effect of carcinogens on cell surface glycoproteins during the different stages of carcinogenesis in epidermis, and the action of retinoids thereon. Recently, Sasak et al. (14) described a detailed investigation of the effects of RA on the cell surface glycoproteins of transformed mouse fibroblasts.

In this report, we describe the changes in glycoproteins shed into the medium during incubation of mouse skin tumors in the course of the different stages of carcinogenesis, treated either chronically or acutely with RA.

1 This study was supported in part by NIH Grant CA13792.
2 Present address: Department of Medicine, University of Massachusetts Medical Center, Worcester, Mass. 01605.
3 Present address: Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn. 37830.
4 Present address: Division of Rheumatology, Washington University School of Medicine, St. Louis, Mo. 63110.
5 To whom requests for reprints should be addressed.
6 The abbreviations used are: RA, retinoic acid; DMBA, 7,12-dimethylbenz(a)anthracene; TPA, 12-O-tetradecanoylphorbol-13-acetate; Con A, concanavalin A; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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promotion, mice from "papilloma" and "carcinoma" groups (Chart 1) were killed in a chloroform chamber. Tumors were excised, pooled in each subgroup (1 to 5 tumors from 7 to 10 mice each), minced with scissors, and put in Erlenmeyer flasks with [6-3H]glucosamine and Krebs-Ringer buffer (60 μCi/10 ml/1 g of tumors). The minced tumors were incubated at the same conditions as epidermis and were filtered through 5-μm Millipore filters. Media were dialyzed extensively and used for DEAE-Sephadex chromatography.

**DEAE-Sephadex Chromatography.** Media, dialyzed against distilled H₂O, and then against 0.01 M sodium phosphate buffer, pH 7.4, with 0.004% EDTA and 0.007% β-mercaptoethanol were applied to DEAE-Sephadex A-50 columns (1 × 30 cm) previously equilibrated against and washed with the same buffer. Glycoprotein peaks were eluted with a linear gradient of 0 to 1.0 M NaCl in the buffer. Radioactivity in fractions (2.80 to 3.2 ml) was determined by liquid scintillation counting. NaCl molarity in fractions was calculated based on conductivity measurements. Glycoprotein peaks used in later experiments were pooled and dialyzed.

**Gel Filtration.** Bio-Gel P-100 columns (1.5 × 50 cm) were equilibrated in the same buffer, as for ion exchange chromatography. Bio-Gel P-4 (1 × 102 cm) and Bio-Gel-P-10 columns (1 × 48 cm) were equilibrated in 0.1 M sodium acetate buffer, pH 5.5.

**Mild Alkaline Hydrolysis.** Hydrolysis was performed as outlined by Schachter and Roden (15) in 0.5 M NaOH at 24° for 30 hr in nitrogen-saturated sealed tubes.

**Con A-Sepharose.** Samples were applied to the columns (0.7 × 6 cm) with the liquid concentration (10 mg/ml) equilibrated in Con A buffer. Radioactivity of 1-ml fractions was counted.

**Uronic Acid Assay.** Uronic assay was performed on 1-ng samples as described previously (1).

**Neuraminidase.** Neuraminidase (type X; Sigma Chemical Co., 250 units/ml; 1 unit defined as 1.0 μmol of N-acetylneuraminic acid liberated per min at pH 5.0 at 37° using N-acetylneuraminic acid-lactose as substrate) was incubated with 1 ml of the glycoprotein solution in 0.02 M sodium acetate buffer, pH 6.0, at 37° for 19 hr with 50 μl of toluene to prevent bacterial contamination. Volume of reaction mixture was 2 ml. The digestion was stopped by boiling for 3 min.

**SDS-PAGE.** Electrophoresis was performed in 10% gels with 3% stacking gel in accordance with the method of Laemmli (9). Cross-linked albumin (Sigma Chemical Co.) was used as a standard, producing bands of molecular weights of 66,000, 13,200, 198,000, and 264,000. After the tracking dye ran to about 1 cm from the end, the slabs were removed and cut in half. One half was stained with Coomassie blue, and the other was prepared for autoradiography by the method of Bonner and Laskey (4).

**RESULTS.**

Before investigating the biochemical consequences of the action of carcinogen, promoter, and RA on the glycoproteins of skin tumors, it was necessary to establish the sequence of histological events during carcinogenesis, to be certain that the papilloma and carcinoma stages had been reached at the times at which glycoprotein synthesis was studied, and that the administered RA had its expected morphological effect.

Chart 1 gives the scheme for the experiment. The first group (Chart 1, a and b) was used to establish the histological effects of RA on skin at early stages of carcinogenesis, when tumors had not yet developed, but latent changes of the epidermal tissue might have occurred. Skin specimens were taken after only 2 weeks of promotion.

The second group (Chart 1, c to e) was used to determine RA effects on papillomas during their development (chronic subgroup), and to compare them with RA action after papilloma development (acute subgroup). About 10 to 12 weeks of pro-motion were required to obtain fully developed papillomas, and this determined the duration of the papilloma part of the experiment.

Some papillomas changed into carcinomas after about 20 weeks of promotion. These mice were used to study the effects of RA on tumor formation throughout promotion (Chart 1, f and g, Chronic I), or on the conversion of papillomas into carcinomas, starting at the stage of fully developed papillomas (Chart 1h, Chronic II subgroup); or on a short-term treatment of the carcinomas Chart 1j, acute subgroup). Controls in each group consisted of animals receiving no RA.

Verma et al. (16) already demonstrated the feasibility of using the mouse as an experimental animal for inhibition of skin tumors by RA. We used a modification of their method. Initially, several concentrations of DMBA in acetone were tried. The 0.1% solution (0.2 ml; 0.78 μmol) was chosen for use in all subsequent experiments, due to the minimal scarring and maximal number of tumors produced at this concentration. The TPA concentration was chosen in accordance with earlier results (16) to ensure adequate tumor yield. A preliminary experiment was done in which 5 groups of 50 mice each were initiated twice by DMBA (0.1% solution of a 0.1% solution in acetone) and promoted via TPA once weekly (0.2 ml of a 0.005% solution in acetone) for a total of 2 months. The number of mice with at least one tumor was 44% ± 9 (S.D.) per group; total number of tumors was 36 ± 15 per group. Tumor size at the end of 2 months ranged from 1.0 to 5.0 mm in diameter and weighed between 10 and 50 mg. Tumors grew on all areas of the back, but particularly on the lumbar region. No usable tumors were produced in the cervical area. These results showed that the mouse model was feasible to produce the papillomas we needed to study RA effects on glycoprotein at different steps of tumor induction.

The optimal concentration of RA (34 nmol/application) was chosen in accordance with earlier experiments (16) for inhibition of skin papilloma formation in mice.

**Histological Observation.**

**Skin.** The skin initiated and promoted twice (Chart 1a; Fig. 1b) showed hyperplasia of the basal cell layer and necrotic cells on the surface, as compared with normal skin (Fig. 1a). RA treatment had little or no effect on these changes (Chart 1b; Fig. 1c).

**Papillomas.** Our experiments, like those of Verma et al. (16), showed that RA inhibited the formation of skin papillomas. The number of animals with tumors in the chronically RA-treated group was smaller at all times of promotion, achieving 35% by 8 weeks.
weeks, compared with 66% in the control group. Chronic RA treatment resulted in a smaller yield of tumors per animal. By 8 weeks, 1 tumor/mouse was observed, as compared to 2.6 in the control group. The chronic group had a smaller number of tumors in all tumor size groups during promotion, in comparison with the same group of control animals (with some deviations at the very beginning of the promotion): 60 papillomas of 1 to 2 mm in diameter, 8 of 2 to 4 mm, and 3 of >4 mm at 10 weeks in controls, and 21 (1 to 2 mm), 2 (2 to 4 mm), and 1 (>4 mm) in RA-treated animals. Papillomas studied histologically after 8 weeks of promotion (Fig. 2a) revealed a papillary shape with a convoluting structure characterized by keratinization, proliferation of the epithelium, and vascularization. Chronic RA treatment (Chart 1d) resulted not only in a smaller size of tumors (Fig. 2c), but also degenerative changes in some instances (Fig. 2d). The tumors after acute RA treatment (Chart 1e) demonstrated softening and necrosis (Fig. 2b).

**Carcinomas.** The animals in the carcinoma group were promoted for 20 weeks, and many developed invasive squamous cell carcinomas. Carcinomas were easily discernible because of their reddish color and fast growth. They showed populations of tumor cells invading the s.c. tissue (Fig. 3a). Chronic RA treatment of fully developed papillomas (Chart 1h, Chronic II subgroup) showed degenerative changes in the already developed carcinomas (Fig. 3b), whereas the chronically treated animals (Chart 1g, Chronic I subgroup) failed to produce carcinomas: they still had papillomas after 20 weeks of promotion. Carcinomas acutely treated with RA (Chart 1i) are shown in Fig. 3, c and d (higher magnification). They are characterized by softening of the tumor surface due to mucus secretion and necrosis.

**Biochemical Observations**

**Isolation of Glycoproteins from Skin and Skin Tumors.** Chart 2 shows the elution profiles of glycoproteins synthesized by papillomas and shed into the media upon incubation of the minced tissues on DEAE-Sephadex. A highly negatively charged peak (eluted with 0.4 M NaCl solution) appeared in the control medium, (i.e., tumors not treated with RA). This was drastically suppressed by acute RA treatment, while the 0.17 M peak was stimulated 3.3-fold. After chronic RA pretreatment, the 0.17 M peak remained unaffected, and the 0.4 M peak decreased slightly compared to the controls. The 0.4 M peak was absent in incubations with promoted epidermis (Chart 3), but the epidermis had the 0.17 M peak which appeared to be unaffected by 2 RA treatments.

Animals from the carcinoma group (Chart 4) also had the 0.4 M peak, which was suppressed by RA treatment. Carcinoma media reveal that the 0.26 M glycoprotein is stimulated by RA.

Table 1 shows the ratios of the label incorporated into glycoproteins from RA-treated tumors to those of the controls. Stimulation of the 0.26 M peak was greatest after the acute treatment (2.5 times greater), somewhat smaller after chronic treatment of developed papillomas (Chart 1, Chronic II, 2 times greater) and even smaller after RA pretreatment from the beginning of promotion (Chart 1, Chronic I). Suppression of the 0.4 M peak was greatest in acute treatment, about the same in a shorter chronic treatment (Chronic II), and insignificant for long chronic treatment. The most profound changes in glycoproteins were caused by acute treatment of the papillomas as well as the carcinomas.

Chart 2. Fractionation of media glycoproteins, produced by papillomas, on DEAE-Sephadex A-50. Papilloma tissue was incubated with [3H]glucosamine as described under "Materials and Methods" (Chart 1, a to e). The incubation media (30,000 to 50,000 dpm) were applied to DEAE-Sephadex A-50 columns (1 x 27 cm). After eluting nonbound material with 0.01 M sodium phosphate buffer, pH 7.4, containing 0.004% EDTA and 0.007% β-mercaptoethanol (2 column volumes), the columns were treated with a linear (0 to 0.8 M) gradient of NaCl in the same buffer. Glycoproteins affected by RA were eluted in 0.17 and 0.4 M peaks. Recovery was 75 to 80%. Radioactivity was determined in 2- to 3-ml fractions. Data were normalized to radioactivity per mg protein of the tissue incubated.

Chart 3. DEAE-Sephadex A-50 fractionation of glycoproteins shed into media by promoted epidermis. Portions of initiated and promoted skin were incubated with [3H]glucosamine as described under "Materials and Methods" (Chart 1, a and b). The incubation media (20,000 to 25,000 dpm) were applied to DEAE-Sephadex A-50 columns (1.5 x 15 cm) and eluted with 2 column volumes of 0.01 M sodium phosphate buffer, pH 7.4, containing 0.004% EDTA and 0.007% β-mercaptoethanol. Glycoproteins bound to the column were desorbed with linear NaCl gradient. Recovery was around 70%. Data were normalized to radioactivity per mg protein of the tissue incubated.

Chronic RA treatment had a greater effect on already formed papillomas than when started at the beginning of promotion.

A small amount of a labeled high-molecular-weight glycoprotein accompanied the glycoproteins shed into the media, described above. This was later identified as fibronectin and forms the subject of another communication (3).
Retinole Acid and Skin Tumor Development

Chart 4. Fractionation of media glycoproteins, produced by carcinomas, on DEAE-Sephadex A-50. Carcinoma tissue was incubated with \( ^3\)H-glucosamine as described under "Materials and Methods" (Chart 1, f to i). The incubation media (70,000 to 100,000 dpm) were applied to the columns of DEAE-Sephadex A-50 (1 x 27 cm). Impurities were removed by elution with 2 column volumes of 0.01 M sodium phosphate buffer, pH 7.4, containing 0.004% EDTA and 0.007% \( \beta \)-mercaptoethanol. The glycoproteins were eluted by a linear 0 to 0.8 M NaCl gradient in the same buffer. Glycoproteins affected by RA treatment were eluted at 0.26 and 0.4 M NaCl. Recovery in the experiments was 70 to 80%. Data were normalized to radioactivity per mg protein of the tissue incubated.

Table 1
Relative values of stimulation of 0.26 M peak and suppression of 0.4 M peak in carcinoma media

<table>
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<th>Treatment</th>
<th>dpm</th>
<th>Control</th>
<th>Chronic I</th>
<th>Chronic II</th>
<th>Acute</th>
</tr>
</thead>
<tbody>
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<td>Fraction 0.26 M</td>
<td>1.00</td>
<td>1.45</td>
<td>2.06</td>
<td>2.47</td>
<td></td>
</tr>
<tr>
<td>Fraction 0.4 M</td>
<td>1.00</td>
<td>0.90</td>
<td>0.28</td>
<td>0.25</td>
<td></td>
</tr>
</tbody>
</table>

peak showed a shoulder of higher molecular weight upon gel filtration (Chart 5A). This disappeared upon alkaline hydrolysis and reappeared as a peak of lower molecular weight. We surmise that the 0.17 M peak had an impurity (about 25%) consisting of an O-linked glycoprotein. The results of alkaline hydrolysis suggest that the main component of the 0.17 M peak and 0.26 and 0.4 M glycoproteins probably had N-glycosidically linked sugar chains.

Since N-linked glycopeptides and glycoproteins, in accordance with their structures and affinities, give distinct elution profiles on Con A-Sepharose, we decided to use this sorbent to study the affinity of our glycoprotein to it. Peaks from DEAE-Sephadex were purified by gel filtration on Bio-Gel P-100. The principal 0.17 M peak (not sensitive to alkaline hydrolysis) and the 0.26 and 0.4 M peaks were applied to Con A-Sepharose columns. The 0.17 and 0.26 M peaks revealed elution patterns (Chart 6A) characteristic for polymannose-type oligosaccharide chains. The 0.4 M peak did not bind to Con A (Chart 6B). Distribution of bound and nonbound material is shown in Table 2.

Chart 5. Gel filtration on Bio-Gel P-100 of glycoproteins before and after alkaline hydrolysis. •, native glycoprotein; O, peaks after mild alkaline hydrolysis (0.5 \( \mathrm{NaOH} \) at 24° for 30 hr). Glycoprotein peaks after DEAE-Sephadex fractionation were dialyzed and were applied either in the native state or after mild alkaline hydrolysis to the Bio-Gel P-100 column (1.5 x 50 cm) equilibrated in 0.01 M sodium phosphate buffer, pH 7.4. Radioactivity in the samples applied was usually 40,000 to 50,000 dpm. One-mi fractions were collected and counted. A, 0.17 M peak from papillomas; B, 0.26 M peak from carcinomas; C, 0.4 M peak from papillomas.

Chart 6. Con A-Sepharose elution patterns of glycoproteins. Samples (dialyzed peaks after DEAE-Sephadex fractionation and alkaline-resistant portion of 0.17 M glycoprotein after Bio-Gel P-100 gel filtration; 6,000 to 10,000 dpm) were applied to the columns (0.7 x 6 cm) equilibrated in Con A buffer (0.1 M sodium acetate, pH 6.05; 0.2 M NaCl; and 0.002 M concentrations each of MnCl\(_2\), MgCl\(_2\), and CaCl\(_2\)) and washed with 10 volumes of this buffer. Bound material was eluted with 0.1 M \( \alpha \)-methylmannoside in Con A buffer (arrows). Aliquots of 1-ml fractions were counted. A, 0.17 M peak from papillomas; B, 0.4 M peak from carcinomas.

Since the 0.4 M peak was not bound to Con A-Sepharose, the possibility was explored that its oligosaccharide structure might be of a complex type (triantennary), either with or without terminal sialic acid. Neuraminidase digestion or mild acid hydrolysis
and the carcinoma stage of skin tumor development? The question, is there a difference in mechanism of RA action at carcinomas to RA treatment, in order to approach an answer to this problem.

Investigated the biochemical response of skin, papillomas, and tumors (2, 13); the mechanisms of action are likely to differ. We observed histologically considerable loss of keratin, as well as necrosis. Torhorst (6) described the effect on mouse skin papillomas of a single, large dose of an aromatic retinoid injected i.p., and noted by Frigg and Torhorst (6).

When phorbol esters are used as promoters, RA inhibits the tendency was observed histologically: although chronic RA treatment caused a smaller number of papillomas to appear than in untreated controls, few other histological changes due to RA treatment were visible, except a reduction of the keratinous layer, thereby reducing tumor size (Fig. 2, a and c), as already noted by Frigg and Torhorst (6). There was a significant decrease in the number of papillomas formed in the RA-treated group compared to the control group.

Furthermore, it was important to determine whether the acute treatment of tumors with RA, as described by Prutkin (13), would have different histological and biochemical consequences from chronic treatment, extending throughout the promotion period.

This report describes the effects of RA application to skin tumors either chronically, starting at various stages of tumor development, through to the time at which the animals were killed; or acutely, with 4 applications 48 hr before killing. The tumors were allowed to develop to either the papilloma or the carcinoma stage. Each stage was observed histologically and with respect to glycoprotein biosynthesis, including initiated and promoted skin. The effect of RA on initiation alone was not investigated, since Verma et al. (16) previously showed that this stage was not affected by RA.

We used relatively high doses of DMBA (200 μg/application) and initiated twice, to ensure a high tumor yield within a shorter time for our biochemical studies.

We observed considerable differences between the glycoproteins released by untreated (control) tumors and RA-treated tumors. Although other glycoproteins were also affected by RA treatment, we concentrated our efforts on the 3 most consistently responsive peaks. For papillomas, one peak (eluted with 0.17 M NaCl) appeared, and another (0.40 M) all but disappeared (Fig. 4, c and d; Chart 2) showed a smaller effect on papillomas than did acute treatment of the papillomas themselves. A similar tendency was observed histologically: although chronic RA treatment caused a smaller number of papillomas to appear than in untreated controls, few other histological changes due to RA treatment were visible, except a reduction of the keratinous layer, thereby reducing tumor size (Fig. 2, a and c), as already noted by Frigg and Torhorst (6).

The acute RA treatment (4 doses in 48 hr) which caused the most striking biochemical changes also showed the most far-reaching morphological changes (Fig. 2b): necrosis, degeneration, and keratinolysis. This is probably an alteration of cell differentiation caused by the excess RA, similar to that observed in many other systems, such as the reversal of keratinization in chick embryo skin, described by Fell and Mellanby (5). At times,
a borderline case could be observed, such as necrosis developing after chronic RA treatment (Fig. 2d).

In an experiment done to show that the glycoproteins of papillomas isolated from the media were actually secreted or shed from the cell surface and were not the result of cell leakage, lactic dehydrogenase of media and cells was assayed. Media contained 15 to 20% of total lactic dehydrogenase, which did not increase with time of incubation, nor was it affected by RA treatment.

The carcinomas produced by 20 weeks of promoter treatment also showed the 0.40 M peak, which is suppressed by RA treatment, particularly the acute treatment (Chart 1, f, g, and i; Chart 4). However, carcinomas lacked the 0.17 M peak of papillomas, and instead showed a pronounced peak eluted at 0.26 M NaCl in response to RA treatment (Chart 4, Chronic I). Interestingly, if RA treatment was started at the papilloma stage instead of the promotion stage (Chart 1h), the glycoprotein released by the resulting carcinoma was also eluted at 0.26 M NaCl (Chart 4, Chronic II), but the peak height was intermediate between chronic treatment (Chart 4, Chronic I) and acute treatment (Table 1; Chart 4, acute). Histologically, RA treatment throughout promotion completely prevented carcinoma formation, although some papillomas were, of course, still produced. Acute RA treatment of carcinomas resulted in the same type of degeneration of the tumor, necrosis, and keratolysis, as with papillomas, with appearance of mucous metaplasia, again emphasizing the probable mechanistic difference in RA effect on promotion and on the differentiation of the fully developed tumors. Similar results were obtained by Matter et al. (12) for mouse skin tumors treated with a single dose of an aromatic retinoid, showing necrosis and impressive mucous metaplasia.

Glycoproteins released by initiated and promoted skin, prior to appearance of papillomas, showed little response to RA treatment (Chart 1, a and b; Chart 3), with the characteristic 0.17 M peak released by treated and untreated skin. The 0.40 M peak characteristic of papillomas and carcinomas was absent. Histologically, there was little response to be observed to RA treatment in initiated and promoted skin, both RA-treated and untreated skin showing merely the hyperplasia caused by the promoter.

The glycoproteins shed into the incubation medium, after purification on DEAE-Sephadex, showed single, symmetrical peaks on gel filtration chromatography (Chart 5). Upon slab gel electrophoresis, the 0.40 M and 0.26 M peaks showed 2 protein-staining bands, and the 0.17 M peak showed a doublet (Fig. 4). All bands stained for glycoprotein. Since all 3 peaks were resistant to mild alkaline hydrolysis (Chart 5), they can be assumed to be glycoproteins with W-linked sugar chains, although the 0.17 M peak showed a shoulder which shifted upon hydrolysis. Sugar composition showed evidence of glucosamine, mannose, galactose, and traces of fucose. No evidence of uronic acid was found in any of the peaks. It was surprising to find that the highly acidic 0.40 M peak released no labeled sialic acid upon neuraminidase treatment. It may, of course, still contain sialic acid, unlabeled because of inefficient incorporation of glucosamine. Alternatively, it is possible that, as has been observed for Morris hepatoma (7), skin tumors have a decreased glucosamine-2'-epimerase activity.

Sasak et al. (14) observed that RA treatment of transformed mouse fibroblasts resulted in increased mannose incorporation into cell surface glycopeptides. In our work, also, the 0.17 M and 0.26 M peaks, which were stimulated by RA treatment, were held by Con A-Sepharose columns, and hence contained some high-mannose chains, whereas the 0.40 M peak, which declined during RA treatment, appeared to be of the complex type, since it was not held by the Con A-Sepharose.

It is necessary to be cautious in interpreting comparisons of the same peaks from different tumors (papillomas and carcinomas, as well as promoted skin), and comparisons of the same peaks from different treatments of the same tumors: one could suppose that these were different glycoproteins, all eluted from DEAE-Sephadex with the same salt concentration. However, behavior on gel filtration and on Con A-Sepharose columns, as well as SDS-PAGE of the 0.40 M peaks from papillomas and carcinomas was identical. The 0.17, 0.26, and 0.40 M peaks from RA-treated and untreated tumors behaved identically by these same 3 criteria. This was so, even though the 0.40 M peak showed 2 bands upon SDS-PAGE.

We have as yet no information as to the meaning of the decline in one peak (0.40 M) at the same time as an increase in another peak (0.17 M) as a result of RA treatment: it could mean that the 0.40 M glycoprotein is not synthesized, or is not processed, or that the 0.40 M glycoprotein changes into the 0.17 M glycoprotein. Other possible changes could be in phosphorylation or sulfation or the amino acid composition. Ongoing work on the structure of the oligosaccharide moieties of these proteins will ultimately answer some of these questions.

REFERENCES


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Fig. 1. a, normal skin; b, control skin, initiated with 2 doses of DMBA (200 μg each; at time zero and 1 week), then promoted with 17 nmol of TPA twice (time, 4 and 5 weeks), and killed (6 weeks). Hyperplasia of the basal cell layer and necrotic cells on the surface can be seen. c, skin, chronic treatment. The same treatment as for b, except that RA (34 nmol) was applied 1 hr before each promotion. Hyperplasia and necrosis similar to b.
Fig. 2.  

a. control papillomas. Initiation with 2 doses of DMBA followed by 8 weekly applications of TPA. Papillomas had a characteristic shape with a convoluting structure, and showed the proliferative part of the epithelium, keratinization, and vascularization. 

b. papilloma after acute treatment. Treatment was the same as for a, except that the tumors were treated with RA doses (34 nmol for 4 doses) at 12-hr intervals, starting 48 hr before death. Regions with necroses and some mucous exudate on the surface were observed; 

c. papilloma chronically treated with RA 1 hr before each promotion. Tumors were smaller but did not display much difference from the control group; 

d. the same treatment as c, but showing degenerative changes.
Fig. 3. a, control carcinomas. The mice were initiated twice with DMBA and promoted for 20 weeks with TPA. They developed invasive squamous carcinoma with populations of tumor cells invading the s.c. layer; b, chronic treatment of carcinomas starting at papilloma stage (see Chart 1h). RA was applied to fully developed papillomas 1 hr before each promotion. One can see degenerative changes and necrotic areas on top of the tumor, which is also invasive; c, acute treatment of carcinomas. The effect was the same as seen for acute treatment of papillomas. Softening of the surface was due to mucous secretion and necrosis; d, higher magnification of c shows invasive populations of cells and necrosis more distinctly.
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