Anionic Polysaccharide Production and Tyrosinase Activation in Cultured Human Melanoma Cells

John Banks, John W. Kreider, V. P. Bhavanandan, and Eugene A. Davidson

Departments of Biological Chemistry and Pathology, The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, Pennsylvania 17033

SUMMARY

A human melanoma cell line established in our laboratory was characterized in terms of tyrosinase activity and anionic polysaccharide production. Tyrosinase levels were diluted during the growth phase and increased after the cell culture became confluent. The anionic polysaccharides produced included hyaluronic acid, heparitin sulfate, and a high-molecular-weight chondroitin 4-sulfate. In contrast, a primary culture of human melanocytes derived from embryonic iris produced much greater amounts of hyaluronic acid, about 30-fold less heparitin sulfate, and a mixture of chondroitin 4-sulfate and dermatan sulfate. Saccharides secreted into the culture medium were generally identical to those remaining cell associated except for the melanoma heparitin sulfate, wherein the latter fraction appeared to be of lower molecular weight.

INTRODUCTION

Human melanoma cells bear a common antigen, cross-reactive between different patients, and unique antigen(s) that are specific for each individual tumor (4, 9, 11, 12). Some preliminary attempts have been made to isolate and characterize membrane-associated melanoma antigens. Partially purified, soluble protein fractions have been obtained from homogenized tumors by gel filtration (6) and electrophoresis (12). It is probable that membrane-associated glycoproteins contribute to the immunogenicity of human melanoma cells.

Melanoma cell cultures provide an opportunity to conduct studies on the characterization of membrane-associated glycoproteins upon a consistent cell population. Human melanoma lines have been established in a number of laboratories (7, 14) including our own.

The present study is a description of some of the biological properties of one of our lines (HM7) and the biochemical characterization of the pronase- and alkali-stable cell-associated and extracellular glycosaminoglycans and glycoproteins labeled by inclusion of tritiated glucosamine and sodium sulfate. It is probable that membrane-associated glycoproteins contribute to the immunogenicity of human melanoma cells.

MATERIALS AND METHODS

Establishment of Melanoma Cell Culture Line. The human melanoma cell line, HM7, was obtained from an excisional biopsy of an axillary nodal metastasis in April, 1974. Chips (1 x 1 mm) of tumors were partially dried onto the surface of a plastic culture flask, incubated with medium for 1 week, and then refed 3 times each week. After a confluent monolayer was obtained, the cells were suspended with 0.04% EDTA and passaged. They have since been passaged at about 2-week intervals in 16-oz prescription bottles.

Establishment of Human Iris Cultures. Human iris melanocytes were obtained from a therapeutically aborted fetus utilizing methodology analogous to that described earlier (19). After aseptic excision, the 2 irises were cultured as explants for 49 hr in 7 ml of complete media containing [3H]glucosamine and Na[35SO4], 20 μCi/ml each, in a 60-mm plastic Petri dish (Falcon Plastics, Oxnard, Calif.). At the end of this time the media were decanted, the cells were harvested by centrifugation, and each fraction was prepared for analysis. Cell counts on the iris explant were not performed due primarily to technical difficulties. In view of the significant saccharide synthesis observed, it seems probable that the bulk of the explant cell population was viable under the conditions of the experiment, although significant cell death cannot be ruled out.

Propagation of Cultures. HM7 cells were routinely propagated in MEM and Earle’s balanced salt solution supplemented with heated (56° for 30 min) fetal calf serum to 10% final concentration, sodium pyruvate, nonessential amino acids, 2 × vitamins, and 100 units penicillin G per ml and 100 μg streptomycin sulfate per ml. All components, with the exception of the antibiotics and the fetal calf serum, were obtained from Grand Island Biological Co., Grand Island, N. Y. Cells were incubated at 36° in a humidified 5% CO2-95% air atmosphere and were propagated in 16-oz glass prescription bottles (Brockway Glass Co., Inc., Brockway, Pa.). The cells became confluent at approximately 6 × 10⁶ cells/bottle and were routinely subcultured to approximately 1 × 10⁶ cells/bottle by suspension with 0.02% ethylene glycol bis(β-aminoethyl ether)-N,N'-tetraacetic acid in calcium- and magnesium-free phosphate-buffered saline, 0.15 M Na⁺:0.005 M K⁺:0.14 M Cl⁻:0.009 M PO₄, pH 7.2. Cultures were routinely tested for bacterial contamination by the use of thioglycolate and tryptose phosphate broth and for yeast
and mold contamination with Sabouraud's medium. No contamination was observed. The possible presence of mycoplasma was not assessed in the cells used for labeling studies although other lines carried in our laboratory have been routinely assayed and found to be free of contamination.

Examination of Karyotype. Petri dishes (60 mm) were seeded with 10° e dye-excluding cells 3 to 5 days before karyotyping experiments. The cultures were treated with colchicine, 0.25 µg/ml (Calbiochem, San Diego, Calif.), for 2 hr, resuspended with 0.25% trypsin in phosphate-buffered saline, and washed twice with MEM. The cell pellet was resuspended in a hypotonic (0.6% sodium citrate:0.06 M KCl) solution, incubated for 30 min at 37°, and then washed twice with MEM. The cell pellet was fixed with methanol:acetic acid (3:1, v/v), refrigerated for 20 min, and washed twice in the fixative. The pellet was broken up with a Pasteur pipet and 2 to 3 drops were placed on a microscope slide. The fixative was evaporated on a warm hotplate and the preparation was stained with 2% Giemsa.

Implantation of Cells into Nude Mice. Human melanoma cells grown to confluency in 16-oz prescription bottles were suspended with 0.1% EDTA, washed, and counted, and 5 × 10° trypan blue-excluding cells were inoculated s.c. into the backs of 10 CRL-HO nude mice (Drug Research and Development Branch, National Cancer Institute, Bethesda, Md.). The mice were housed under ordinary ambient laboratory conditions but with tetracycline added to the drinking water.

Cell Labeling and Sizing. Two confluent bottles (16 oz) of human melanoma cells were fed with 0.1% EDTA, washed, and counted, and 5 × 10° trypan blue-excluding cells were inoculated s.c. into the backs of 10 CRL-HO nude mice (Drug Research and Development Branch, National Cancer Institute, Bethesda, Md.). The mice were housed under ordinary ambient laboratory conditions but with tetracycline added to the drinking water.

Analysis of Anionic Polysaccharides. Saccharide fractions were isolated from culture medium and cell-associated material by fractional salt elution from CPC precipitates of pronase-digested and alkali-treated samples as described by Satoh et al. (19).

Liquid chromatography in 0.5 M CaCl₂ utilizing previously calibrated 10-240 80/120 beads (Electro-Nucleonics, Inc., Fairfield, N. J.) was done as described previously (19) and in the indicated figures. Recoveries for these runs were greater than 90% and standards were always included.

Amino sugar analyses of 5,000 to 20,000 ³H dpm of the labeled samples were done by hydrolysis in 6 N HCl for 24 hr at 100° in a vacuum. Recovery was 70 to 80% for polysaccharide samples with the losses occurring during hydrolysis. The hydrolysates were dried in a vacuum over P₂O₅ and NaOH and were then chromatographed on a Beckman 120C amino acid analyzer using a stream-splitting attachment.

For liquid scintillation counting of samples, the volume of the sample was brought to 1 ml, and 10 ml of a scintillation cocktail (1) were added. The counting efficiency of tritium in this system was 15 to 16%, and, of ³⁵S, was 46%, utilizing an Intertechnique SL36 liquid scintillation spectrometer. Cross-over of ³⁵S counts into ³H window was usually 13.5%, while ³H count crossover into the ³⁵S window was negligible and was not corrected. No additional quenching was observed with any of the solvents at the concentration used for the experiments. For stream-splitting analyses, 4-ml fractions were collected and counted utilizing 10 ml of 3a40 complete scintillation cocktail (Research Products International Corp., Elk Grove Village, Ill., 60007).

d-Glucosamine-6[^³H]hydrochloride, 7.3 Ci/m mole, and carrier-free Na₂³⁵SO₄, 80 to 800 mCi/m mole, were obtained from New England Nuclear, Boston, Mass. The enzymes and other components were obtained from previously described sources (19) with the exception of leech hyaluronidase, which was obtained from Biotics, Arlington, Mass. Heparinase and heparinase were isolated from an adapted Flavobacterium heparinum strain obtained from Dr. A. Linker. The enzyme purifications were followed through the initial hydroxylapatite column elution (15). At this point, contamination of the heparinase and heparinase peaks by glucuronidase and chondroitinase was considered low enough to pool appropriate tubes for enzyme assays.

Conditions for the enzyme assays were 0.25 unit chondroitinase ACII or ABC, 24 hr at 37° in 0.1 M phosphate buffer, pH 7.8, or in 1 to 5 diluted enriched Tris buffer (18). The total volume for the enzyme digest was 500 µl; 250 µg of a chondroitin 4:6 chondroitin sulfate mixture was completely hydrolyzed when run in parallel with the sample that contained less than 15,000 ³H dpm. These enzymes had no significant heparitinase activity. For the heparitinase assays, the samples with HS or heparan sulfate were digested for 24 hr in 0.1 M sodium acetate buffer, pH 7, containing 1 mM calcium acetate. The temperatures for incubation were 30° for heparinase and 43° for heparitinase. Contaminating chondroitinase accounted for less than 10% of the activity. Samples for leech hyaluronidase digestion were incubated in McIlvaine's standard buffer at 37° for 6 hr in a 400-µl volume. Leech hyaluronidase, 125 µg, was added every hr for the 1st 3 hr to ensure complete digestion of HA.

For paper chromatography of chondroitinase ACII- and/or ABC-digested samples, the volume of the enzyme digest was 50 µl in enriched Tris buffer as described by Saito et al. (18). Chondroitin 4-sulfate and chondroitin 6-sulfate standards were digested with the enzymes to detect any sulfatase activity. No significant activity was found. Radioactive chromatograms were cut into 1-inch strips after drying and added to a counting vial containing 2 ml of deionized H₂O. After overnight shaking, 15 ml of a scintillation cocktail were added, and after thorough mixing the samples were counted. Elution of applied ³H and ³⁵S dpm under these conditions was greater than 90%.
**Tyrosinase Assay.** Tyrosinase activity in situ of living cells in culture was determined by a recent modification (16) of the Pomerantz method (17). Briefly, tritiated L-tyrosine (New England Nuclear) was added to the cultures at a final concentration of 1 μCi/ml. After 24 hr, a 200-μl aliquot of supernatant was removed. The aliquot was adsorbed with Norit A and the soluble fraction was counted in Aquasol (New England Nuclear) in a liquid scintillation spectrometer.

Cellulose acetate electrophoresis was carried out in 0.2 M calcium acetate, pH 7.0, at 5 ma for 3 hr or in pyridine formic acid buffer, pH 3.0, at 10 ma for 30 min.

**RESULTS**

**Characteristics of HM7 Cell Monolayers.** The doubling time of the HM7 cells after 20 passages decreased to approximately 40 hr from a mean value of 84 hr after 5 passages but has not changed significantly in several recent experiments. This is somewhat longer than the average value reported by Liao et al. (14) for several human melanoma lines, but, since our growth medium and substratum differ, direct comparison cannot be made. Viability, as measured by trypan blue exclusion, was better than 80%. The cells of the HM7 line were considerably variable in size. Relatively small cells with scanty cytoplasm predominated, but there were also occasional cells 6 to 10 times the diameter of the smallest cells. The large cells were amelanotic and contained single nuclei. The observed morphological heterogeneity may in part derive from the fact that this line has not been cloned. However, we believe that some of the differences noted may be related to the maturational state of the cells.

With continued growth and the attainment of confluency, the HM7 monolayer developed multilayered foci characteristic of transformed cell lines. Cells in these foci sometimes presented amber-brown pigmented granules, similar to those described in other melanotic human melanoma cell lines (14).

A frequency distribution of cell volume was obtained with the Coulter counter and showed a mean cell volume of 1980 cu μm² (Chart 1).

**Implantation of HM7 Cells into Nude Mice.** Nude mice inoculated with HM7 cells developed small (2 to 3 mm), plaque-like, s.c. nonpigmented nodules. The nodules were not elevated and did not spread beyond the injection sites. Microscopic sections of the nodules demonstrated pleomorphic amelanotic cells with conspicuous nucleoli and nuclear cytoplasmic evaginations. Death of all animals occurred as a consequence of intercurrent bacterial infection.

**Relationship of Cell Population Density and Tyrosinase Activity.** The production of 3H₂O is a measure of the in situ functional activity of tyrosinase (16). During the replicative phase of culture growth, the tyrosinase activity progressively decreased each day until the 9th culture day, when the monolayer became confluent and tyrosinase levels were minimal (Chart 2). With the attainment of confluency, tyrosinase activity increased until it was restored to its former level by the end of the experiment at 17 days.

**Evaluation of HM7 Karyotype.** An enumeration of the numbers of chromosomes in spread metaphases showed a very broad distribution of values. However, about two-thirds of the metaphase cells contained 40 to 60 chromosomes. Although the chromosomes could not be paired in consistent patterns, most of those studied were metacentric, a characteristic of human chromosomes.

**Saccharide Fractionation and Characterization.** Summary data of ³H and ³⁵S incorporation at the final stages in the standard workup procedure (19) are shown in Table 1. From 6.6 to 26% of the incorporated ³H and ³⁵S is found in CPC-precipitable material (Table 2). After dialysis to remove residual CPC, the nonprecipitable human melanoma media fraction was examined by high voltage electrophoresis for the presence of keratan sulfate; none was found. The ³H-labeled material migrated only slightly from the origin, whereas the ³⁵S-labeled material little of which remained, migrated completely from the strip in a manner identical to that of inorganic sulfate.

**The 0.4 M NaCl Eluate.** Fractionation of the 0.4 M NaCl eluates from the media on CPG-10 240 80/120 glass beads resulted in the patterns shown in Chart 3. Incubation of the samples from the melanoma or the iris culture with bacterial or leech hyaluronidase resulted in complete degradation of the media saccharides and degradation of about 90% of the material in the cell-associated fraction. These results indicate that both the cell and media fraction contained HA almost exclusively. The cell-associated fraction from the...
human melanoma was resolved by preparative chromatography on a glass bead column. The lower-molecular-weight fraction contained both glucosamine (80%) and galactosamine (20%) as well as sialic acid. The latter component contained somewhat more than one-half of the radioactivity in this fraction and indicates that the major if not exclusive materials present are sialoglycopeptides. These results were confirmed by electrophoresis and are summarized in Table 3.

The 0.8 M NaCl Eluate. Fractionation of the 0.8 M NaCl eluates on CPG-10 240 80/120 glass bead columns resulted in the patterns shown in Charts 4 and 5. Incubation of the human melanoma media fraction with heparitinase resulted in digestion of 80% of the $^3$H dpm and 95% of the $^{35}$S dpm (Chart 4). The remaining 20% of the $^3$H dpm was a 1:1 mixture of chondroitin 4:sulfate and HA based on amino sugar content, cellulose acetate electrophoresis, and susceptibility to leech hyaluronidase and chondroitinase ACII. Following digestion of the media fraction with chondroitinase ACII, 9% of the $^3$H dpm and 7% of the $^{35}$S dpm; incubation with heparitinase resulted in hydrolysis of 52% of the $^3$H dpm. These results agree well with the amino sugar analyses indicating a 1:1 mixture of glucosamine and galactosamine and were confirmed by cellulose acetate electrophoresis. The human iris sample differed in that 90% of the material was a chondroitin sulfate, presumably chondroitin 4-sulfate. The presence of a small amount of derman sulfate could not be ruled out since derman sulfate was present in both the 0.8 and 2.0 M NaCl eluates. The identification of the glucosamine-containing compo-

Table 1

Incorporation of precursor label into macromolecular products

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Stage</th>
<th>$^3$H dpm/10^6</th>
<th>$^{35}$S dpm/10^6</th>
</tr>
</thead>
<tbody>
<tr>
<td>HM7 media</td>
<td>CPC supernatant</td>
<td>1657</td>
<td>367</td>
</tr>
<tr>
<td>HM7 cell associated</td>
<td>CPC supernatant</td>
<td>270</td>
<td>27</td>
</tr>
</tbody>
</table>

$^3$H dpm $\times 10^{-3}$ $^{35}$S dpm $\times 10^{-3}$

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Stage</th>
<th>$^3$H dpm/10^6</th>
<th>$^{35}$S dpm/10^6</th>
</tr>
</thead>
<tbody>
<tr>
<td>HM7 media</td>
<td>CPC supernatant</td>
<td>11,684.6</td>
<td>2,585.4</td>
</tr>
<tr>
<td>HM7 cell associated</td>
<td>CPC supernatant</td>
<td>1,906.6</td>
<td>189.6</td>
</tr>
<tr>
<td>H.I.M. media</td>
<td>CPC supernatant</td>
<td>3,322</td>
<td></td>
</tr>
<tr>
<td>H.I.M. cell associated</td>
<td>CPC supernatant</td>
<td>1,300</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Stage refers to labeled nondialyzable material remaining in solution after addition of CPC to 0.2% CPC (final concentration) in 3 mM NaCl and addition of carrier mucopolysaccharides (19) and celite. This material appears to be mainly glycopeptide in nature.

Table 2

Summary of CPC elution results

<table>
<thead>
<tr>
<th>Salt eluate fraction</th>
<th>0.4</th>
<th>0.8</th>
<th>1.2</th>
<th>2.0</th>
<th>Totals</th>
<th>% precipitated$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$^3$H</td>
<td>$^{35}$S</td>
<td>$^3$H</td>
<td>$^{35}$S</td>
<td>$^3$H</td>
<td>$^{35}$S</td>
</tr>
<tr>
<td>A. HM7$^b$ media</td>
<td>22.8</td>
<td>1.5</td>
<td>26.0</td>
<td>18.6</td>
<td>30.6</td>
<td>25.5</td>
</tr>
<tr>
<td>HM7 cell associated</td>
<td>16.2</td>
<td>2.2</td>
<td>27.2</td>
<td>3.6</td>
<td>11.7</td>
<td>4.8</td>
</tr>
<tr>
<td>B. H.I.M. media</td>
<td>656.7</td>
<td>.17</td>
<td>47.5</td>
<td>1.4</td>
<td>45.8</td>
<td>3.6</td>
</tr>
<tr>
<td>H.I.M. cell associated</td>
<td>111.1</td>
<td>1.9</td>
<td>25.5</td>
<td>1.6</td>
<td>13.0</td>
<td>.9</td>
</tr>
<tr>
<td>C. HM7 media</td>
<td>160.9</td>
<td>10.3</td>
<td>183.6</td>
<td>131.4</td>
<td>215.9</td>
<td>179.6</td>
</tr>
<tr>
<td>Cell associated</td>
<td>114.4</td>
<td>1.4</td>
<td>191.5</td>
<td>25.7</td>
<td>82.7</td>
<td>33.8</td>
</tr>
</tbody>
</table>

$^a$ $^3$H and $^{35}$S dpm in the CPC-precipitated fraction divided by the sum of the non-CPC-precipitated and the CPC-precipitated dpm.

$^b$ The number of HM7 cells used was 7.05 x 10^6 in two 16-oz bottles.

The 0.8 M NaCl Eluate. Fractionation of the 0.8 M NaCl eluates on CPG-10 240 80/120 glass bead columns resulted in the patterns shown in Charts 4 and 5. Incubation of the human melanoma media fraction with heparitinase resulted in digestion of 80% of the $^3$H dpm and 95% of the $^{35}$S dpm (Chart 4). The remaining 20% of the $^3$H dpm was a 1:1 mixture of chondroitin 4:sulfate and HA based on amino sugar content, cellulose acetate electrophoresis, and susceptibility to leech hyaluronidase and chondroitinase ACII. Following digestion of the media fraction with chondroitinase ACII, 9% of the $^3$H dpm and 7% of the $^{35}$S dpm; incubation with heparitinase resulted in hydrolysis of 52% of the $^3$H dpm. These results agree well with the amino sugar analyses indicating a 1:1 mixture of glucosamine and galactosamine and were confirmed by cellulose acetate electrophoresis. The human iris sample differed in that 90% of the material was a chondroitin sulfate, presumably chondroitin 4-sulfate. The presence of a small amount of derman sulfate could not be ruled out since derman sulfate was present in both the 0.8 and 2.0 M NaCl eluates. The identification of the glucosamine-containing compo-

The 1.2 M NaCl Eluate. Fractionation of the melanoma 1.2 M NaCl eluate on a CPG-10 240 80/120 glass bead column resulted in the patterns shown in Chart 6. Incubation of the melanoma fraction with chondroitinase ACII resulted in hydrolysis of approximately 58% of the $^3$H dpm and 87% of the $^{35}$S dpm; incubation with heparitinase resulted in hydrolysis of 52% of the $^3$H dpm. These results agree well with the amino sugar analyses indicating a 1:1 mixture of glucosamine and galactosamine and were confirmed by cellulose acetate electrophoresis. The human iris sample differed in that 90% of the material was a chondroitin sulfate, presumably chondroitin 4-sulfate. The presence of a small amount of derman sulfate could not be ruled out since derman sulfate was present in both the 0.8 and 2.0 M NaCl eluates. The identification of the glucosamine-containing compo-
susceptibility by the dialysis assay. However, when incubation with the enzyme was followed by product analysis by Bio-Gel P2 or paper chromatography, it was obvious that the bulk of the material was incompletely hydrolyzed. The maximum amount of chondroitinase ACII-susceptible material was 62% by these methods. Following chondroitinase ABC incubation, however, the sample was completely digested to disaccharides; the main unsaturated disaccharide present was \( \Delta \)-disaccharide-4-sulfate (18). These results agree well with the amino sugar analyses, and it may be concluded that this fraction contains both chondroitin 4-sulfate and dermatan sulfate (Table 6).

A summary of the incorporation data for the melanoma and iris melanocyte anionic saccharides and their relative amounts is given in Table 7.

**DISCUSSION**

The HM7 line was composed of a morphologically heterogeneous population of cells including a preponderance of small amelanotic cells and occasionally much larger forms. We observed similar changes in the B16C3 melanoma cell line and produced evidence supporting the conclusion that the heterogeneity was the result of the murine B16 melanoma cells maturation and differentiation pattern (13). Although a similar interpretation for the heterogeneity of the...
The activity of tyrosinase in this system is dependent upon the replicative activity of the cells. This observation is similar to that which we found in B16C3 cells (J. W. Kreider, unpublished observations) but with some important differences. In the B16C3 line, tyrosinase activity was not detectable during the replicative phase and appeared only after confluency was attained. In the HM7 line, tyrosinase activity was present at the time of cell planting and declined in inverse proportion to replicative activity. Such dilution of tyrosinase activity with cell replication has been observed in chick melanocyte cultures and has been interpreted as the result of the redistribution of a fixed amount of preformed tyrosinase among an increasing number of newly produced melanocytes (25). This seems to be a reasonable explanation for the results we have described here for the HM7 line. With the attainment of confluency, additional tyrosinase activity is demonstrable and continues to increase each day for the results we have described here for the HM7 line.

HM7 line is heuristically appealing, we have not yet examined this possibility. The activity of tyrosinase in this system is dependent upon the replicative activity of the cells. This observation is similar to that which we found in B16C3 cells (J. W. Kreider, unpublished observations) but with some important differences. In the B16C3 line, tyrosinase activity was not detectable during the replicative phase and appeared only after confluency was attained. In the HM7 line, tyrosinase activity was present at the time of cell planting and declined in inverse proportion to replicative activity. Such dilution of tyrosinase activity with cell replication has been observed in chick melanocyte cultures and has been interpreted as the result of the redistribution of a fixed amount of preformed tyrosinase among an increasing number of newly produced melanocytes (25). This seems to be a reasonable explanation for the results we have described here for the HM7 line. With the attainment of confluency, additional tyrosinase activity is demonstrable and continues to increase each day in a relatively fixed number of cells. This suggests de novo enzyme synthesis, but we have not directly examined this point. In contrast, in the B16C3 mouse melanoma cell line, tyrosinase activity appears only after confluency and rapidly diminishes to 0 within a few days. The loss of activity in the B16C3 may be due to a saturation of the melanosomes with accumulated melanin (22). Since the HM7 cells produce very little melanin, such saturation of the melanosomes probably does not occur within the duration of the experiment.

Implantation of HM7 cells into athymic nude mice produced small plaque-like nodules of persistent tumor cells that did not grow progressively. Although a number of human neoplastic cell lines have formed tumors in athymic mice (7), failure to do so cannot be interpreted as evidence that the lines are not neoplastic since other human tumor cell lines implanted in nude mice have also failed to produce progressive growths (23). This could be attributable to inadequate nutritional conditions for these human tumor cells in the athymic mice. Microscopic examination of the persistent nodules in our experiment demonstrated cells with typical human melanoma morphology that were actively invading between the muscle bundles of the underlying panniculus carnosus.

It is not probable that the HM7 line has been contaminated by admixture with murine melanoma cells. The kinetics of growth in vitro is very different. The doubling time of the B16C3 line is 19 to 29 hr, while that of the HM7 line is 40 to 72 hr. The kinetics of tyrosinase activation also is remarkably distinctive, the mean cell volumes are at least 2-fold different, and the morphology of both lines is unique. Melanin produced by the B16C3 cells is coal-black in color, while that produced by the HM7 is amber-brown. The chromosomes of murine cells generally are predominantly acrocentric, while those of the HM7 line are metacentric.

The polysaccharides produced by the HM7 human melanoma line differ in several ways from those made by human embryonic iris melanocytes. The percentage of HA made by the HM7 cells is significantly reduced, while the proportion of HS and C4S is significantly increased, and the HM7 cells contain no detectable deramatan sulfate while the human iris melanocytes produce significant quantities. It is not possible to attribute these differences to the neoplastic state alone, as the comparisons are not just between neoplastic versus normal melanocytes. The cells studied also differ as adult versus embryonic, integumentary versus ocular, and established cell line versus primary explant. There are no control cell culture lines of normal adult melanocytes. The use of primary explants of human embryonic iris, while inadequate in the respects detailed above, provides the only available control for these studies.

The HS secreted into the medium by the melanoma is apparently incorporated in molecules of higher molecular weight than those previously observed in mouse melanoma (19). The significance of this is not clear and may be a
known. Lectin-binding sites increase in number after hyaluronidase treatment of Rous sarcoma virus-transformed CEF cells (6). Treatment of HeLa cells with chondroitinase ABC produced a marked reduction in electrophoretic mobility, indicating that these molecules are associated with the cell surface (24). However, it is difficult to determine if these molecules are anchored into the plasma membrane and/or are cell surface associated through ionic or protein core interactions. In addition, the presence of polysaccharides in the nucleus has been reported (3).

The reduction of HA synthesis and the increase in HS and CSO₄ synthesis may represent compensatory responses. These molecules (HS, CSO₄) are markedly different from HA in physical parameters, and one might reasonably expect some changes in surface properties to occur, especially if the amount associated with the cell surface changes significantly. The distribution of these components on the surface as well as their variation during the cell cycle are also of interest.

The recent report of an alkali-stable common melanoma antigen being excreted by the majority of human melanoma patients (6) raises the possibility that the protein component of 1 of the saccharide proteoglycans functions as an antigen; alternative sources may be glycoproteins shed from the cell surface.

REFERENCES

Human Melanoma Saccharides

Anionic Polysaccharide Production and Tyrosinase Activation in Cultured Human Melanoma Cells

John Banks, John W. Kreider, V. P. Bhavanandan, et al.


Updated version
Access the most recent version of this article at:
[http://cancerres.aacrjournals.org/content/36/2_Part_1/424](http://cancerres.aacrjournals.org/content/36/2_Part_1/424)

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.