Cell Surface Properties of B16 Melanoma Variants with Differing Metastatic Potential


ABSTRACT

Several cell surface properties of three B16 melanoma variant lines, which exhibit differing lung-colonizing capacities, have been examined. Equal numbers of cells from the B16-F10 Lr and B16-F1 lines, injected i.v. into syngeneic mice, produced significantly fewer lung nodules than did cells of the B16-F10 line. These wide differences in biological behavior could not, however, be ascribed to major qualitative differences in the cell surface properties studied.

The 3 cell lines displayed similar quantitative and qualitative patterns of exposed surface proteins. The quantity of radioactively labeling of the major sialoglycoprotein (78,000 daltons) was inversely proportional to the capacity of cells to produce lung tumor colonies. Lectin-binding assays revealed that B16-F10 Lr cells bound 50% less concanavalin A than did the B16-F1 or B16-F10 cells; the B16-F10 cells bound 40% less wheat germ agglutinin than did the B16-F1 or B16-F10Lr cells. Levels of 5′-nucleotidase activity were lower in B16-F10 cells than in B16-F1 and B16-F10Lr cells. At the same time, there was no reduction in levels of the cytoplasm-associated enzyme, acid phosphatase. No differences in membrane lipid fluidity among the cell lines were detected by either fluorescent polarization or photobleaching techniques. The kinetics of adhesion of B16-F10 Lr and B16-F10 to a variety of substrates were very similar. The present data suggest that wide differences in metastatic behavior of B16 melanoma cells need not be correlated with major qualitative changes in a single cell surface biochemical or morphological characteristic.

INTRODUCTION

The process of metastasis involves the release of cells from the primary tumor, dissemination to distant sites, arrest in the microcirculation of organs, extravasation and infiltration into the stroma of those organs, and survival and growth into new tumor colonies. The outcome of metastasis depends on both host factors and tumor cell properties, which may vary among tumor systems (15). Since the interaction of metastatic cells with their environment is mediated to a considerable extent by the cell surface, it seems likely that cell surface properties could influence the metastatic potential of tumor cells. Changes in the cell surface that accompany neoplastic transformation have been studied in great detail. In fact, almost all characteristics of the transformed cell membrane appear altered when compared to those of the untransformed counterpart (21, 24, 33, 47). The relationship of any specific cell surface change to the outcome of experimental metastasis, however, is unknown.

In order to identify those properties of tumor cells that allow them to interact favorably with their host and to establish metastatic growths, it is advantageous to study variants of the same tumor with differing capacities to form experimental metastases. The isolation of tumor variants can be achieved by several methods. The first involves isolation and propagation of variant lines from metastases. In such diverse murine tumor systems as melanoma (12), fibrosarcoma (44), methylcholanthrene-induced sarcoma (26), lymphosarcoma (7), Lewis lung carcinoma (18), and lymphoma (27), variants with different metastatic potentials have been selected. A second method for isolation of tumor variants involves in vitro selection of cells for specific properties, such as detachment from a monolayer (5), resistance to lysis by syngeneic lymphocytes (14), and resistance to lectin-mediated toxicity (48), and then to determine whether the selection affects metastatic behavior in vivo. A third more recent and complementary approach has been to establish in vitro a large number of clones or lines from parent tumors such as the B16 melanoma (6, 12, 13, 16), a UV-induced fibrosarcoma (28), a mammary tumor (10), and a methylcholanthrene-induced fibrosarcoma (43). Using this approach, the behavior in vivo and in vitro of such clones can be studied.

In recent years, we have concentrated on the B16 melanoma, syngeneic to the C57BL/6 mouse, as a tumor system. By manipulating various host and tumor variables, we have attempted to determine the relative contributions of several factors to the survival and growth of blood-borne tumor cells. We have described the selection in vivo (12) and in vitro (14) of variant sublines of the B16 melanoma with different capacities for experimental metastasis. Several biochemical differences between the cells of low and high lung-colonizing potential have been reported. Among these were electrophoretic mobility and surface glycoproteins (19). However, comparative analysis of surface proteins labeled by lactoperoxidase iodination and glycopeptides released by mild trypsin-pronase digestion failed to demonstrate any significant differences between high- and low-metastatic B16 cell variants (34, 48).

The present report concerns our analysis of the functional, structural, morphological, and dynamic properties of the cell membrane components and their correlation with the lung-colonizing behavior of 3 variants of the B16-F series. The results indicate that no qualitative differences in major components of the cell membrane among the lines can be demonstrated. However, subtle quantitative and perhaps cumulative changes in the cell surface may contribute to the success or failure of blood-borne tumor cell survival and growth in vivo.

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MATERIALS AND METHODS

Animals

Specific-pathogen-free C57BL/6 mice were obtained from the Animal Production Area of the Frederick Cancer Research Center. For each experiment, all mice were age and sex matched.

Tumor Cell Variants

The B16-F1 cell line (low incidence of lung colonization) was derived from pulmonary metastases produced by i.v. injection of B16 melanoma cells obtained from The Jackson Laboratory, Bar Harbor, Maine. The B16-F10 cell line (high incidence of lung colonization) was selected by successive passaging of lung tumor colonies for 10 in vivo-in vitro selections as described in detail previously (12). B16-F10 (lymphocyte resistant, low incidence of metastasis to the lung) is a variant cell line selected from B16-F10, after tumor cell monolayers were subjected to cytotoxic lymphocytes 6 times in vitro (14).

Tumor Cultures

All cultures were maintained on plastic in Eagle’s minimal essential medium supplemented with 10% fetal bovine serum, sodium pyruvate, nonessential amino acids, L-glutamine, and 2-fold vitamin solution (Flow Laboratories, Inc., Rockville, Md.). Antibiotics were not included in the medium for routine maintenance of the cultures. Cell cultures were grown at 37° in a humidified atmosphere containing 5% CO₂. The cell lines were examined for and found to be free of Mycoplasma and the following murine viruses: reovirus type 3; pneumonia virus of mice; K virus; Theiler’s virus; Sendai virus; lymphocytic choriomeningitis virus; ectromelia virus; and lactate dehydrogenase virus (Microbiological Associates, Bethesda, Md.). The following lectins were radioactively labeled by the 125I-chloramidine-T method (23): SBA, UEA1 (Vector Laboratory, Burlingame, Calif.), Con A, WGA (Miles Yeda, Rehovot, Israel), and WBA. The radioactively labeled lectins were separated from free iodine by gel filtration on a Bio-Gel P-2 column. Specific radioactivity of the lectins ranged from 3 to 10 x 10⁶ cpm/mg protein.

For quantitative studies with radioactively labeled lectins, 0.5 x 10⁶ tumor cells were exposed to increasing concentrations of a given lectin for 30 min at 24°. The specific binding of a lectin to the cell surface was calculated by subtracting the amount bound in the presence of a specific inhibitor (to lectin binding) from the amount of lectin bound in the absence of its inhibitor. The specific inhibitors were 0.1 M α-methyl-D-mannopyranoside for Con A, 0.1 M N-acetylgalactosamine for WGA, 0.1 M N-acetylgalactosamine for SBA, 0.1 M L-fucose for UEA1, and 0.1% fetuin for WBA. Lectin binding in the presence of specific inhibitors constituted less than 10% of total binding.

Neuraminidase Treatment

B16 cells suspended in PBS were incubated for 30 min at 37° with protease-free Vibrio cholerae neuraminidase (50 units/ml) purchased from Calbiochem, La Jolla, Calif., and then washed in buffer to remove the free sialic acid and the enzyme. When comparison between untreated and neuraminidase-treated cultures was desired, the untreated cells were incubated in PBS for the same duration as the enzyme treatment. No loss of cell viability could be detected following neuraminidase treatment.

Surface Labeling of Glycoproteins

Galactose residues of cell surface glycoproteins were radio-labeled before or after neuraminidase treatment. Cells were incubated with 50 μg of galactose oxidase for 180 min at 37° and then centrifuged, washed in, and reduced with 0.5 mCi NaB³H₄ (New England Nuclear, Boston, Mass.) for 30 min at 24°. Cells were washed and processed for electrophoresis. For surface labeling of sialic acid residues of glycoproteins, 2 x 10⁶ cells suspended in 1 ml of phosphate-buffered NaCl (0.13 M NaCl in 0.02 M potassium phosphate, pH 7) were incubated with 5 μCi Na¹⁴C lactate at 4° for 15 min (19); 0.2 ml of 0.1 M glyceral was then added, and the cells were incubated for an additional 10 min. After being washed with the buffer, the suspensions were reduced with [¹⁴]borohydride, and the cells were processed for electrophoresis. Labeling procedures did not lead to decreased cell viability.

Electrophoresis

The labeled cells were solubilized with 1% Triton X-100, 0.4 M KI-0.05 M Tris-HCI, pH 9.0, and centrifuged at 10,000 x g for 15 min to remove insoluble material. An aliquot was resolved by electrophoresis separation on a 5 to 20% gradient of sodium dodecyl sulfate-polyacrylamide gel (29). Fluorography was car-

3 The abbreviations used are: PBS, phosphate-buffered saline (0.01 M phosphate buffer, pH 7.2-0.15 M NaCl); SBA, soybean agglutinin; UEA1, Ulex europaeus agglutinin I; Con A, concanavalin A; WGA, wheat germ agglutinin; WBA, wax bean agglutinin; DPH, 1,6-diphenyl-1,3,5-hexatriene; Dil, 3,3′-dioctadecyldiocarbocyanine.
ried out by the method of Bonner and Laskey (3). Gels were fixed, stained and destained, and then incubated with 22% dimethyl sulfoxide in dimethyl sulfide, dried, and exposed to RP Royal X-omat film (Kodak) at -70°. Interpretation of labeling could be made by superimposition of the developed fluorogram on the Coomassie blue-stained protein bands.

**Assay for Enzyme Activities**

All assays were performed on attached monolayer cell lines (38). The determinations of enzyme activity are expressed in relation to mg protein. Cells of semiconfluent monolayers were washed 3 times with 2-ml aliquots of 0.9% NaCl solution, drained, and exposed to 0.6 ml of glass-distilled water. The culture plates with lysed cells were frozen at -24°.

**Acid Phosphatase.** After thawing, buffer (0.4 M sodium acetate, pH 5, 0.3 ml) and substrate (0.2 M β-glycerophosphate, pH 5, 0.3 ml) (Fisher Scientific Company, Fairlawn, N. J.) were added, and the plates were incubated at 37° for 2 hr. The reaction was terminated by the addition of 0.4 ml of cold 20% trichloroacetic acid. After 15 min in the cold trichloroacetic acid, the solution was centrifuged, and an aliquot of the supernatant fluid (0.5 ml) was analyzed for P1 release according to the method of Ames and Dubin (1). A correction for free P1 in cells and for nonenzymic substrate hydrolysis was included.

**5'-Nucleotidase.** Essentially the same procedure as adopted for acid phosphatase was used. Buffer and substrate consisted of 0.5 ml of 0.2 M Tris-HCl, pH 8.5, containing 20 mM MgCl2 and 20 mM AMP.

**Protein Determination.** Triplicate cell cultures treated and washed identically to the method used for cultures in the corresponding assays were dissolved in 0.1 N NaOH, and their protein content was measured according to the method of Lowry et al. (31).

**RESULTS**

**In Vivo Lung Colonization Properties**

B16-F1, B16-F10, and B16-F10Lr cell lines were grown in vitro from frozen stocks. The lung colonization capacity of the 3 cell lines was tested several times over the period during which the various in vitro studies were carried out. Data from representative experiments show that the relative lung colonization capacities of the 3 cell lines remained constant for 12 weeks of continuous in vitro cultivation (Table 1). In all tests, cells from B16-F1 or B16-F10Lr formed fewer pulmonary metastases than did equal numbers of injected B16-F10 cells. Moreover, cells from B16-F1 also formed extrapulmonary metastases in organs such as liver, lymph nodes, ovary, and adrenal gland. At 18 days after i.v. injection, no extrapulmonary metastases were found in mice given injections of cells of B16-F10 or B16-F10Lr.

These findings are in agreement with data demonstrating that the low lung colonization of B16-F1 and high lung colonization capacities of B16-F10 cells were maintained in cell lines even after 18 weeks of continuous incubation in vitro. Similarly, cells obtained from B16-F1 or B16-F10 serially transplanted s.c. for 12 months (35 s.c. transplants) retained their respective low or high lung colonization capacities. Collectively then, we are confident that in vitro studies of tumor surface properties, which may correlate with lung colonization in vivo, can be carried out on these cell lines incubated in vitro for up to 12 consecutive weeks. Nonetheless, we maintained the cell lines for no longer than 60 culture days before replacing the cultures from frozen stocks.

**Labeling of Galactose**

Labeling of glycoprotein galactose residues was carried out by borohydride reduction, before or after treatment of cells with neuraminidase to remove sialic acid and oxidation with galactose oxidase (Fig. 1). Only traces of labeled NaB3H4 incorporation could be detected in the absence of neuraminidase treatment in all 3 cell lines. The glycoprotein profile on the gel reveals that less radioactivity was incorporated into the

![Figure 1: Fluorography pattern of sodium dodecyl sulfate-polyacrylamide gel electrophoresis of B16 melanoma variants labeled in galactose residues of cell surface glycoproteins. +, after neuraminidase and galactose oxidase treatment; −, after neuraminidase and galactose oxidase treatment without neuraminidase.](image-url)

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Cell no.</th>
<th>Median of pulmonary metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>B16-F1</td>
<td>12,500</td>
<td>2 (1-7)</td>
</tr>
<tr>
<td></td>
<td>25,000</td>
<td>3 (2-13)</td>
</tr>
<tr>
<td></td>
<td>50,000</td>
<td>12 (1-79)</td>
</tr>
<tr>
<td>B16-F10</td>
<td>12,500</td>
<td>12 (7-18)</td>
</tr>
<tr>
<td></td>
<td>25,000</td>
<td>100 (59-145)</td>
</tr>
<tr>
<td></td>
<td>50,000</td>
<td>170 (125-204)</td>
</tr>
<tr>
<td>B16-F10Lr</td>
<td>50,000</td>
<td>2 (0-7)</td>
</tr>
</tbody>
</table>

* Ten mice/group.  
* Cells were harvested for i.v. injection from a semiconfluent culture with 2 mM EDTA.  
* Pulmonary metastases counted with a dissecting microscope 18 days after i.v. tumor cell injection.  
* Numbers in parentheses, range.
B16-F10 major glycoprotein than into the B16-F1 and F16-F10Lr major glycoprotein. The decrease in labeling is best seen in the direct labeling of sialic acid.

**Labeling of Sialic Acid**

The selective labeling of cell surface sialoglycoproteins achieved by mild periodate oxidation of the sialic acid and reduction with [3H]borohydride is illustrated in Chart 1. Densitometric tracings of the gels show no qualitative differences in the pattern of surface labeling among the 3 B16 melanoma cell lines. On the other hand, the degree of labeling of the major sialoglycoprotein (78,000 daltons) appeared to be inversely proportional to the degree of lung colonization in vivo, high at B16-F10 and low at B16-F10. Similarly, the B16-F10Lr 78,000-dalton glycoprotein is more intensely labeled than that of the B16-F10, following the galactose oxidase method of labeling.

**Lectin Binding**

The composition of carbohydrate-containing receptors that can specifically bind lectins could serve as an indicator of the similarity of cell surface components. The binding of radioactively labeled Con A, WGA, SBA, and WBA to the surface of B16-F, B16-F10, and B16-F10Lr is illustrated in Chart 2. The number of Con A molecules bound to the surface of B16-F1 and B16-F10 cells is identical. In B16-F10Lr cells, 50% fewer Con A molecules are bound. A different pattern of binding is observed with WGA. The high-lung-colonizing cell line B16-F10 had about 40% fewer WGA-binding sites than did the low metastatic cell lines B16-F1 and B16-F10Lr. Free SBA-binding sites on the cell surface were barely detectable, but a 30-min treatment with neuraminidase exposed the receptor sites (Chart 2). Here again, the B16-F10 cells bound 2.5-fold fewer SBA molecules than did the cells of B16-F1 or B16-F10Lr. The number of WBA surface-bound molecules revealed that the 3 cell lines had the same number of binding sites. No binding of UEA1 could be detected with the 3 cell lines, suggesting that the B16 melanoma has no exposed L-fucose with which the lectin can interact.

**Enzyme Activities**

The 3 cell lines were analyzed for the specific activity of the plasma membrane enzyme marker, 5'-nucleotidase, and for the specific activity of the lysosomal enzyme marker, acid phosphatase. The results in Table 2 show a significant reduction in the specific activity of 5'-nucleotidase in the B16-F10 cell line as compared with B16-F1 and B16-F10Lr cell lines. The decrease in 5'-nucleotidase activity was not associated with a similar decrease in cytoplasmic enzyme activities, since acid phosphatase activity was similar in the 3 cell lines (Table 2).

**Gangliosides**

Gangliosides were extracted from lyophilized cell pellets and identified as described previously (9, 45). The 3 melanoma cell variants exhibited a very simple ganglioside pattern, which was almost identical to that observed when the cells were separated on thin-layer chromatography plates. They exhibited a very prominent Gm3 and only minor traces of Gm2 and Gm1 (not shown).

**Fluorescence Measurements**

**Fluorescence Polarization.** The 3 cell lines were harvested from confluent and semiconfluent conditions and were assayed for membrane fluidity of lipid regions by means of fluorescence polarization analysis of DPH embedded in membranes of intact viable cells (25, 41). No differences were detected in the degree of fluorescence polarization of the 3 DPH-labeled cell lines, grown to the same cell densities.

**Fluorescence Photobleaching Recovery.** Fluorescence photobleaching measurements (39) of diffusion of the lipid probes DII and rhodamine-conjugated WGA on the plasma membrane of single cells of B16-F10 and B16-F10Lr demonstrated no differences in either diffusion constants or mobile...
Surface Properties of Metastatic Cells

Chart 2. Specific binding of iodinated lectins to B16-F1 (○, ◇), B16-F10 (△, ▲), and B16-F10Lr (■, ■) in 125I-SBA binding curve. ○, △, □, cells pretreated with neuraminidase; ◇, ▲, △, without neuraminidase treatment. Experiments were done in quadruplicate. Bars, S.D.

Table 2
Specific activities of 5'-nucleotidase and acid phosphatase in B16 melanoma cell lines

<table>
<thead>
<tr>
<th>Cells</th>
<th>5'-Nucleotidase activity (nmol P_i/mg protein)</th>
<th>Acid phosphatase activity (nmol P_i/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B16-F1</td>
<td>142.2 ± 2.1*</td>
<td>1066.6 ± 15.9</td>
</tr>
<tr>
<td>B16-F10</td>
<td>90.9 ± 1.5</td>
<td>919.5 ± 8.1</td>
</tr>
<tr>
<td>B16-F10Lr</td>
<td>176 ± 3.5</td>
<td>1046 ± 11.5</td>
</tr>
</tbody>
</table>

* Mean ± S.E. computed from 6 different samples.

DISCUSSION

In this study, we have examined some aspects of cell surface composition and properties of 3 B16 melanoma variant cell lines with differing capacity to form experimental pulmonary metastases following i.v. injection. The incidence of pulmonary metastasis produced by cells of B16-F10 line was at least 10-fold that of B16-F1 or B16-F10Lr (Table 1). This difference in biological behavior in vivo was not reflected by comparable major qualitative alterations in the cell surface characteristics that we studied.

The process of metastasis is complicated, and the production of visible metastases is dependent on the ability of malignant cells to survive and complete a number of sequential steps. The injection of tumor cells into the circulation bypasses the first steps of the process, i.e., invasion and detachment. However, all the subsequent steps such as transport and survival in the circulation, arrest in the capillary bed of an organ, extravasation into the parenchyma, establishment of a microenvironment, escape from host defense mechanisms, and tumor cell multiplication in the distant organ must all be completed for clinical metastasis to occur. Failure to complete any one of these steps will lead to the elimination of a tumor cell as a potential progenitor of a secondary growth (42).

Reports from several laboratories have now demonstrated that the in vivo metastatic capacity of the B16-F lines correlates with fractions within the 20 to 30% S.D. of the measurements. The diffusion of the DII molecules in the membrane plane was ~150 x 10^{10} sq cm/sec, while the diffusion of rhodamine-WGA was ~3 x 10^{10} sq cm/sec. These values were similar in both cell lines.

Morphological Observation

Treatment of the cells with 2 mM EDTA to prepare cells for i.v. injection induced cell rounding and detachment from the substratum. The surface of the detached cells is rough, and the cells have a moderate number of flaps and ridges with extended thin filopodia. The cells of the B16-F10 cell line appeared to be somewhat smaller than the cells of the B16-F1 or B16-F10 lines. Thus, the differences in cell arrest in the lung are not due to gross variations in the morphology or size among the 3 B16 melanoma cell lines.
with their ability to aggregate with platelets (20), a phenomenon that could be due to differences in prostaglandin production (17). The cell lines also differ in their ability to clump with syngeneic lymphocytes (13) and endothelial cells (35, 48) and in their response to hormones (36). Control of many of these interactions probably resides at the tumor cell surface, and it is not unreasonable to postulate that differences in cell surface composition may account for the disparate behavior of the variant cell lines. In fact, some isolated cell surface differences between the cell lines have been described (4, 50), but no correlation between these differences and divergent metastatic behavior has been possible. Since no one property of tumor cells may determine the eventual outcome of metastasis, we attempted to survey a wide range of tumor cell surface characteristics and then compared the overall profile of one cell line with the others. We wish to emphasize 2 points regarding the experimental procedures: (a) this study represents a collaborative effort of 5 laboratories, in which each concentrated on assays that they use routinely and with which they are most familiar; (b) the cell lines were coded for all in vitro assays, and thus the studies represent blind assays.

We have used several different techniques of labeling surface constituents since most techniques, if used in isolation, have some drawbacks (8). Protein composition of the cell surface was studied by protein tyrosine iodination with lactoperoxidase (32). Analysis of surface-labeled proteins failed to demonstrate any appreciable difference in the surface-labeling pattern of the 3 B16-F lines (data not shown). These results, which failed to demonstrate any difference between B16-F and B16-F10 cell lines, are in agreement with an earlier report (34).

Studies of cell surface carbohydrates used 3 techniques: (a) labeling of galactose residues; (b) surface labeling of sialic acid residues; and (c) lectin binding. Analysis of cell surface glycoproteins by gel electrophoresis and fluorography prior to and after neuraminidase treatment demonstrated that most of the terminal galactose residues in the carbohydrate chains of glycoproteins are sialated, since no appreciable labeling was detected prior to the cleavage of the sialic acid. At least 11 distinct bands of surface glycoproteins ranging from 14,000 to 200,000 daltons, with a major glycoprotein at 78,000 daltons, could be demonstrated. Direct labeling of the sialic acid and analysis of the labeled sialoglycoprotein by gel electrophoresis gave a pattern similar to the one obtained with the galactose oxidase method. Both techniques failed to demonstrate any qualitative differences among the B16-F1, B16-F10, and B16-F10<sup>Lr</sup> cell lines.

Lectin binding of the cell lines differed. B16-F1 and B16-F10 cells exhibited similar binding of Con A. This is in agreement with studies reporting similar agglutination of these 2 cell lines by Con A (34). However, the poorly metastatic cell line, B16-F10<sup>Lr</sup>, had a significantly lower number of surface Con A receptors than the 2 other lines. The B16-F10<sup>Lr</sup> cells are resistant to in vitro lysis mediated by syngeneic lymphocytes, which is presumably due to the deficiency of lymphocyte-binding receptors (13). Whether the receptors for Con A and cytotoxic syngeneic lymphocytes are related is unclear. The B16-F10 cells were found to have about a 50% reduction in WGA or SBA binding as compared with B16-F1 and B16-F10<sup>Lr</sup> cells. SBA agglutinin, which binds to terminal galactose residues, barely bound to the surface of the 3 cell lines prior to the removal of sialic acid. This finding correlated with the results that we obtained by surface labeling with galactose oxidase. Moreover, the fact that after neuraminidase treatment fewer SBA-binding sites were exposed on the F10 cell membrane than on the other 2 cell lines also agrees with the data obtained by the galactose oxidase labeling. The latter technique demonstrated that surface galactose accessible for labeling was reduced on B16-F10 cells and that B16-F10 cells also have less surface sialic acid accessible for labeling. We did not find that B16-F10 cells have a 2-fold increase in neuraminidase-accessible sialic acid surface molecules (4, 50). The 3 cell lines had the same number of WBA-binding sites, reflecting the fact that the sugar residues, which are in proximity to the protein core, are the same in all 3 cell lines.

Plasma membrane gangliosides are believed to be receptors for numerous biologically active agents such as toxins and hormones. Moreover, changes in ganglioside pattern may accompany transformation (21). We found no qualitative differences in the ganglioside composition of the 3 cell lines. The ganglioside pattern was found to be a simple one, consisting mainly of GM<sub>2</sub> with traces of GM<sub>1</sub> and GM<sub>3</sub>. These findings agree with a previous report in which the ganglioside profile of B16-F1 and B16-F10 cells was studied (50).

We next examined the relationship of protein dynamics to membrane lipid fluidity. Fluorescence polarization analysis of DPH when embedded in membrane lipids was used to monitor the degree of microviscosity of the surface membrane of B16-F10, B16-F1, and B16-F10<sup>Lr</sup> tumor cells. We detected no differences in lipid microviscosity among cells of the 3 variant lines. The fluorescence polarization analysis, which examines populations of cells, is an averaging method. In contrast, fluorescence photobleaching recovery measures the diffusion of the lipid probe DII and rhodamine-conjugated WGA in the plasma membrane of individual cells. The measurements were performed at a given lectin concentration on the same day. Nonetheless, no detectable differences in either diffusion constant or mobile fractions were observed between cells of B16-F10 and B16-F10<sup>Lr</sup> lines.

The adenosine-generating enzyme 5'-nucleotidase is a plasma membrane-bound enzyme of mammalian cells. Different levels of this enzyme can distinguish among different subpopulations of cells both in normal and transformed systems (2, 30). The specific activity of 5'-nucleotidase in the highly metastatic melanoma cell line (B16-F10) was decreased by about 50% as compared with the 2 low-metastatic cell lines (B16-F1 and B16-F10<sup>Lr</sup>). The observed reduction of enzyme activity was limited to the plasma membrane, since it was not accompanied by a similar reduction of an intracellular enzyme, acid phosphatase. The B16 melanoma variants had a relatively low specific activity of 5'-nucleotidase as has been observed in other transformed cells (37).

Collectively then, the present data as well as that of others (34, 48) are unable to demonstrate a major alteration in the plasma membrane characteristics of B16 melanoma cells that might be associated with their metastatic potential in vivo. The production of a metastasis requires a cell to survive many destructive events. It is entirely possible that what appears to be a subtle variation in a cell surface property measured in vitro could be responsible for a dramatic alteration in malignant behavior in vivo. Certainly, our study illustrates some of the problems inherent in evaluating a complex in vivo process such as metastasis by using in vitro correlates which may measure...
only one cellular property (of many) requisite for the metastatic cell. Nonetheless, it is possible that subtle variations in composition (47) or arrangement of cell surface constituents could indeed be responsible for the ability of tumor cells to interact with their host, survive, and develop into metastases.

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