Differential Expression of Endogenous Lectins on the Surface of Nontumorigenic, Tumorigenic, and Metastatic Cells

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ABSTRACT

A monoclonal antibody that was found to recognize endogenous galactoside-specific lectins of various tumor cells by immunoblot analysis was used for quantitative analyses of cell surface lectin on nontumorigenic, tumorigenic, and metastatic cells of diverse histological types and origin. Indirect immunofluorescent staining of viable cells followed by analysis with a fluorescence-activated cell sorter revealed marked differences in the amount of surface lectins between untransformed and malignant cells. While lectin was either absent or present in a very low density on the surface of normal cells, neoplastic cells were invariably stained by the antilectin antibodies. Furthermore, among related tumor cell variants of the K-1735 melanoma and UV-2237 fibrosarcoma tumor systems, cells exhibiting a higher lung-colonizing potential also expressed higher levels of cell surface lectin. These results suggest that the presence of a lectin on the cell surface may be related to neoplastic transformation and progression toward metastasis.

INTRODUCTION

The metastatic process involves the hematogenous or lymphatic spread or both of tumor cells from the primary tumor to distant organs. The arrest of some of these tumor cells in the capillary beds is greatly influenced by their interaction with and adhesion to other neighboring cells and to circulating host cells (1-4). Indeed, the incidence of experimental metastasis is correlated with the number and size of circulating tumor cell emboli (5-7). Although it is clear that cellular interactions are influenced by cell surface components, the molecular basis for tumor-tumor and tumor-normal cell adhesion is only now being unraveled.

Endogenous surface lectins have been implicated in mediation of cognitive intercellular adhesion by binding with carbohydrate-containing surface membrane components on adjacent cells (8-12). We have found that extracts of a wide variety of human and murine neoplastic cells contain galactoside-specific lectins (13). Subsequent studies suggested that these lectins may be related to the colony-forming ability of certain tumor cells in semisolid medium and in the lungs of mice (14, 15). These results prompted us to hypothesize that the expression of cell surface lectins may contribute to the ability of tumorigenic cells to produce metastases. If this were the case, the expression of the surface lectin should increase in cells with increasing malignant potential.

The quantitation of cell surface lectins awaited the development of suitable analytic procedures. Recently, we produced a monoclonal antibody that reacts with the endogenous galactoside-specific lectin of various tumor cells, including melanoma, fibrosarcoma, neuroblastoma, and carcinoma cells (16). In the present study, we used this mAb for a quantitative analysis of endogenous lectin on the surface of nontumorigenic, tumorigenic, and metastatic cells of rodent and human origins. Both indirect immunofluorescence labeling and FACS analyses provide a strong support for our hypothesis.

MATERIALS AND METHODS

Cells and Culture Conditions. The B16-F1 cell line was derived from pulmonary metastases produced by the i.v. injection of wild-type B16 melanoma cells (17). The B16-F1-NA was derived from B16-F1 cells after 4 cycles of aggregation with asialofetuin and successive selections for nonaggregated cells (14). The UV-2237-15 cloned cell line was obtained from the sixth in vitro passage as previously described (18). The UV-2237-IP3 variant cell line was selected from the parental UV-2237 tumor after 3 successive passages of i.p. tumor cells as previously described (19). The K-1735-MI cell line was derived from a spontaneous lung metastasis produced by the K-1735 parental tumor (20). The K-1735-MI selected cell line was obtained from the parental cell line as previously described (21). The distinctive lung implantation capabilities of all these cell lines were confirmed by the i.v. injection of 10⁶ cells into syngeneic recipients. All of the cell lines maintained their previously reported lung-colonizing capabilities. The murine melanomas B16 and K-1735 and the UV-2237 fibrosarcoma cell lines were obtained from Drs. M. L. Kripke and I. J. Fidler (The University of Texas M. D. Anderson Hospital and Tumor Institute at Houston, TX). The 3T3 fibroblasts and SV40-polyoma virus-transformed 3T3 cells (SVPy-3T3) were obtained from Dr. A. Ben-Ze'ev (The Weizmann Institute of Science, Rehovot, Israel). The SV40-transformed human fibroblast line (SV80) and human foreskin fibroblast line were obtained from Drs. Y. Groner and M. Revel (The Weizmann Institute). Adult skin fibroblasts line 302 was obtained from Dr. L. Kopelovich (Memorial Sloan-Kettering, New York, NY). The human carcinoma cell line HeLa-S3 was supplied by the American Type Culture Collection (Rockville, MD). The NRK cells infected with a temperature-sensitive mutant (LA23) and a wild-type (B77) strain of RSV were obtained from Drs. B. Geiger and A. Ben-Ze'ev (The Weizmann Institute). All of these cell lines were grown in monolayers on plastic in Dulbecco's modified Eagle's medium (GIBCO) containing 10% heat-inactivated fetal bovine serum (Bio-Lab, Israel), nonessential amino acids, and antibiotics. The cells were maintained at 37°C in a humidified atmosphere of 7% CO₂ and 93% air. To ensure reproducibility, all experiments were carried out with cultures grown for no longer than 4 weeks after recovery from frozen stocks.

Polyacrylamide Gel Electrophoresis and Immunoblot Analysis of Proteins. The proteins from similar numbers of cells (0.5 x 10⁶) were separated on 12.5% acrylamide gels according to Laemmli (24) and were transferred electrophoretically to nitrocellulose paper (Schleicher & Schuell, Inc.) according to Gershoni and Palade (23), utilizing a transfer apparatus with gradient electric field (25) and hemoglobin solution (1% in PBS-NaNa₂) as the protein quencher. The 5D7 mAb (1:50 dilution of ascites fluid) was used as the first antibody, and the second antibody was 125I-labeled goat anti-mouse IgG (Melloy). Antibodies. Antilectin mAbs were developed from mice vaccinated with 5D7 hybridoma (16). Fluorescein-labeled rabbit anti-mouse immunoglobulin was purchased from Nordic Immunological Laboratory. Rhodamine-
labeled goat anti-mouse immunoglobulin was obtained from Dr. B. Geiger.

Immunofluorescence. The FACS analysis was carried out as described previously (25). In brief, the cells were harvested from cultures in their exponential growth phase by washing the monolayer with warm Ca+2- and Mg+2-free PBS and overlaying the cells with a solution of 2 mM EDTA in the same buffer for 3 min at 37°C. The cells were washed and resuspended in PBS. Cell suspensions (3 × 10^6 cells/ml) in 0.1 ml PBS containing 0.02% NaN_3 were incubated for 30 min with 20 μl ascites fluid at 4°C (optimal concentration). After two washes with PBS, the cells were incubated with an optimal dilution (1:60) in azide PBS of fluorescein-labeled rabbit anti-mouse immunoglobulin antibodies for 30 min at 4°C. After two washes with PBS, the cells were analyzed in the FACS II (Becton, Dickinson & Co.). A scatter window was set to eliminate dead cells and cell debris. The frequency and fluorescence profile of the stained cells was determined by using a laser output of 400 mV and a photomultiplier at 450 V, and fluorescent gains of 1 to 16. For control stainings, the cells were either labeled with the second antibody alone (background) or with antilectin mAbs 4G323 or 6G81, which are known to inhibit the hemagglutination activity of solubilized tumor cell lectins but to fail to recognize exposed cell surface antigenic sites (16). The profile of their fluorescence staining was similar to that of the background in all cells tested.

In Vitro Transformation. Primary embryonal fibroblasts were established from 12-day-old Sprague-Dawley rat embryos. Secondary Sprague-Dawley embryo fibroblasts were plated at a density of 10^4 cells/90-mm dish and transfected by the calcium phosphate procedure with a mixture of the following DNAs: 10 μg pL8 containing the adenovirus 2/EIA and part of the EIB region, 10 μg of pEJ6.6 containing an activated human c-ras' gene, and 5 μg BALB/c liver DNA. Three days later, cells were trypsinized and 2 × 10^3 cells were plated into semisolid 0.33% agar in 60-mm dishes. Colonies 1 to 2 mm in diameter were detected after 14 days, removed with Pasteur pipet, and placed in 3542 Costar tissue cluster wells. One of the clones that developed to a cell monolayer was propagated and transferred to vessels of increasing size. Following 3 in vitro passages, the cell monolayers were harvested, washed, counted, and used in the in vivo and in vitro experiments.

RESULTS

The membrane expression of the cell surface endogenous galactoside-specific lectin was subjected to quantitative analyses with the FACS. This examination determines both quantitative and qualitative expression of the antigen on cell surfaces. The fluorescence distribution of B16 melanoma cell variants stained with mAb 5D7 and the relative percentage of positively stained cells are shown in Fig. 1. Although the fluorescence staining of B16-F1 cells was relatively low, it was still more intense than the staining of F1-NA, a cell variant that we selected from B16-F1 for reduced homotypic aggregation. Moreover, the B16F1-NA has reduced capacity for tumor colony formation in agarose and markedly reduced production of lung metastases, as compared with the B16-F1 cells (14). That mAb 5D7 binds to the endogenous lectin molecules has been determined previously by solid phase radioimmunoassay and by inhibition of hemagglutination (16). To further establish the identity of the target antigen with which mAb 5D7 reacts we performed immuno blot analyses. As shown in Fig. 2, mAb 5D7 recognizes the affinity-purified B16 melanoma lectins (Fig. 2, Lane Ib) that comigrate with the affinity-purified lectins, attesting to the specificity of the mAb. Similar results were obtained with purified lectin (Fig. 2, Lane Ia) or total cell extracts (Fig. 2, Lane IIb) of the UV-2237-IP3 fibrosarcoma, and with extracts of K-1735 melanoma (Fig. 2, Lane IIIb) or HeLa-S3 carcinoma (Fig. 2, Lane IIIc) cells.

Because B16 melanoma cell variants usually display a low density of the cell surface antigen, and since B16-F10, a high lung-colonizing cell variant, did not differ significantly from B16-F1 cells (data not shown), we examined 2 different tumor systems (K-1735 melanoma and UV-2237 fibrosarcoma) expressing significantly higher cell surface lectin density (16). We noted a more intense labeling and a higher proportion of fluorescently labeled cells in the metastatic cell variants, K-1735-M1 and UV-2237-IP3 and 15 fibrosarcoma cells stained with 5D7 antilectin antibodies. Inset: percentage of fluorescent cells at each gain of the FACS. b.g., background fluorescence.

Fig. 1. Fluorescence distribution of B16-F1 and NA melanoma cells, K-1735-M1 and D melanoma cells, and UV-2237-IP3 and 15 fibrosarcoma cells stained with 5D7 antilectin antibodies. Inset: percentage of fluorescent cells at each gain of the FACS. b.g., background fluorescence.
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Fig. 2. Immunoblot analysis of mAb 5D7 specificity. Lane Ia, autoradiogram of lectin purified by affinity chromatography on immobilized asialofetuin from an extract of [35S]methionine-labeled B16-F1 melanoma cells. Immunoblot of lectins purified by affinity chromatography from extracts of the following cells: B16-F1 melanoma (Lane Ib); UV-2237-IP3 (Lane Ila). Immunoblots of total cell extracts of: UV-2237-IP3 (Lane Iib); B16-F1 (Lane IIIa); K-1755 (Lane IIIb), and HeLa-S3 (Lane IIIc). Ordinate, migration of 14C-methylated proteins of molecular weights of 14,300 (lysozyme); 30,000 (carbonic anhydrase); 46,000 (ovalbumin); and 69,000 (bovine serum albumin) which were purchased from Amersham. Arrows on right, location of endogenous lectins of molecular weights of 34,000 and 14,500, respectively.

transformed SVPy-3T3 cells (Fig. 3). Although the BALB/c-3T3 cells are untransformed, they have an indefinite life span in culture and therefore may not be normal. For this reason, we examined lectin expression on the surface of untransformed cells that senesce in culture and have a limited life span. The binding of the antilectin antibodies to the surface of human adult skin fibroblasts and human foreskin fibroblasts was almost negligible (Fig. 4). In contrast, the mAb reacted strongly with the SV40 virus-transformed human fibroblast cell line SV80 and with the human cervical carcinoma cell line HeLa (Fig. 4).

When cultured at 33°C (permissive temperature), the LA23-infected NRK cells (temperature-sensitive mutant of RSV) exhibit a transformed phenotype, whereas when grown at 39°C (nonpermissive temperature), the cells exhibit a normal morphology (26, 27). The spatial distribution of lectin molecules on the surface of LA23-infected cells at the nonpermissive and permissive temperatures is depicted in Fig. 5, A and B, respectively. Staining of viable LA23/NRK cells at 39°C was faint and in the form of small microclusters distributed over the entire surface of the cells (Fig. 5A). In sharp contrast, staining of the same cells grown at the permissive temperature revealed the appearance of intense fluorescence microclusters distributed randomly at the cell circumference (Fig. 5B). The mAb 5D7 binding to the surface of the same NRK cells which were infected with B77 (wild-type RSV) and cultured at 39°C (Fig. 5C) or at 33°C (Fig. 5D) revealed the same basic pattern of membrane fluorescence as observed with LA23-infected cells when cultured at the permissive temperature, a result that is also similar to that found for other tumor cells (16). From the quantitative comparison of the fluorescence distribution of LA23-infected cells grown at 39°C or 33°C and stained with mAb 5D7, a clear-cut difference was observed in the membrane expression of the endogenous lectin (Fig. 6). The cells grown at the permissive temperature reacted positively with these antibodies, in contrast to the almost negligible binding of the antilectin antibodies to cells grown at the nonpermissive temperature (Fig. 6). The B77-infected cells expressed the cell surface lectin at high density at both temperatures. Thus, FACS analysis corroborates the observations made using the immunofluorescence microscope.

To further establish the possible association between the transformed phenotype and the expression of cell surface lectins, we next compared the binding of antilectin antibodies by normal diploid fibroblasts and their newly transformed coun-

Fig. 3. Fluorescence distribution of 3T3 and SVPy-3T3 cells stained with 5D7 antilectin antibodies. Inset: immunofluorescence localization of the cell surface tumor cell lectin on suspended 3T3 and SVPy-3T3 cells. b.g., background fluorescence.

Fig. 4. Fluorescence distribution of SV40-transformed human fibroblasts, HeLa cervical carcinoma cells, foreskin fibroblasts, and adult skin fibroblasts (line 302) stained with 5D7 antilectin antibodies. b.g., background fluorescence.
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Fig. 5. Immunofluorescence localization of tumor cell surface lectin on: LA23-NRK cell (A and B); B77-NRK cells (C and D). Cells were grown as monolayers on glass coverslips at 39°C (A and C) and 33°C (B and D) for 48 h and were indirect immunofluorescence stained with 5D7 antibodies and rhodamine-labeled goat anti-mouse immunoglobulin and visualized as previously described (16). x 950.

Fig. 6. Fluorescence distribution of LA23- and B77-infected NRK cells stained with 5D7 antilectin antibodies. Cells were cultured for 48 h at the designated temperature prior to the analysis. o.g., background fluorescence.

DISCUSSION

Endogenous vertebrate lectins can be divided into 2 types: one type can be solubilized only with detergents and behaves as an integral component of cellular membranes (10, 11), and the other can be extracted without detergents and is defined as a soluble lectin (12, 28, 29). These lectins also share some basic properties: their extraction into aqueous solutions is enhanced by lactose, and they bind galactosides. The lectins require a reducing agent for sugar binding and hemagglutinating activities, but do not require divalent cations. The cellular location of these molecules may determine their function. Membrane-associated lectins, which are exposed on the cell surface, can serve as receptors for soluble extracellular glycoconjugates or can mediate intercellular interactions by binding complementary glycoconjugates on the surface of adjacent cells (8, 10–12). The presence of galactoside-specific lectins in various human and murine tumor cells suggested to us that such molecules could influence the pathogenesis of tumor metastasis by promoting the formation of multicell emboli in the circulation (13, 14).

We have recently described the development of mAb 5D7 which binds the affinity-purified B16 melanoma lectin as revealed by solid phase radioimmunoassay. The functions of the endogenous lectin including hemagglutination (16) and asialofetuin-mediated homotypic aggregation were inhibited by mAb 5D7. Indirect immunofluorescence staining of viable tumor cells revealed that mAb 5D7 binds to the cell surface (16). The present study has demonstrated by immunoblot analysis that mAb 5D7 binds specifically to affinity-purified lectin molecules.
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Fig. 7. Phase-contrast photomicrographs of SPDF (A–C) and SPDF-T (A’–C’) fibroblasts. A and A’, sparse monolayer; B and B’, confluent cultures; C and C’, colony growth in semisolid medium (0.5% agarose).

Fig. 8. Fluorescence distribution of normal (N) and transformed (T) Sprague-Dawley fibroblasts stained with 5D7 antilectin antibodies. Inset: percentage of fluorescent cells at each gain of the FACS. b.g., background fluorescence.

exhibiting molecular weights of 14,500 and 34,000, respectively. These 2 proteins were also recognized by mAb 5D7 on blots of total cellular proteins. In a previous analysis of chloramine-T-oxidized 125I-labeled affinity-purified lectin of B16 melanoma cells a M, 68,000 component was also detected (16). Since this protein is not detected, following affinity purification of metabolically labeled lectin it is plausible to assume that the iodinated M, 68,000 protein was an aggregate of the lower molecular weight lectin molecules.

The lodgment, attachment, and growth of blood-borne neoplastic cells depend largely on cell-cell adhesion, which is a cell surface-mediated phenomenon (1, 2, 5–7). It is possible that in vivo cell surface lectins recognize and bind galactosyl residues on different side chains of the same serum glycoprotein molecule. Thus, the glycoprotein can serve as a cross-linking bridge between adjacent cells and subsequently lead to the formation of cell aggregates. Alternatively, cell surface lectins may interact directly with cell surface carbohydrate residues on adjacent tumor or normal host cells, leading to the formation of tumor cell emboli. Furthermore, both types of interactions may occur simultaneously. The increased endogenous lectin density on the surface of the metastatic UV-2237 fibrosarcoma of K-1735 melanoma cells (Fig. 1) may support the hypothesis that the cell surface lectins play a role in expression of the metastatic phenotype of some tumor cells, possibly as mediators of intercellular recognition for embolization and adhesion. The inability to demonstrate this phenomenon in all of the B16 melanoma variants suggests that in this tumor system the expression of high density cell surface lectin may not be a rate-limiting factor for the expression of the metastatic capability.

The acquisition of metastatic properties by tumor cells is considered to be a late event in the progression of normal cells
to malignancy following neoplastic transformation (1, 2, 4). Preceding events in tumor progression include acquisition of anchorage independence and alterations in cell attachment and spreading, loss of normal growth control, and tumorigenicity (30, 31). We questioned whether cellular transformation is accompanied by the appearance of the galactoside-specific lectin on the cell surface. This possibility was explored by testing the binding of the antilectin mAb to normal cells and to their transformed counterparts. Comparing the relative number of cells expressing the cell surface lectin antigen and the fluorescence distribution of established untransformed and transformed cell variants stained with mAb 5D7, we observed a marked difference in the membrane expression of the antigen. The antilectin mAb seemed to react strongly with the transformed cell variants, whereas normal cells presented negligible or relatively low densities of the cell surface lectin.

Most of the tumorigenic cells tested in this study were established in culture and propagated in vitro for relatively extended periods that may have led to expression of surface lectins in the transformed cells. For this reason, we analyzed the expression of cell surface lectin in LA23-infected NRK cells in which the transformed phenotype can be modulated by shifting the culture temperature. Apparently, the acquisition of the transformed phenotype induces concomitant expression of cell surface lectin. This expression is most probably not a temperature artifact since the B77-infected NRK cells strongly express the antigen at both 39°C and 33°C. Further, we transferred normal rat diploid fibroblasts with oncogenes and established a variant at both 39°C and 33°C. Further, we transfected normal rat diploid fibroblasts with oncogenes and established a variant at both 39°C and 33°C. Further, we transfected normal rat diploid fibroblasts with oncogenes and established a variant at both 39°C and 33°C.


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