Differential Expression of Cell Surface Antigens and Glial Fibrillary Acidic Protein in Human Astrocytoma Subsets


ABSTRACT

We have characterized five distinct cell surface antigens of human astrocyomas and correlated their expression with the expression of glial fibrillary acidic protein (GFAP) and four previously defined cell surface markers of astrocyomas. One of the newly studied antigens, A4, which was originally detected on rat central nervous system (but not peripheral nervous system) neurons, is expressed on GFAP* human astrocytoma cells, but not on GFAP* astrocyomas or a wide range of other neuroectodermal, epithelial, and hematopoietic cells. Antigens F19 (M, 140,000/90,000 glycoprotein) and F24 (M, 90,000 glycoprotein) also show restricted distribution and are expressed on subsets of neuroectodermal and mesenchymal cells. Antigens G253 (M, 95,000 glycoprotein) and S5 (M, 120,000 glycoprotein) are more widely distributed on the cultured cell panel. The distribution of these antigens was determined on a series of 22 astrocytoma cell lines and in normal brain tissue and the results were compared with the distribution of 5 additional glial cell markers: GFAP and two other antigens A010 (M, 110,000 glycoprotein); AJ8 (M, 100,000 glycoprotein); LK26 (M, 35,000 glycoprotein); and Thy-1. Distinct patterns of expression on cultured astrocytomas and in neural tissues were identified for all antigenic systems studied, and cell surface expression of antigen A4 was found to correlate closely with GFAP phenotype of cultured astrocytomas. The antigens described in this study provide new markers to study normal glial differentiation and to correlate the phenotypes and biological behavior of distinct subsets of astrocytomas.

INTRODUCTION

Distinct pathways and stages of cellular differentiation are associated with specific patterns of cell surface antigen expression. This principle was first established through analysis of normal and malignant cells of hematopoietic origin and has been extended recently to other cell lineages (1-4). The finding of an ordered progression of surface phenotype changes during normal differentiation has permitted classification of leukemias and lymphomas (2, 5) and malignant melanomas (3) into subtypes that show antigenic similarity with normal cells at distinct stages of hematopoietic or neuroectodermal differentiation. For most other human neoplasms, however, little is known about coordinate patterns of antigen expression and their correlation with normal cellular differentiation.

We have previously defined several restricted cell surface antigens of human astrocyomas (6). Two antigens, AJ8 and A010, showed reciprocal (although overlapping) patterns of expression on a panel of cultured astrocytomas; the AJ8*/A010- and AJ8-/A010+ phenotypes were found to correlate with expression in these cell lines of GFAP, the best-known molecular marker of glial differentiation. The present study extends the serological analysis of glial tumors to include several newly defined antigenic systems. The most restricted of these antigens, designated as A4, was first described in the rat (7) and serves as a marker that distinguishes central (A4+) and peripheral neurons (A4-). We now show that A4 is expressed on a proportion of human astrocytomas and that A4 expression is closely correlated with the expression of GFAP. Expression of six additional surface antigenic systems was also found to identify astrocytoma subsets. Our findings support the notion that distinct patterns of cell surface antigen expression characterize subsets of glial neoplasms and that different tumor phenotypes may correspond to antigenic phenotypes of normal cells at distinct stages of glial differentiation.

MATERIALS AND METHODS

Cell Lines and Cell Culture. Nineteen human astrocytoma cell lines (SK-MG and SK-GS series) were established from tumor specimens of different patients obtained from North Shore University Hospital and New York University Hospital; all tumors were diagnosed as grade III or grade IV astrocytomas by routine pathological evaluation. Stock cultures of newly established cell lines were stored in liquid nitrogen at early passage levels (passages 5-20); in the present study, cell lines of early and late passage levels were analyzed. Additional cell lines were obtained from the collection of Dr. J. Fogh (Sloan-Kettering Institute) and from the human tumor cell line bank of our laboratory. Cell lines were routinely tested for Mycoplasma contamination and only Mycoplasma-free cultures were used for analysis.

Monoclonal Antibodies and Serological Procedures. MAB G253 was derived from a (BALB/c x C57BL/6)F1 mouse immunized with SK-GS-1 cells, MAB S5 from a mouse immunized with SK-MG-17 cells, and MABs F24 and F19 from a mouse immunized with lung fibroblasts, following published fusion, hybrid selection, and cloning procedures (8). The Ig subclass, as determined by double diffusion in agar with anti-immunoglobulin heavy chain-specific antisera (Bionetics, Kensington, MD), was found to be IgG2a for MAB G253 and IgG1 for MABs F19, F24, and S5. Generation and initial characterization of MABs A4, A010, AJ8, K117, and LK26 have been described previously (6, 7, 9, 10). A second antibody to the A4 antigen, MAB C5 (IgG), was also used in this study and results for cell lines and tissue typing were identical to those obtained with MAB A4.

MHA rosetting assays for the detection of surface antigens on cultured cells have been described (11, 12). Briefly, 200-300 cells/well were seeded into Falcon 3034 Microtest II plates (Falcon Labware, Oxnard, CA), and cultured for 24-48 h prior to serological analysis. Microcultures were incubated with serial dilutions of antibody for 1 h. After repeated washes, cells were incubated for 45 min with indicator cells, prepared by conjugating purified rabbit anti-mouse Ig (Dako Corp., Santa Barbara, CA) or goat anti-mouse IgM (Dako) with human erythrocytes using 0.01% chromium chloride. Finally, plates were washed and reactivity was scored microscopically by determining the proportion of individual target cells that showed erythrocyte rosetting at each antibody dilution step tested; the highest antibody dilution giving rosette formation was defined as the reciprocal titration end point. MHA assays were also evaluated for the percentage of cells within antigen-positive cultures showing rosette formation, but no heterogeneity in antigen expression was observed. Unrelated MABs were used as negative controls in all MHA assays; they gave no rosette formation.
Immunocytochemical Procedures. Cultured astrocytoma cells were fixed with acetone:methanol (1:1) or acetone:formaldehyde (3.7% in phosphate-buffered saline) and tested for GFAP expression using a rabbit antiserum (1:500 dilution; Dako) and a MAb to GFAP, purchased from Labsystems (Chicago, IL), in indirect immunofluorescence assays (13); both reagents gave identical results with the astrocytoma cell lines and in our hands do not cross-react in immunocytochemical assays with vimentin or other intermediate filament proteins. Normal brain tissue was obtained at autopsy and quick-frozen in isopentane precooled in liquid nitrogen. Frozen 7-μm sections were cut, mounted on gelatin-coated slides, air-dried, and fixed in cold acetone or ethanol. Avidin-biotin immunoperoxidase tests were carried out as described (14). MAbS were used as undiluted hybridoma culture supernatants (A010, F24, F19, AJ8, and S5) or ascites fluid (1:1000 dilution; G253, C5, and A4). Biotinylated horse anti-mouse Ig was used as second antibody, followed by avidin-biotin complex (Vector Labs, Burlington, CA). Negative control experiments were carried out with unrelated MAbS of IgG and IgM class and did not give any staining.

Immunohistochemical Procedures. Cells were metabolically labeled with [35S]methionine or [3H]glucosamine (New England Nuclear, Boston, MA) and extracted with 0.5% Nonidet P40 in Tris buffer as described (6). Lysates were incubated with MAbS, followed by rabbit anti-mouse Ig antibody. Immune complexes were isolated with Staphylococcus aureus, washed, extracted and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography as described (6).

RESULTS

Nine distinct cell surface antigenic systems (Table 1) defined by MAbS were examined by MHA assays and radioligand precipitation tests with cultured cells and by immunohistochemical analysis of normal and malignant tissues. Results of MHA assays with five MAbS (A4, S5, F19, F24, and G253) on a large panel of cultured human cells are presented in Table 2; results of immunoprecipitation experiments are shown in Fig. 1. The serological characterization of the four additional antigenic systems (AJ8, A010, K117, and LK26) has been reported (6, 9, 10). In the present study, we have determined the previously unknown biochemical nature of the AJ8 (M, 100,000 glycoprotein) and A010 (M, 110,000 glycoprotein) astrocytoma antigens (Fig. 1). MAb K117 recognizes human Thy-1 and MAb LK26 recognizes a M, 35,000 cell surface glycoprotein. Immune complexes were isolated with Staphylococcus aureus, washed, extracted and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography as described (6).

Table 1  Cell surface antigens of human astrocytoma defined by mouse MAbS

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Antibody subclass</th>
<th>Immunizing cell</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A4</td>
<td>M, 110,000 glycoprotein</td>
<td>IgM</td>
<td>Central nervous system neurons</td>
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</tr>
<tr>
<td>A010</td>
<td>M, 95,000 glycoprotein</td>
<td>IgG</td>
<td>SK-A02 astrocytoma</td>
<td>6</td>
</tr>
<tr>
<td>G253</td>
<td>M, 90,000 glycoprotein</td>
<td>IgG2a</td>
<td>SK-GS-1 astrocytoma</td>
<td></td>
</tr>
<tr>
<td>F24</td>
<td>M, 140,000/90,000 glycoproteins</td>
<td>IgG1</td>
<td>Lung fibroblasts</td>
<td></td>
</tr>
<tr>
<td>F19</td>
<td>M, 120,000 glycoprotein</td>
<td>IgG1</td>
<td>SK-MG-1 astrocytoma</td>
<td>6</td>
</tr>
<tr>
<td>AJ8</td>
<td>M, 25,000 glycoprotein (Thy-1 antigen)</td>
<td>IgG1</td>
<td>SK-MG-2 astrocytoma</td>
<td>9</td>
</tr>
<tr>
<td>S5</td>
<td>M, 120,000 glycoprotein</td>
<td>IgG2a</td>
<td>Lu-75 choriocarcinoma</td>
<td>10</td>
</tr>
<tr>
<td>K117</td>
<td>M, 25,000 glycoprotein (Thy-1 antigen)</td>
<td>IgG2a</td>
<td>Lung fibroblasts</td>
<td></td>
</tr>
<tr>
<td>LK26</td>
<td>M, 35,000 glycoprotein</td>
<td></td>
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A summary of the serological analysis of a wide range of normal and malignant human cell types for A4 expression.

Table 2 shows that A4 expression is highly restricted to cell lines derived from glial tumors. Of 22 astrocytoma cell lines tested, 7 typed A4+, the rest A4-. In contrast, 86 of 88 additional cell lines derived from other neuroectodermal tumors (neuroblastoma, retinoblastoma, medulloblastoma, melanoma), sarcomas, epithelial cancers, leukemias and lymphomas, were A4-. The only two exceptions were a sarcoma cell line (A2394) and the SK-LC-6 cell line. SK-LC-6 was derived from a lung tumor, but its antigenic phenotype (AJ225+/K117+/F19+/A4+) clearly distinguishes it from 20 other lung cancer lines, and also from other epithelial cancers examined in our laboratory (6); it resembles the antigenic phenotype of neuroectodermal tumors. All cultured normal cell types tested including fetal and adult fibroblasts, melanocytes, and kidney epithelial cells were A4-.

We attempted to characterize the A4 antigen in immunoprecipitation experiments with [3H]glucosamine- and [35S]methionine-labeled extracts of SK-GS-1, U251MG, SK-MG-2, and SK-LC-6 cells. However, no specific components were detected in immunoprecipitates with MAb A4. The A4-reactive epitopes on U251MG and SK-LC-6 cells were destroyed by boiling (5 min) but were resistant to treatment with trypsin, Pronase, and neuraminidase. These findings are in agreement with the characteristics of the A4 antigen in the rat (7).

F24 Cell Surface Antigen. MAb F24 detects a glycoprotein of M, 90,000 (not shown) expressed on a subset of astrocytoma cell lines and on cultured fibroblasts. Low levels of F24 expression were also found on a proportion of melanoma cell lines and the Hs913T fibrosarcoma line. Other neuroectodermally derived cell types (neuroblastomas, retinoblastoma, medulloblastoma) did not express F24, nor did 10 cell lines derived from human sarcomas (including 2 fibrosarcomas, HT1080 and 8387) and a large number of cell lines derived from epithelial cancers and hematopoietic malignancies. (Results are summarized in Table 2.)

F19 Cell Surface Antigen. MAb F19 detects a glycoprotein complex with subunits of M, 140,000 and 90,000, respectively (Fig. 1). The distribution of F19 on a large panel of cell lines is presented in Table 2. F19 was found on most astrocytomas, sarcomas, a subset of melanomas, and on cultured normal fibroblasts. All neuroblastomas and retinoblastomas, normal and malignant epithelial cells, and hematopoietic malignancies tested were F19-.

S5 Cell Surface Antigen. MAb S5 recognizes a glycoprotein of M, 120,000 (not shown) with an intermediate type distribution on the cultured cell panel (Table 2). Most astrocytomas and a proportion of melanomas are strong S5 expressors whereas other neuroectodermally derived cells lack the antigen. Normal and malignant cells of mesenchymal origin are also

\[ ^3 \] W. J. Rettig, unpublished observations.
Table 2 Reactivity of MAbs to human cell surface antigens with a panel of over 100 independently derived human tumor cell lines and short-term cultures of normal cells

Summary of MHA assays using serial, 5-fold dilutions of antibody (starting dilution 1:500 of nu/nu serum or ascites) to determine highest antibody dilution (titration end point) giving rosette formation (see Fig. 2). Results of titration experiments are indicated as follows: •, strong reactivity with reciprocal titers of 5 × 10^(-1) to 10^5; ○, weak reactivity with reciprocal titers of 5 × 10^(-2) to 5 × 10^(-1); ◦, no reactivity at starting dilution of MAb. Each symbol represents typing results with a single tumor cell line.

<table>
<thead>
<tr>
<th>Cultured cell type</th>
<th>Monoclonal antibody</th>
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<tbody>
<tr>
<td></td>
<td>A4</td>
</tr>
<tr>
<td>Neuroectoderm-derived cells</td>
<td></td>
</tr>
<tr>
<td>Astrocytoma</td>
<td>⬤⬤⬤⬤⬤</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>⬤⬤⬤⬤⬤</td>
</tr>
<tr>
<td>Retinoblastoma</td>
<td>⬤⬤⬤⬤⬤</td>
</tr>
<tr>
<td>Medulloblastoma</td>
<td>⬤⬤⬤⬤⬤</td>
</tr>
<tr>
<td>Melanoma</td>
<td>⬤⬤⬤⬤⬤</td>
</tr>
<tr>
<td>Sarcomas</td>
<td>⬤⬤⬤⬤⬤</td>
</tr>
<tr>
<td>Normal fibroblasts</td>
<td>○○○○○</td>
</tr>
<tr>
<td>Epithelial cells</td>
<td></td>
</tr>
<tr>
<td>Normal kidney epithelial cells</td>
<td>○○○○○</td>
</tr>
<tr>
<td>SV40-positive keratinocytes</td>
<td>○○○○○</td>
</tr>
<tr>
<td>Epithelial cancers</td>
<td>⬤⬤⬤⬤⬤</td>
</tr>
<tr>
<td>Hematopoietic cells</td>
<td></td>
</tr>
<tr>
<td>Leukemias/lymphomas</td>
<td>⬤⬤⬤⬤⬤</td>
</tr>
<tr>
<td>Epstein-Barr virus-positive B-cells</td>
<td>⬤⬤⬤⬤⬤</td>
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</table>

**Fig. 1.** Fluorogram of immunoprecipitates obtained with MAbs to cell surface antigens using extracts of [3H]glucosamine-labeled fibroblasts (lanes A and B), U251MG (lane C), SK-MG-7 (lane D), or SK-GS-1 astrocytoma cells (lanes E and F). Immunoprecipitates were separated on sodium dodecyl sulfate-polyacrylamide gels under reducing conditions. MAbs used for immunoprecipitation tests were: lane A, F19; lane C, A010; lane D, AJ8; lane E, G253; lane F, A4; and lane B, unrelated control MAb SV63 (10). Molecular weights of immunoprecipitated components are indicated on the right (molecular weight × 10^3).**

S5*. Normal kidney epithelial cells and a large proportion of cell lines derived from epithelial cancers as well as hematopoietic malignancies are negative for S5 expression. Only 3 of 36 epithelial cancer lines (choiociarcinoma GCC-SV and renal cancers SK-RC-1 and SK-RC-18) and one of 9 leukemia lines tested (null cell leukemia NALL-1) were strongly S5 expressors.

G253 Cell Surface Antigen. MAb G253 detects a glycoprotein of M, 95,000 (Fig. 1) that also shows an intermediate type distribution (Table 2). Normal cultured kidney epithelial cells were strongly G253+, skin fibroblasts were weakly reactive, and most cell lines derived from neuroectodermal tumors, epithelial cancers, and sarcomas were strongly G253+. Leukemias and lymphomas, Epstein-Barr virus-transformed B-cells, and other cell lines growing in suspension culture (retinoblastomas and neuroblastomas) were G253*.

Thy-1 and LK26 Expression on Astrocytoma Cell Lines. MAb K117 recognizes human Thy-1 antigen, a M, 25,000 cell surface glycoprotein expressed on normal and malignant cells of neuroectodermal and mesenchymal origin (9). Typing of the astrocytoma panel showed that all cell lines included in this study were strongly reactive with MAb K117. With regard to LK26, two astrocytoma lines (U373MG and SK-MG-9) were found to express this antigen at moderate levels and three additional lines (SK-MG-4, SK-MG-11, and T98) showed only weak reactivity. MAb LK26 was raised against a human choriocarcinoma cell line and has been shown to react with cultured epithelial cells (10) and with a small range of normal epithelial tissues.* More significantly, we show in this study that a proportion of glial cells in normal adult brain are LK26* (see below).

Coordinate Expression of GFAP and Surface Antigens on Glial Tumor Cells. The panel of 22 astrocytoma cell lines was typed for expression of the nine cell surface antigens included in this study and for expression of GFAP. Fig. 2 shows examples of MHA titration experiments with MAbs A4, A010, G253, F19, and AJ8 on four representative astrocytoma cell lines. A characteristic of these five surface antigens and of antigens S5 and F24 is that they are expressed on distinct subsets of the glial tumor panel (Table 3). A close correlation was observed between A4 and GFAP expression, with 21 of 22 cell lines showing

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* P. Garin Chesa and W. J. Rettig, manuscript in preparation.
Fig. 2. Titration of MAb reactivity with human astrocytoma cell lines by MHA rosetting assay. Serial 5-fold dilutions of MAbs (range: 1/1,000–1/3,125,000 of nu/nu sera or ascites fluid) were tested for rosette formation of indicator cells (erythrocytes coated with secondary antibody) with the target cells; at each dilution step, the proportion of target cells showing rosette formation was determined microscopically.

concordance for these 2 markers: 6 lines were GFAP+/A4+, 15 lines were GFAP+/A4−, and one line was GFAP−/A4−. A010 expression correlated with both GFAP and A4 expression: 7 of the 11 A010+ cell lines were A4+ (of these, 6 were also GFAP+), whereas none of the 11 A010− cell lines expressed either A4 or GFAP.

We have compared our typing results for AJ8 and A010 expression in the astrocytoma panel (Table 3) with the results described by Cairncross et al. (6) and good general agreement was observed. A direct comparison of the AJ8 and A010 typing reagents used in the two studies established that the reagents (nu/nu sera and ascites fluid) used in the present study contain approximately 10-fold higher concentration of specific MAbs than those used by Cairncross et al. (6); thus, it is not surprising that we now find weak or moderate reactivity with some cell lines previously typed as unreactive or weakly positive with the respective MAb. (We disagree, however, with the results reported by Cairncross et al. (6) for the SK-MG-9 cell line which we found to be consistently AJ8+/A010−, both by MHA assay and by radioimmunoprecipitation tests.) Furthermore, we have compared early and late passages of SK-MG and SK-GS astrocytoma lines for surface antigen expression and no differences were observed, suggesting considerable phenotypic stability of long-term cultured astrocytomas. In this and previous studies (3, 6, 8–10) we have evaluated the reciprocal titration end points determined in MHA assays with different cell lines as a measure
of quantitative differences in antigen expression. In agreement with this interpretation, we have found that in radioimmuno-precipitation assays MAb A010 precipitates the characteristic M, 110,000 glycoprotein from SK-GS-1, SK-MG-2, and U251MG cell extracts but not from extracts of T98, SK-MG-16, or SK-MG-1 cells; in contrast MAb AJ8 precipitates the M, 100,000 glycoprotein from SK-MG-7, SK-MG-22, and SK-MG-13 cells but not from SK-GS-1, SK-MG-2, U251MG, SK-MG-23, T98, or SK-MG-5 cells. Similarly, all other MAbS (except MAb A4; see above) were successfully used to precipitate the respective glycoproteins from several high-expressor cell lines (identified by MHA titration experiments) but failed to precipitate any detectable antigen from cell lines that are unreactive or weakly reactive by MHA assays.

Antigen Expression in Normal and Malignant Human Brain Tissues. In order to examine antigen expression in normal neural cells in vivo, MAbS were tested with frozen sections of human brain tissues by the avidin-biotin immunoperoxidase method. Tissue samples were taken from several regions of normal brain, including cerebral cortex, thalamus, basal ganglia, hypothalamus, medulla oblongata, and cerebellum. MAb A4 showed strong staining of gray matter in all areas tested, labeling both cell bodies with a cytoplasmic pattern and neuropil (Fig. 3, A and B); in addition, MAb A4 stained cell processes in white matter (Fig. 3, A and B). The strong reactivity of MAb A4 with neuronal cells made it difficult to determine whether glial cells also express A4 antigen. MAb A010 showed a more restricted staining pattern in normal brain. Neurons in several areas tested, including cerebellum, medulla oblongata, and globus pallidus showed A010 staining localized to neuronal cell bodies and cell processes (Fig. 3, D and E). In contrast to the uniform neuronal reactivity seen for A4, A010 reactivity was detected only in subpopulations of CNS neurons. (The A010 distribution in the CNS will be described in detail elsewhere.) No A010 reactivity was seen with glial cells.

MAbs G253 and LK26 showed reactivity with glial cells but not neurons, as judged by the characteristic morphology of the antigen-expressing cells (Fig. 3I) in all areas of the brain tested. However, distinct staining patterns distinguish these two antigenic systems. MAb G253 labeled a large proportion of glial cells and blood vessels whereas reactivity with MAb LK26 was restricted to a small subpopulation of glial cells; these LK26+ glial cells have not yet been further characterized. MAb AJ8 showed strong reactivity with glial cell bodies and cell processes (Fig. 3H), but did not seem to label any neurons. No staining was seen with MAbS S5, F19, and F24 in any of the normal brain tissues tested.

To determine whether any of the cell surface antigens detected on cultured astrocytoma cells but not on normal glial cells in vivo are expressed on astrocytoma cells in vivo, frozen tissues from four high-grade astrocytomas were tested by immunoperoxidase procedures with the respective MAbS. Three tumors were found to be A4+/A010- (Fig. 3, C and F) and did not express any of the other markers and the fourth tumor was LK26+ and negative for the other markers.

DISCUSSION

GFAP is the most extensively studied molecular marker of mature astrocytes; it is thought to be acquired during late stages of glial differentiation (15). We have now defined a human cell surface antigen, A4, which shows concordant expression with GFAP on a large panel of cultured glial tumors. The A4 antigen, initially described in the rat as a neuronal antigen that distinguishes neurons of the central nervous system (antigen positive) from those of the peripheral nervous system (antigen negative), is highly restricted to human astrocytoma cell lines. Furthermore, it is predominantly expressed on GFAP+ cell lines, permitting classification of cultured astrocytomas into GFAP+/A4- and GFAP+/A4+ subsets. Immunohistochemical tests with normal human brain were carried out to examine whether normal astrocytes also express A4. However, neurons of the central nervous system uniformly express A4 and the strong neuronal staining made it difficult to determine whether glial...
Fig. 3. Distribution of cell surface antigens of cultured human astrocytomas in normal adult brain and astrocytoma tissues. Immunoperoxidase staining of frozen sections, hematoxylin counterstain. A, globus pallidus tested with MAb A4, low-power magnification to show strong uniform reactivity in areas of gray matter (solid arrowheads) and discrete filamentous staining in white matter (open arrowhead); B, caudate nucleus tested with MAb A4 (arrowheads as in A); C, high-grade astrocytoma tested with MAb A4, strong membrane staining; D, medulla oblongata tested with MAb A010, low-power magnification to show neuronal staining in the principal nucleus of the inferior olivary complex (gyrated pattern); no staining of surrounding neuropil; E, globus pallidus tested with MAb A010, higher-power magnification to show A010 staining of individual neuronal cell bodies and processes (arrowheads); F, high-grade astrocytoma tested with MAb A010, strong membrane staining; G and H, cerebellum tested with MAb AJ8, staining of a small proportion of cells in the granular layer (G) and of radial fibers in the molecular layer (H); I, cerebral cortex tested with MAb G253, reactivity with glial cell. Original magnifications: A, B, and D, x 40; C and E–H, x 200; I, x 400.

cells in general or a proportion of glial cells also express this antigen. Therefore, it is significant that we have demonstrated A4 expression in a proportion of astrocytoma tissues, indicating that the A4+ phenotype of cultured astrocytomas is not merely a tissue culture artifact. We have previously described a M, 145,000 surface antigen of human astrocytomas, detected by MAb AJ225, which also shows a highly restricted distribution (6). Our serological findings clearly distinguish this antigen from A4. Other astrocytoma antigens, described by us and others (6, 16, 17) can also be distinguished from A4 by their broader representation on neuroectodermal cells. Similarly, F19, F24, S5, and G253 appear to be distinct from the previ-
ously described antigens. AJ8 shares many characteristics with common acute lymphoblastic leukemia antigen (18), and studies in our laboratory indicate that the two antigens are identical.7

The biochemical nature of both rat and human A4 molecules has only partially been determined. However, the difference in subcellular localization between GFAP, a cytoskeletal component, and A4, a cell surface component, clearly distinguishes these two markers. Thus, GFAP and A4 seem to be coordinately expressed as part of a glial differentiation program. Based on the distribution of GFAP and A4 alone, two major subsets are defined on the astrocytoma cell panel, one being GFAP+/A4+ and one being GFAP−/A4−; by including additional surface antigenic markers, such as A010, AJ8, and S5, several intermediate phenotypes can be identified. Among these cell surface antigens, A4/A010 and AJ8/S5 tend to be expressed on reciprocal subsets of the astrocytoma cell panel. Cell surface antigenic systems that are expressed only on subsets of certain types of malignancies have been described by several investigators (for a review, see Ref. 19). Relatively few tumors have been shown to express different antigenic systems in coordinate concordant or reciprocal patterns (2, 3, 6, 10), and these may parallel antigenic phenotypes of normal cells at distinct stages of cellular differentiation. However, malignant transformation of subsets of glial cells and preservation of their characteristic antigenic patterns is only one possible mechanism to explain the antigenic diversity observed in the present study. With regard to the S5, F19, and F24 antigens which are expressed on distinct subsets of cultured astrocytomas but not detected on glial cells in vivo, it will be important to determine whether their expression is related to the increased proliferative activity or the transformed state of the tumor cells. Alternatively, S5, F19, and F24 expression on astrocytoma cell lines may be induced by specific tissue culture conditions. If this is true, an additional explanation is required for the clear distinction between antigen-positive and antigen-negative lines. In the case of the A010 antigen, which is also expressed on a number of cultured astrocytomas but not by normal glial cells in vivo, we have already shown in this study that antigen expression is not limited to cultured astrocytomas but is also found in a proportion of astrocytomas in vivo. Similarly, A4 is expressed both on cultured astrocytomas and in astrocytoma tissues and possibly also on normal glial cells (20). In order to determine the contributions of differentiation- and transformation-related mechanisms in generating specific tumor phenotypes it will be necessary to compare the patterns of antigen expression seen in normal fetal and adult brain with the antigenic phenotypes of a larger series of astrocytoma tissues. This typing may lead to a classification of glial tumors not only with respect to their cellular origin but also to their biological properties.

ACKNOWLEDGMENTS

We are grateful to Drs. J. Ransohoff and R. Carras for providing tumor specimens. We acknowledge the expert technical assistance of S. Walker and G. Lark and excellent secretarial help provided by J. Rios.

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Differential Expression of Cell Surface Antigens and Glial Fibrillary Acidic Protein in Human Astrocytoma Subsets


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