Correlation of Chondroitin Sulfate Proteoglycan Expression on Proliferating Brain Capillary Endothelial Cells with the Malignant Phenotype of Astroglial Cells

Martin Schrappe, F. George Klier, Robert C. Spiro, Thomas A. Waltz, Ralph A. Reisfeld, and Candeece L. Gladson

Research Institute of Scripps Clinic [M. S., F. G. K., R. C. S., T. A. W., R. A. R.], La Jolla, California 92037, and University of California, San Diego Medical Center

ABSTRACT

Human glioblastomas (five of five), the most malignant astroglial-derived tumors, specifically express a chondroitin sulfate proteoglycan that is recognized by monoclonal antibody 9.2.27 and localized to the glioneuronal cell surface, proliferating endothelial cells, and the perivascular extracellular matrix within the tumor bed. In contrast, the expression of this proteoglycan in normal adult neocortex and white matter is limited to the smooth muscle of small arteries, while normal glia, endothelial cells, and endothelial cell basement membranes are nonreactive. Moreover, two anaplastic astrocytomas, representing medium-grade astroglial-derived tumors, fail to react with monoclonal antibody 9.2.27. In culture, glioblastoma and capillary brain endothelial cells specifically synthesize a 250-kDa core protein and a high-molecular-mass chondroitin sulfate proteoglycan, recognized by monoclonal antibody 9.2.27. These data suggest a correlation between the expression of this chondroitin sulfate proteoglycan on proliferating brain capillary endothelial cells and the malignant phenotype of astroglial cells. The prominent perivascular localization of chondroitin sulfate proteoglycan makes it a marker for both proliferating brain capillary endothelial cells and the most malignant transformed astroglial cells, thus providing an ideal target for the immunotherapy of glioblastoma.

INTRODUCTION

PGs are involved in a diverse array of biological processes including cell adhesion and the control of cell proliferation (1). These molecules contain a core protein of variable size and a variable number of covalently linked sulfated glycosaminoglycan chains, i.e., chondroitin sulfate, dermatan sulfate, heparan sulfate, or keratan sulfate. PGs have been identified in the ECM or BM, the cell membrane, and as intracellular granules (1, 2). The ECM, or interstitial tissue, contains widely distributed CSPGs (3) of small molecular mass, while those of larger molecular mass are localized primarily to cartilage (4) and to the developing or mature brain of various species (5-7).

In situ, the human adult brain contains chondroitin sulfate proteoglycan in the normal grey and white matter neuropil, as well as in the tumor parenchyma of low-grade astroglial-derived tumors, such as astrocytoma (8, 9). In contrast, such high-grade astroglial-derived tumors as GM contain little or no tumor cell-associated chondroitin sulfate, but characteristically, the latter is detected in the capillary endothelial proliferations of such tumors (8-11). The core protein for this CSPG, which localizes to proliferating endothelial cells in glioblastoma tumors, has not previously been identified.

We and others described a CSPG defined by mAb 9.2.27 that is expressed on human malignant melanoma (12-14). We decided to investigate the reactivity of normal brain and astroglial-derived tumors with mAb 9.2.27 for the following two reasons. (a) This molecule is expressed on neuroectoderm-derived tissues, and (b) a previous report identified a similar sized CSPG with a different antibody on glioblastoma cells in culture (15).

We report here that the CSPG recognized by mAb 9.2.27 is a marker of both proliferating brain capillary endothelial cells and the most malignant transformed astroglial cells. This CSPG is localized to the extracellular matrix, and its core protein is synthesized by proliferating human capillary brain endothelial cells.

MATERIALS AND METHODS

Cell Lines. Four glioblastoma cell lines, A172, U87MG, U373MG, and T98G, were obtained from the American Type Culture Collection, Rockville, MD. Two additional glioblastoma cell lines, D247MG and U251MG, and the medulloblastoma cell line, DAOY, were kindly provided by Dr. D. Bigner, Duke University, Durham, NC. Another glioblastoma cell line, A1207, was a gift from Dr. S. Aaronson, NIH, Bethesda, MD. The human melanoma cell line, M21, is a subclone which was propagated in this laboratory from the UCLA-SO-M21 cell line originally provided by Dr. D. L. Morton, University of California, Los Angeles. The cells were grown in either Dulbecco’s modified Eagle’s medium (A172, T98G, D247MG, U251MG, and A1207) or Eagle’s minimal essential medium (U87MG, U373MG, and DAOY) and were supplemented with 10% fetal calf serum (Whittaker Bioproducts), 2 mM glutamine, and 50 μg/ml of gentamycin. All cell lines were routinely screened for the presence of Mycoplasma contamination.

Karyotypic analysis of glioblastoma cell lines A172 and U251MG at the Chromosome Laboratory of the University of California, San Diego, revealed the same markers as those initially described (16-18). Brain capillary endothelial cells were a kind gift from Dr. Jay Nelson, Research Institute of Scripps Clinic, and were grown as described (19).

Tissues. Immunoperoxidase staining of tumor specimens was performed on freshly frozen biopsy material obtained from either Scripps Clinic or the University of California, San Diego. Routine histopathological investigations were performed on formalin-fixed and paraffin-embedded samples of these same tumor tissues. The normal brain specimens were obtained from autopsy or surgical biopsy at the margin of a glialoma tumor resection.

Antibodies. mAb 9.2.27 (IgG2a) was produced in this laboratory, as described previously (20). Affinity-purified mAb 9.2.27 was produced by BioTechnetics, San Diego, CA. Supernatants of P3-X63-Ag8 myeloma cells were used as negative control, and mAb W6/32 directed to a framework structure of HLA Class I antigens served as a positive control. Reagents used for tissue staining included mAb to human fibronectin (antifibronectin; Sigma, St. Louis, MO), mAb to α-smooth muscle actin (ENZO, New York, NY), rabbit antibody to GFAP (Sigma, St. Louis, MO), and mAb to von Willebrand factor that was kindly provided by Dr. Zaverio Ruggeri (Scripps Clinic).

Received 3/14/91; accepted 7/2/91.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported in part by the Preus Foundation for Brain Tumor Research and by NIH Grant CA42508. This is the Research Institute of Scripps Clinic Manuscript 64581MM.

2 Recipient of a fellowship of the Deutsche Forschungsgemeinschaft, Federal Republic of Germany.

3 To whom requests for reprints should be addressed, at Research Institute of Scripps Clinic, Dept. of Immunology, IMM13, 10666 N. Torrey Pines Road, La Jolla, CA 92037.

4 Supported by PHS SK12AG00353.

5 The abbreviations used are: PG, proteoglycan; mAb, monoclonal antibody; CSPG, chondroitin sulfate proteoglycan; GFAP, glial fibrillar acidic protein; GM, glioblastoma; BM, basement membrane; ECM, extracellular matrix; PBS, phosphate-buffered saline, pH 7.4; FITC, fluorescein isothiocyanate; FACS, fluorescence-activated cell sorter; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; IgG, immunoglobulin G; BSA, bovine serum albumin; ABC, avidin-biotin complex; ELISA, enzyme-linked immunosorbent assay; MIF, mean intensity of fluorescence.

Downloaded from cancerres.aacrjournals.org on April 19, 2017. © 1991 American Association for Cancer Research.
Immunoassay. Monoclonal antibody 9.2.27 was adsorbed for 1 h at 4°C to Protein A-Sepharose beads (Sigma). Detergent cell extracts (0.5 ml), equivalent to approximately 0.2 to 5.0 x 10^6 cells, were incubated overnight at 4°C with 200 

RESULTS

Expression of the Proteoglycan Recognized by mAb 9.2.27 on Glioblastoma Cells. Five glioblastoma tumors, two anaplastic astrocytomas, one medulloblastoma, one meningioma, and one primary central nervous system lymphoma were analyzed with mAb 9.2.27 by the indirect immunoperoxidase technique. In 5 of 5 GM tumors, intense reactivity of proliferative endothelial cells and perivascular ECM was identified, as well as focal weak glioma cell staining (Fig. 1A). Normal cerebral grey and white matter (4 of 4) failed to react with mAb 9.2.27, including the endothelial cells and capillary basement membranes (Fig. 1C). However, mAb 9.2.27 shows some reactivity with smooth muscle cells in small arteries. Medium-grade anaplastic astrocytoma (0 of 2) also failed to react with mAb 9.2.27, as did one primary central nervous system lymphoma and one meningioma. The medulloblastoma showed endothelial cell and perivascular ECM staining with mAb 9.2.27; however, tumor cell reactivity was not clearly detected (data not shown). All five GM tumors and the two anaplastic astrocytoma reacted positively with rabbit antibody to human gial fibrillary acidic protein (data not shown), a marker of the intermediate filament specific for astroglial-derived cells (21). A mAb directed against von Willebrand factor, a marker of endothelial cells, and an antifibronectin mAb reacted positively with endothelial cells. The anti-fibronectin mAb also reacted positively with the perivascular ECM (data not shown), as was previously described (21, 30). In addition, a mAb directed against α-smooth muscle actin detected only rare pericytes in the endothelial cell proliferations of the GM tumors (Fig. 1B), while reacting strongly with many pericytes in the capillaries of normal white matter (Fig. 1D). In this regard, α-smooth muscle actin has been reported to be a marker of pericytes and smooth muscle (31). In view of these reaction patterns, mAb 9.2.27 directed against CSPG appears to be a marker of capillary brain endothelial cell proliferation, as well as a potential marker of the most malignant astroglial-derived tumors.

To achieve a more detailed analysis of the localization of this CSPG, immunoelectron microscopy with mAb 9.2.27 (Fig. 2) was performed. The glioblastoma tumor tissues showed staining of glioma tumor cells, endothelial cells, endothelial basement membranes, and perivascular ECM. Fig. 2a demonstrates that mAb 9.2.27 is strongly reactive with structures on the outer surface of the capillary basement membrane and in the subendothelial space of small capillaries in this tumor. This finding suggests reactivity of 9.2.27 with components of the ECM. In another sample of a much smaller capillary (Fig. 2c), strong reactivity was observed on the capillary wall. Due to the size of this capillary, the exact nature of the capillary wall could not be determined. In this case, there were also reactivity with structures in the periendothelial space and strong binding to the cell surface of an adjacent glioma cell (Fig. 2, c and e). In contrast, mAb 9.2.27 failed to react with endothelial cells, endothelial cell basement membrane, or structures in the periendothelial space of capillaries found in normal brain (Fig. 2b). Compared with this control tissue, there is distinct reactivity of mAb 9.2.27 with the surface of tumor cells; however, this
Fig. 1. Specific expression of the 9.2.27 proteoglycan by glioblastoma tumors. Indirect immunoperoxidase staining of GM tumor and normal adult cerebral white matter with mAb 9.2.27 (10 μg/ml) demonstrates positive reactivity of endothelial and tumor cells in the GM tumor (A), while the normal brain glial and endothelial cells are nonreactive (C). Serial sections of GM tumor and normal adult brain reacted with anti-α-smooth muscle actin antibody (commercial dilution) show no detectable pericyte in the GM tumor tissue (B), but pericytes are identified in the capillaries of normal adult cerebral white matter (D). Arrowheads mark endothelial cells or vessels, and arrows mark GM tumor cells.
staining is in some areas weaker than that observed in the perivascular space. It appears that the staining of cells adherent to capillaries is increased on that part of the cell surface which is closest to the basement membrane. A control antibody directed against GFAP, which is expressed intracellularly in normal and transformed glial cells (21), was reactive with endothelial cells and the basement membrane, but showed strong reactivity with glial filaments in normal glial and tumor cells (Fig. 2, d, f, and g).

**Differential Reactivity of Human Glioma Cell Lines with mAb 9.2.27.** GM cell lines A172, A1207, D247MG, and U87MG and the medulloblastoma cell line DAOY reacted strongly by ELISA with mAb 9.2.27 (20, 27). However, three GM cell lines, U251MG, U373MG, and T98G, failed to react with this antibody. An identical pattern of reactivity was found, when the same panel of cell lines was examined with mAb 9.2.27 by indirect immunofluorescence and FACS analysis (Table 1; Fig. 3). Thus, mAb 9.2.27 clearly identified positive cell populations, with a rather uniform antigen profile, on 4 of 7 GM cell lines, i.e., A172, A1207, D247MG, and U87MG, and on the medulloblastoma cell line, DAOY. Among these GM cell lines, A172 and A1207 revealed a greater increase in fluorescence signal after reaction with mAb 9.2.27 than did D247MG, DAOY, and U87MG. Under microscopic examination, the immunofluorescence staining with mAb 9.2.27 showed the typical punctuated and pericellular surface staining characteristic for this CSPG (14). Three of the GM cell lines, U251MG, U373MG, and T98G, were not reactive with this antibody under these conditions, but could be stained with control mAb W6/32 reactive.

![Image of cell lines and staining patterns](image-url)

**Table 1 FACS analysis of human glioma cell lines**

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>A172</th>
<th>A1207</th>
<th>D247MG</th>
<th>DAOY</th>
<th>U87MG</th>
<th>T98G</th>
</tr>
</thead>
<tbody>
<tr>
<td>mAb 9.2.27</td>
<td>24</td>
<td>13</td>
<td>10</td>
<td>1</td>
<td>8</td>
<td>1</td>
</tr>
</tbody>
</table>

*Reactivity with mAb 9.2.27 (at 10 μg/ml) is defined by the increase in the MIF. Thus, MIF=1 indicates the lack of increase in fluorescence signal when compared with the signal of the negative control of each cell line (secondary antibody only). The data represent the average of several experiments performed for each cell line.*
CHONDROITIN SULFATE PROTEOGLYCAN EXPRESSED BY GLIOBLASTOMA

Fig. 3. Expression of the 9.2.27 proteoglycan on the surface of the glioblastoma cells. Reactivity profiles of three GM (A172, U87MG, and T98G) cell lines with mAb 9.2.27 as determined by FACS analysis. A, A172, MIF = 24; B, U87MG, MIF = 8; C, T98G, MIF = 1 (see Table 1). -----, mAb 9.2.27; -----, control with secondary antibody only.

with the framework of Class I major histocompatibility complex antigens (data not shown).

In order to determine the number of binding sites per cell and to assess the binding affinity of mAb 9.2.27, three of the GM cell lines (A172, A1207, and U87MG) and the medulloblastoma cell line (DAOY) that reacted positively with this mAb were used for saturation binding studies with mAb 9.2.27 labeled with 125I (Table 2). Scatchard plots derived from the data of these binding studies indicated a high affinity of mAb 9.2.27 with a dissociation constant (Kd) of 0.12 nM (Fig. 4).

The GM cell line, A172, which previously showed the strongest reactivity with mAb 9.2.27 by FACS analysis, also expressed the highest number of antigenic sites, i.e., 400,000 sites/cell. Of the more weakly staining cell lines, the medulloblastoma cell line, DAOY, expressed approximately 100,000 sites/cell, while GM cell lines, A1207 and U87MG, revealed 140,000 and 40,000 antigen sites, respectively. In comparison, M21 melanoma cells, which were previously shown to react strongly with mAb 9.2.27, express approximately 600,000 antigen sites/cell, and the affinity of this antibody for M21 cells was essentially the same as that determined for glioma cells, i.e., Kd = 0.15 nM (32).

Biosynthesis of the 9.2.27 Proteoglycan by Glioblastoma Cells. The 9.2.27 antigen complex of melanoma cells has previously been shown to consist of a 240-kDa precursor that is converted to a 250-kDa glycoprotein and a high-molecular-mass chondroitin sulfate proteoglycan, both of which are expressed on the cell surface (12, 25). To determine the biosynthesis pattern of the 9.2.27 antigen on human glioma cells, five GM cell lines and one medulloblastoma cell line were biosynthetically labeled with [35S]methionine, and detergent lysates were immunoprecipitated with mAb 9.2.27 (Fig. 5). The results of several analyses of these immunoprecipitates on SDS-PAGE demonstrated the presence of a high-molecular-mass proteoglycan and specific 240- and 250-kDa forms of the proteoglycan core protein in three GM cell lines (A172, A1207, and U87MG), while the medulloblastoma cell line, DAOY, synthesized only the 250-kDa form of the core protein. Since approximately equal amounts of 35S-labeled lysates were used in these immunoprecipitations, it appears that A172 and A1207 contain more 9.2.27 reactive antigen than DAOY and U87MG. Among the glioma cell lines tested, U251MG, U373MG, and T98G were not reactive with mAb 9.2.27 (data not shown). These results are, therefore, completely consistent with our findings from the ELISA and FACS analysis, which indicated that these three cell lines did not react with mAb 9.2.27.

To determine the composition of the glycosaminoglycan chains, detergent lysates of 9.2.27-positive glioma cell lines were digested with chondroitinase ABC, immunoprecipitated with mAb 9.2.27, and analyzed by SDS-PAGE (Fig. 5). The lysates of the three glioblastoma cell lines A172, A1207, and U87MG showed a significant decrease in the high-molecular-mass fraction after chondroitinase ABC treatment with a concomitant increase of the 250-kDa form of the core protein, indicating the chondroitin sulfate nature of the GM proteoglycan.

Table 2 Antibody dissociation constants and binding sites of several glioma cell lines

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Kd (nM)</th>
<th>Sites (x10^6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A172</td>
<td>0.14</td>
<td>40</td>
</tr>
<tr>
<td>A1207</td>
<td>0.17</td>
<td>14</td>
</tr>
<tr>
<td>U87MG</td>
<td>0.22</td>
<td>4</td>
</tr>
<tr>
<td>DAOY</td>
<td>0.1</td>
<td>10</td>
</tr>
</tbody>
</table>

* Binding studies with 125I-labeled mAb 9.2.27 to determine dissociation constant Kd and number of antigen sites, as derived from Scatchard plot analysis.
can. The medulloblastoma cell line, DAOY, was not sensitive to enzyme treatment as expected from the absence of the high-molecular-mass form.

**Biosynthesis of the 9.2.27 Proteoglycan by Brain Capillary Endothelial Cells.** The results from immunohistochemistry and immunolectron microscopy suggest that proliferating capillary endothelial cells in GM tumors express antigen reactive with mAb 9.2.27. Therefore, brain capillary endothelial cells were also investigated by immunoprecipitation with mAb 9.2.27. The results of these experiments indicate that, prior to chondroitinase ABC treatment, brain capillary endothelial cells synthesize a fraction of high molecular mass recognized by mAb 9.2.27 (Fig. 6, Lane 4) as do A172 GM cells (Lane 1). After chondroitinase ABC treatment, a component of approximately 250 kDa is seen in lysates of brain capillary endothelial and A1207 GM cells (Lanes 5 and 2, respectively). The molecular mass of 250 kDa corresponds with that of the core protein previously found expressed by melanoma cells (12, 14, 20). The IgG control antibody fails to immunoprecipitate a component of 250 kDa or a high-molecular-mass fraction for either cell line (Lanes 3 and 6).

**DISCUSSION**

We have identified the core protein for a CSPG expressed on proliferating brain capillary endothelial cells, as well as in the perivascular ECM, and on the surface of the most malignant astroglial-derived tumor cells of GM tumors. Other investigators have reported this CSPG to be absent from normal adult neocortex, white matter, and cerebellum (13), and we confirmed this by immunohistochemical analyses, which indicated the lack of mAb 9.2.27 reactivity with glia, endothelial cells, neurons, the neuropil, or the perivascular ECM and endothelial cell basement membrane of adult neocortex and white matter. These findings are further substantiated by results obtained from immunoelectron microscopy, which failed to show mAb 9.2.27 reactivity with nontumorous cerebral white matter adjacent to a GM tumor. The absence of mAb 9.2.27 reactivity with two anaplastic astrocytomas, which lacked endothelial cell proliferation, suggests that expression of the CSPG detected by this antibody may correlate with tumor grade.

Other investigators have reported a large CSPG with a similar sized core protein in the developing rat neuropil, which becomes intracytoplasmic in mature rat cerebellar neurons and in astrocytes (5, 7). We cannot completely rule out an intracytoplasmic CSPG reactivity with mAb 9.2.27 in normal adult cerebral or cerebellar neurons, since immunoelectron microscopy, more sensitive than immunohistochemistry, was performed only on a very small sample of white matter that does not contain neurons. Also, immunohistochemistry may fail to detect a small number of intracytoplasmic molecules in neurons and grey matter glia. Alternatively, in this case, the epitope recognized by 9.2.27 may be inaccessible to this antibody. In addition, the CSPG recognized by mAb 9.2.27 is thought to be different from that reported by Norling et al. (33) in nontransformed
glial and GM cells in culture. Thus, this previously described CSPG appears to be immunologically similar to bovine cartilage (33), while mAb 9.2.27 fails to recognize cartilage in situ (14). Also, the degree of chondroitin sulfate substitution is higher for the CSPG recognized by mAb 9.2.27 than that described by Norling et al. (33), resulting in a reduction of molecular mass after chondroitinase ABC treatment of 150 kDa versus only 40 kDa, respectively.

The fact that the same CSPG is detected on the endothelial cell and perivascular ECM of glioblastoma and medulloblastoma tumors and is absent from normal adult neocortex and white matter suggests that the CSPG recognized by mAb 9.2.27 may be a marker of proliferative brain capillary endothelial cells. The appearance of this CSPG in endothelial cell proliferations of glioblastoma coincides with the stimulation of angiogenesis, long noted historically (10, 11). In this regard, at least 70% of the proteoglycan associated with the subendothelial extracellular matrix is of the heparan sulfate type (34). Angiogenic molecules, such as basic fibroblast growth factor, are thought to be bound to heparan sulfate within the endothelial ECM and released upon perturbation of the basement membrane (35, 36). Within glioblastoma tumors, the stimulation of angiogenesis is likely due in part to basic fibroblast growth factor, which has been demonstrated in proliferating endothelial and glialoma cells (37, 38). However, a correlation between angiogenesis and expression of CSPG on endothelial cells has not been shown previously. Therefore, the identification of a CSPG recognized by mAb 9.2.27 in the ECM of proliferating but not quiescent brain capillary endothelial cells is indeed a novel finding.

Our finding of the CSPG recognized by mAb 9.2.27 in the capillary endothelial basement membrane and the perivascular ECM suggests that this antigen is likely derived from endothelial cell synthesis, especially since it is synthesized by these cells in vitro and is expressed on their surface and in their ECM in situ. Alternatively, fibroblasts or pericytes could be a source of this CSPG in the perivascular ECM; however, previous testing of a variety of tissues with mAb 9.2.27 did not reveal fibroblast expression (13), and in our experiments, only rare pericytes were identified in GM endothelial cell proliferations with a mAb to α-smooth muscle actin (Fig. 1) (31). This is consistent with the observation that only rare pericytes were identified by electron microscopy in the endothelial cell proliferations of glioblastoma tumors (39).

It is of interest that the CSPG recognized by mAb 9.2.27 and synthesized by malignant astroglial cells and proliferating brain capillary endothelial cells is expressed at two different locations, i.e., on the surface of glialoma cells and in the ECM of endothelial cells. In this regard, it is noteworthy that primary cultures of umbilical vein endothelial cells do not express CSPG recognized by mAb 9.2.27 when examined by indirect immunofluorescence.6 It is likely that, when CSPG is expressed in the extracellular matrix, it is either cleaved from the endothelial cell membrane or is secreted from the endothelial cell. In this regard, there is a precedent for cleavage of cell membrane proteoglycans in mammary tumor cells (2). Thus, spent media of GM cells expressing CSPG recognized by mAb 9.2.27 contain a truncated form of the 250-kDa core protein, as well as proteoglycan components that react specifically with this antibody, similar to what was previously observed in supernatants of M21 melanoma cells.7 However, the spent media of the capillary brain endothelial cells remain yet to be investigated with mAb 9.2.27.

We and others previously reported that the CSPG recognized by mAb 9.2.27 is present on the surface of melanoma cells (12–14, 20) and melanocytes (13); however, there is thus far no report on the reactivity of this antibody with endothelial cells in melanomas (13, 14). This may be because of a more prominent stimulation of angiogenesis in glioblastoma than in melanoma. In addition, relatively strong staining of melanoma tumors with mAb 9.2.27 was observed (12–14, 20), as compared with the weaker immunohistochemical staining of glioma cells in glioblastoma tumors (Fig. 1). This may be due to fewer antigenic sites for mAb 9.2.27 on glioblastoma tumor cells since, in culture, GM tumor cells contain from 40,000 to 400,000 binding sites for mAb 9.2.27 (Table 2) as compared with the >600,000 sites detected on melanoma cells (32).

In summary, the CSPG recognized by mAb 9.2.27 is expressed on proliferating brain capillary endothelial cells and is localized to the perivascular extracellular matrix. In addition, normal glial cells fail to express this molecule, which is in contrast to the expression of CSPG on melanocytes in melanoma tumors and on the most malignant transformed cells of both cell lineages. The prominent perivascular localization of this chondroitin sulfate proteoglycan makes it a marker for both proliferating brain capillary endothelial cells and the most malignant transformed astroglial cells, thus providing an ideal target for the immunotherapy of glioblastoma.

**REFERENCES**


Correlation of Chondroitin Sulfate Proteoglycan Expression on Proliferating Brain Capillary Endothelial Cells with the Malignant Phenotype of Astroglial Cells


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/51/18/4986