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Expression of oligodendrocyte progenitor cell antigens by gliomas: Implications for the histogenesis of brain tumors

YIGAL SHOSHAN*†‡, AKIKO NISHIYAMA*§, ANSI CHANG*, SVERRE MÖRK*, GENE H. BARNETT†, JOHN K. COWELL*†, BRUCE D. TRAPP*, AND SUSAN M. STAUGAITIS*†¶

*Department of Neurosciences, The Lerner Research Institute, and †Department of Neurosurgery, Cleveland Clinic Foundation, Cleveland, OH 44195

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ABSTRACT **The early events in neoplastic transformation can be understood only by comparison of the neoplastic cell with its nontransformed counterpart. The most common central nervous system gliomas traditionally are thought to arise from mature astrocytes and oligodendrocytes. We examined the possibility that gliomas arise from a population of glia that has properties of oligodendrocyte progenitors. These glial cells express the NG2 chondroitin sulfate proteoglycan and the α receptor of platelet-derived growth factor *in vivo*. We identified NG2 and the α receptor of platelet-derived growth factor expression in tissue from seven of seven oligodendrogliomas, three of three pilocytic astrocytomas, and one of five glioblastoma multiforme. These data provide evidence that glial tumors arise from glial progenitor cells. Molecules expressed by these progenitor cells should be considered as targets for novel therapeutics.**

In 1926, Bailey and Cushing classified glial neoplasms on the basis of the morphological similarities of the tumor cells to nonneoplastic cells (1). Although the classification and nomenclature have evolved over the years, this general principle is still applied. The most common gliomas, astrocytoma [including glioblastoma multiforme (GBM)] and oligodendroglioma (OLIGO), are defined as being composed of neoplastic astrocytes and oligodendrocytes, respectively (2, 3). The implication of such definitions is that these neoplasms originate from mature cell types. The alternative possibility, that gliomas arise from a dividing progenitor cell, has been raised on numerous occasions (4–12); however, the testing of this hypothesis has been limited by the lack of phenotypic markers that identify specific progenitor cell populations in tissue sections.

One progenitor that has attracted much interest is the O-2A progenitor (13). *In vitro* studies have shown that this cell is responsive to the platelet-derived growth factor (14–16) because of its expression of the α receptor (PDGFα-R) (17). O-2A progenitors also express the NG2 chondroitin sulfate proteoglycan (18–20). Both NG2 and PDGFα-R can be detected reliably in tissue sections (18). Although these antigens are expressed on a variety of nonneuroectodermal cells (21–23), glia that coexpress these antigens represent a unique cell population. These cells are abundant throughout the neuroaxis (18, 24, 25) and show evidence of DNA synthesis even in adulthood (24). NG2 is not expressed by mature oligodendrocytes, astrocytes, or microglia (18, 26). Some $NG2^+$ cells also express oligodendrocyte markers in a spatial and temporal pattern that closely precedes myelination, indicating that these cells are oligodendrocyte progenitors (24, 27); however, $NG2^+$ cells may have additional functions (20). $NG2^+$ glia recently have been demonstrated in adult human brain tissue sections (see *Results*). In pathological conditions such as multiple sclerosis, individual cells are stained more intensely by NG2 and PDGFα-R than cells in normal adult brain (A.C. and B.D.T., unpublished observation).

The response of different gliomas to therapy shows a correlation with cell lineage. For example, patients with anaplastic oligodendroglioma (AOLIGO) have a higher response rate to chemotherapy (28) than do patients with tumors thought to be derived from astrocytes. Most recently, this chemoresponsiveness has been correlated with specific genetic changes (29). These observations warrant renewed investigation into the cellular origin of various glial tumors by using these more recently characterized cell lineage markers. In the present study we have used immunohistochemistry and immunoblotting to detect NG2 and PDGFα-R in OLIGO and different types of astrocytoma.

MATERIALS AND METHODS

Antibodies. Antibodies from the following sources were used: monoclonal anti-human melanoma-associated chondroitin sulfate proteoglycan [mAb 9.2.27, 1:2,000; R. Reisfeld, Scripps Research Institute, La Jolla, CA, (30, 31)]; rabbit 553 anti-rat NG2 antibody (1:2,000; W. B. Stallcup, Burnham Institute, La Jolla, CA); rabbit anti-human PDGFα-R [R7, 1:2,000; C.-H. Heldin, Ludwig Institute, Uppsala, Sweden (32)]; rabbit anti-glial fibrillary acidic protein (GFAP, 1:5,000, Dako); monoclonal anti-GFAP (1:2,500, Dako); monoclonal anti-myelin basic protein (MBP, 1:2,000; Dako); and leukocyte common antigen (LCA, 1:40; Dako).

Tissue Processing. Brain tumor samples were obtained by biopsy from 16 patients. These studies were approved by the Institutional Review Board of the Cleveland Clinic Foundation, and the specimens were obtained after representative sampling for diagnostic purposes. Original slides were reviewed by two neuropathologists (S.M.S. and S.M.). The diagnoses were: four OLIGO, three AOLIGO, three pilocytic astrocytoma (PA), one fibrillary astrocytoma (A), and five GBM. The tissues collected were snap-frozen in liquid nitrogen and stored at −70°C. Portions of five of the tumors were fixed immediately in 4% paraformaldehyde at 4°C overnight and cryoprotected in 0.1 M phosphate buffer, pH 7.6, containing 20% glycerol at 4°C until used for sectioning (27).

Histochemical Studies. Unfixed, frozen samples were thawed in 4% paraformaldehyde overnight at 4°C and cryoprotected. Sections (30 μm thick) were obtained by using a

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Abbreviations: PDGFα-R, α receptor of platelet-derived growth factor; GBM, glioblastoma multiforme; OLIGO, oligodendroglioma, AOLIGO, anaplastic oligodendroglioma; GFAP, glial fibrillary acidic protein; MBP, myelin basic protein; LCA, leukocyte common antigen; PA, pilocytic astrocytoma; A, fibrillary astrocytoma.

‡Present address: Department of Neurosurgery, Hadassah University Hospital, Jerusalem, Israel 91120.

§Present address: Department of Physiology and Neurobiology, University of Connecticut, Storrs, CT 06269.

¶To whom reprint requests should be addressed at: Department of Neurosciences #NC30, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195. E-mail: staugas@ccf.org.

sliding microtome (Leica SM 2000R) and maintained in cryostorage solution (27). Sections adjacent to those used for immunostaining were dried onto slides at 50°C for 30 min, stained with hematoxylin/eosin, and compared with the sections used for diagnosis. Only those samples that contained tumor were included in the study. One AOLIGO (no. 10), one GBM (no. 4), and the A (no. 24) consisted of gray matter infiltrated by variable numbers of neoplastic cells. One specimen from a GBM (no. 57) showed low-grade histology only. The high-grade areas of the AOLIGO were focal in the paraffin sections and could not readily be identified in the specimens used in our studies.

Immunohistochemistry. Sections were rinsed in PBS and treated with 10% Triton X-100 and 3% H_2O_2 for 30 min at room temperature. Sections were stained by the avidin–biotin complex procedure as described previously (27). Tissue sections were incubated overnight at 4°C in primary antibody diluted in PBS containing 3% normal goat serum. Diluent without primary antibody served as a negative control. Sections were photographed by using an Axiophot microscope (Zeiss).

Five OLIGO were analyzed further by double-immunofluorescence labeling. The antibody combinations examined were: NG2 and PDGFα-R, GFAP, or LCA and PDGFα-R and GFAP. Sections were processed as described above, and the primary antibodies were applied to the sections simultaneously for 1–5 days. Sections were incubated with a mixture of biotinylated anti-mouse (1:500; Vector Laboratories) and fluorescein-conjugated anti-rabbit antibody (1:500; Jackson ImmunoResearch) for 2 hr at room temperature, followed by incubation in Texas red-conjugated avidin D (1:500; Vector Laboratories) for 1 hr at room temperature. For NG2 and LCA double-labeling, biotinylated anti-mouse IgG2a and fluorescein-conjugated anti-mouse IgG1 (Southern Biotechnology Associates) were used. Sections were rinsed, mounted in Vectashield (Vector Laboratories), and analyzed by confocal microscopy (Aristoplan; Leica).

Immunoprecipitations and Western Blots. Cultured cells and frozen tissues were used for biochemical studies. MG63 human osteosarcoma cells and B49 rat glioma cells express NG2, whereas U251MG human glioma cells do not express NG2 (19). To verify the presence of tumor tissue, frozen samples ($\approx$1 cm^3) were sectioned on a cryostat (Leica) along their peripheral surfaces and stained with hematoxylin/eosin. Extracts from the occipital cortex of an epileptic patient and normal gray and white matter from a multiple sclerosis patient were used as nontumor controls. Cells and tissues were lysed in 50 mM Tris·HCl, pH 8/150 mM NaCl/1% NP40/1 mM PMSF/10 μg/ml leupeptin/2 μg/ml aprotinin for 30 min at room temperature. To remove glycosaminoglycan chains from NG2, 0.1 unit/ml chondroitinase ABC (EC 4.2.2.4; ICN) was added during the extraction. For immunoprecipitation, chondroitinase-treated extracts were precipitated with mAb 9.2.27 by using protein A-Sepharose. Total extracts and immunoprecipitates were resolved on 4–12% polyacrylamide gradient gels under reducing conditions. Equal amounts of protein from tissue extracts, as determined by the Lowry method, were loaded in each well. After electrophoresis, proteins were transferred to PVDF Immobilon membranes (Millipore, MA) in a buffer containing 25 mM Tris·HCl, 192 mM glycine, and 10% methanol. The membranes were blocked for 2 hr at room temperature in 5% nonfat dry milk in PBS and then incubated with the rabbit 553 anti-rat NG2 antibody (1:2,000) or R7 anti-human PDGFα-R antibody (1:1,000) diluted in blocking solution at 4°C overnight. After four washes, the membranes were incubated with horseradish peroxidase-labeled goat anti-rabbit immunoglobulins (1:10,000; Bio-Rad). Labeled bands were visualized by using ECL detection reagents (Amersham).

RESULTS

Characterization of NG2 Antibodies. The mAb 9.2.27 recognizes the core protein of a proteoglycan originally characterized in malignant melanoma (30). The amino acid sequence of this proteoglycan has 84% identity with that of rat NG2 and is considered to be the human homologue of NG2 (33, 34). Because mAb 9.2.27 does not recognize denatured NG2, the 553 rabbit anti-rat NG2 antibody was used for Western blots. To demonstrate that these two antibodies recognize the same molecule, the 553 antibody was used to probe a Western blot containing immunoprecipitates from MG63 cells that had been formed with mAb 9.2.27. As shown in Fig. 1*a*, lane 5, mAb 9.2.27 precipitated a 300-kDa species that was detected by the 553 antibody and had the same electrophoretic mobility as the NG2 core protein in total extracts from MG63 (lane 4) and B49 cells (lanes 2 and 3). The U251MG cell line, which does not express NG2, was used as a negative control.

NG2 Proteoglycan and PDGFα-R Immunohistochemistry. $NG2^+$ cells are not detectable in normal human brain under the conditions that routinely demonstrate NG2 immunoreactivity in gliomas. Prolonged incubations of normal tissues with increased antibody concentrations reveals an abundant population of process-bearing cells in both gray and white matter (Fig. 1*b*). The cytoplasmic morphology of these cells is identical to that observed in rodent $NG2^+$ cells (18), and they are morphologically and immunohistochemically distinct from human astrocytes and microglia (A.C. and B.D.T., unpublished results; see also Figs. 2 and 3).

All OLIGO and PA demonstrated intense NG2 immunoreactivity on neoplastic cells (Table 1). This is best illustrated in sections representing the interface between neoplastic foci and normal-appearing tissue (Fig. 1*c*). The neoplastic cells of OLIGO are small and round by routine histology (Fig. 1*d*). Cells with the same morphology show predominantly cell-surface staining with antibodies to NG2 and PDGFα-R (Fig. 1 *e* and *f*). In sections in which the tumor mass was adjacent to infiltrated brain parenchyma, neoplastic cells showed a few short, thin processes. In tumor 10, which contained only infiltrated gray matter, scattered $NG2^+$ cells with short processes were observed. The morphology of these cells was distinct from resident $NG2^+$ cells in normal brain (Fig. 1*b*) and also from that observed in MS lesions and at the edge of high-grade neoplasms composed of $NG2^-$ cells (data not shown). In PA, intense membrane staining was seen in the microcystic areas of the tumor. More diffuse NG2 immunoreactivity was seen in the compact bipolar areas of the tumor. One GBM (no. 54) showed numerous $NG2^+$ tumor cells; the other GBM and the A showed no NG2 immunoreactivity. NG2 immunoreactivity was seen in capillaries of many of the tumors irrespective of diagnosis (Table 1). Capillary staining was seen only in regions containing tumor. Tumors with $NG2^+$ capillaries also contained areas with $NG2^-$ capillaries.

Five of seven OLIGO, three of three PA, and one of five GBM contained a large number of cells that stained with anti-human PDGFα-R antibody (Fig. 1*f*). The two OLIGO that did not show immunoreactivity on tissue sections showed clear, strong bands on a Western blot (see below). The lack of staining in these cases may be due to overfixation of the specimen or other technical reasons. The morphology of the PDGFα-R^+ tumor cells was similar to that of $NG2^+$ tumor cells (Fig. 1 *e* and *f*).

Double-immunofluorescence labeling by using antibodies against human NG2 and PDGFα-R was performed on tumors 2, 3, 30, 39, and 44. Confocal laser-scanning microscopy revealed extensive colocalization of these two antigens (Fig. 2 *a–c*).

GFAP, MBP, and LCA Immunostaining. GFAP reactivity was detected in normal glia and tumor cells of most of the OLIGO examined in this study. Some of the $GFAP^+$ cells in

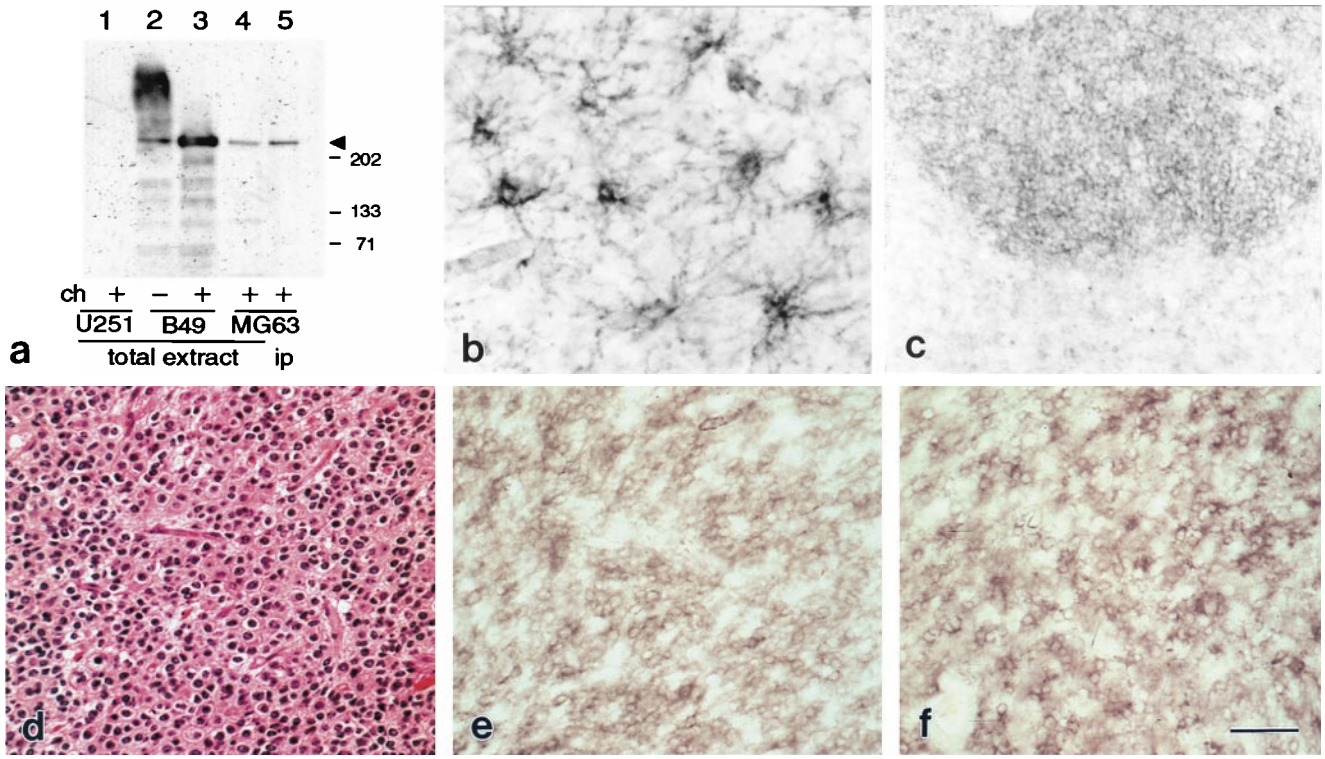

FIG. 1. (*a*) mAb 9.2.27 and rabbit 553 anti-rat NG2 antibodies recognize the same NG2 core protein. Extracts from human U251MG, MG63 and rat B49 cells lines before (−) and after (+) chondroitinase ABC (ch) treatment were subjected to Western blot by using the rabbit 553 anti-rat NG2 antibody. Lane 5 shows immunoprecipitates from MG63 cells that were formed with mAb 9.2.27. (*b*) mAb 9.2.27 recognizes complex process-bearing cells in human cerebral cortex resected for intractable epilepsy. (*c–f*) OLIGO express NG2 and PDGFα-R. At low magnification, NG2 immunostaining (*c*) demarcates a hypercellular neoplastic area. High magnification of hematoxylin/eosin-stained tissue (*d*) demonstrates densely packed round cells typical of OLIGO. The majority of neoplastic cells show a membrane-labeling pattern with NG2 (*e*) and PDGFα-R$^+$ (*f*) antibodies. Tumor 30. [Bar = 35 μm (*b*), 120 μm (*c*), and 50 μm (*d–f*).]

the OLIGO had long, ramified processes with large cell bodies, suggesting that they are reactive astrocytes (Fig. 3*a*). Some OLIGO contained GFAP$^+$ cells with one or two short processes and nuclei identical to those of neoplastic GFAP$^-$ cells (Fig. 3*b*). The morphology is similar to the glial fibrillary oligodendrocytes described previously in OLIGO (35). Double-labeling of five OLIGO with antibodies to GFAP and NG2 or GFAP and PDGFα-R failed to reveal colocalization in all tumors examined (Fig. 2 *d–f*).

Immunohistochemistry for MBP was performed in five OLIGO. Brain adjacent to tumor showed immunoreactivity in myelinated axons (Fig. 3*c*). Residual myelinated fibers within the tumor mass also were stained (Fig. 3*c*). MBP antibodies did not label neoplastic cells in any of the OLIGO examined.

Antibodies to LCA were used to identify bone marrow-derived cells including microglia in three OLIGO. Variable numbers of ramified and amoeboid microglia were present within the tumor tissues (Fig. 3*d*). Double-labeling by using antibodies to NG2 and LCA showed no colocalization of these antigens (Fig. 2 *g–i*), indicating that these antibodies recognize nonoverlapping populations of cells.

Western Blot Analysis. Immunoblot analysis of solubilized extracts from brain tumors was carried out to confirm the presence of PDGFα-R and NG2. The R7 anti-human PDGFα-R antibody recognized a major component of approximately 180 kDa in extracts from MG63 human osteosarcoma cells, the expected size for the mature PDGFα-R protein, and a faster migrating band at 140 kDa (Fig. 4 *Upper*), as described previously (19). No clear band was observed in the nonneoplastic human brain extracts (Fig. 4 *Upper*). Detectable levels of PDGFα-R were identified in all tumor samples. Intense bands were detected in four of seven OLIGO, two of two PA, one of five GBM, and one of one A (Table 1). There was no significant difference in the range of band intensities or the average intensity seen in the different tumors (Fig. 4 *Upper*).

Blots also were probed with rabbit anti-rat NG2 antibody. A 300-kDa NG2-immunoreactive band was detected in chondroitinase-treated extracts from MG63 cells and two of seven OLIGO, two of two PA, and three of five GBM (Fig. 4 *Lower*). These data support the interpretation that the immunoreactivity observed in the tissue sections represents the NG2 core glycoprotein. We also observed strong bands migrating at 60–100 kDa. It is possible that these bands represent degradation products of NG2 because of delays in freezing of the tissue at the time of surgery. The NG2 identified in Western blots of tissue extracts represents the sum of the NG2 produced by the tumor cells and by endothelial cells. Therefore, the amount of NG2 on the blots is not directly proportional to levels of tumor cell-derived NG2.

DISCUSSION

All OLIGO and PA examined in this study consisted of cells with surface antigens characteristic of oligodendrocyte progenitor cells. There are several possible interpretations of these results: these tumors arise from NG2$^+$/PDGFα-R$^+$ cells, they arise from a less mature progenitor cell that acquires NG2 and PDGFα-R immunoreactivity, or they arise from mature cells that acquire NG2 and PDGFα-R immunoreactivity as they dedifferentiate. We prefer the first interpretation for the following reasons. In the adult mammalian brain, NG2$^+$/PDGFα-R$^+$ progenitor cells are abundant, are located throughout the neuroaxis, and retain the ability to divide. Cell division is an important characteristic for tumorigenicity because it perpetuates alterations in growth-regulating genes. In experimental animals, for example, proliferating subventricu-

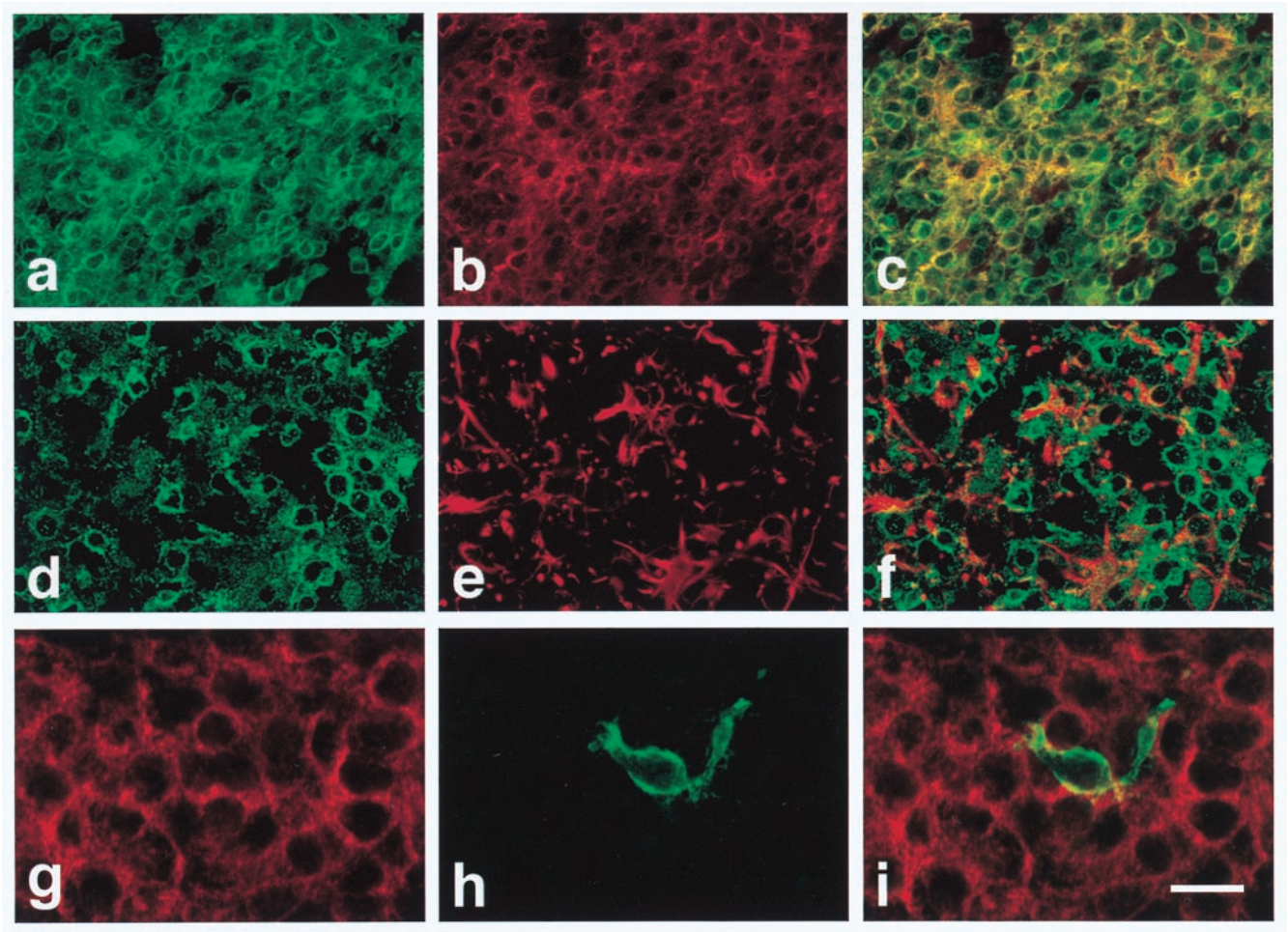

FIG. 2. OLIGO cells coexpress NG2 and PDGFα-R but not GFAP or LCA. (*a–c*) Sections from an OLIGO double-labeled with antibodies to PDGFα-R (*a*, green) and NG2 (*b*, red). Merged images demonstrate colocalization of NG2 and PDGFα-R (*c*, yellow). (*d–f*) Double-labeling with antibodies to PDGFα-R (*d*, green) and GFAP (*e*, red). No colocalization is observed in the merged image (*f*). (*g–i*) Double-labeling with antibodies to NG2 (*g*, red) and LCA (*h*, green). No colocalization is observed in the merged image (*i*). Tumor 3. [Bar = 40 μm (*a–c*), 30 μm (*d–f*), and 20 μm (*g–i*).]

lar zone cells are more susceptible to chemically and virally induced oncogenesis than less proliferative brain regions (8, 9). $NG2^+/PDGF\alpha\text{-}R^+$ cells, therefore, have a greater predisposition to oncogenesis than the postmitotic oligodendrocyte. Collectively, these observations support the hypothesis that $NG2^+/PDGF\alpha\text{-}R^+$ cells are the precursors of these gliomas. Whether or not these neoplasms arise from a progenitor to the $NG2^+/PDGF\alpha\text{-}R^+$ cell can be tested only when markers for such progenitors are discovered. Dedifferentation of oligodendrocytes to cells with progenitor phenotypes has not been described *in vitro* or *in vivo*; therefore, it is unlikely that OLIGO arise from mature oligodendrocytes that lose their myelin markers while regaining characteristics of their precursors. Our results confirm previous reports (36–38) that mature oligodendrocyte markers are not expressed by the neoplastic cells in OLIGO. Recently, mRNA encoding oligodendrocyte-specific proteins was reported in OLIGO (39), however our results and other studies (40) indicate that these myelin protein gene products are located within preexisting myelinating oligodendrocytes.

A provocative finding of this study was the detection of high levels of NG2 and PDGFα-R in PA. Although OLIGO and PA are viewed as distinct clinical and pathological entities, they share several interesting properties (3). Both are typically slowly growing tumors and have better prognoses than fibrillary astrocytomas. Both PA and low-grade OLIGO are cytologically benign but often histologically heterogeneous. PA characteristically have a biphasic histology composed of compact areas containing bipolar cells and microcystic areas composed of stellate cells. Some PA can even have areas histologically identical to OLIGO (3). Our results show that antibodies to NG2 and PDGFα-R labeled the round cells of OLIGO and both the stellate cells and bipolar cells of PA. These observations suggest that $NG2^+$ cells have the potential give rise to neoplasms with distinctive clinical and pathological phenotypes, perhaps as a result of different environmental influences within the brain parenchyma or different acquired genetic alterations. In support of environmental influences, PA are most commonly located in the cerebellum, optic nerve, and hypothalamus, whereas OLIGO usually are located in the cerebral hemispheres. PA also typically occur at an earlier age than OLIGO (3). Genetic changes are rarely described in PA, whereas loss of heterozygosity in chromosomes 1p and 19q are common in OLIGO (41–43). These genetic alterations may predispose OLIGO to histological progression, which is rare in PA.

NG2 immunoreactivity was observed in four of five GBM examined; however, in three of these cases, the staining was restricted to capillary endothelial cells. The expression of NG2 by endothelial cells most likely accounts for the NG2 seen in our Western blots of GBM. Strong staining of capillaries and weak staining of tumor cells with antibody to NG2 has been reported previously (31). In our series, one of five GBM showed strong immunoreactivity for both NG2 and PDGFα-R

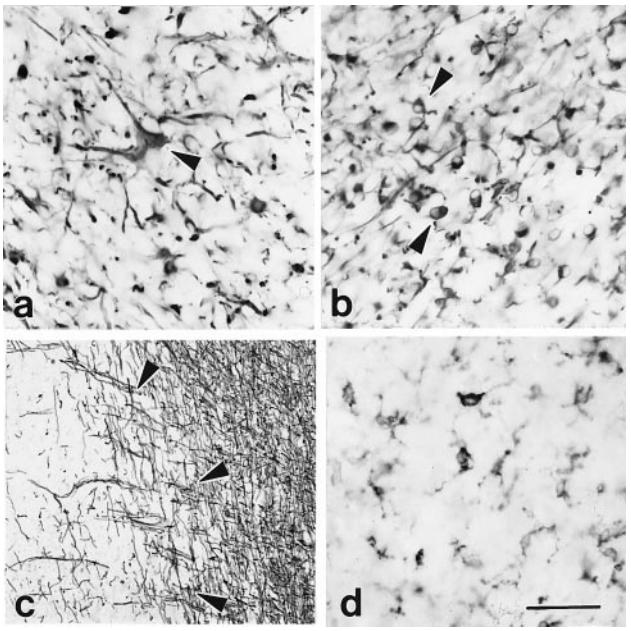

FIG. 3. OLIGO contain reactive astrocytes, neoplastic GFAP$^+$ cells, residual myelin, and activated microglia. GFAP$^+$ cells were present in all OLIGO (*a* and *b*). Many had the appearance of reactive astrocytes (*a*, arrowhead). Occasional cells had one or two short GFAP$^+$ processes (*b*, arrowheads) and may reflect neoplastic cells. (*c*) MBP antibodies stained myelin in normal brain adjacent to OLIGO (*Right*) and residual myelin within the tumor (*Left*), but not neoplastic cells. Arrowheads denote the border between tumor and adjacent normal brain. (*d*) LCA$^+$-activated microglia were seen frequently within the neoplasms. (*a* and *b*) Tumor 3. (*c* and *d*) Tumor 30. [Bar = 70 μm (*a* and *b*), 100 μm (*c*), and 60 μm (*d*).]

on the majority of tumor cells. This GBM was histologically indistinguishable from the others examined. High expression of PDGFα-R was detected on Western blots of two additional astrocytomas (one low grade and one GBM). Although we did not observe PDGFα-R immunoreactivity on tissue sections from these tumors, we believe this was a result of overfixation. High expression of PDGFα-R mRNA in a variety of glial neoplasms is well documented in the literature (12, 44–48), and the possibility that endogenous PDGFα-R$^+$ cells are the precursors to gliomas has been noted previously (12, 48). Detection of both NG2 and PDGFα-R on a subset of GBM, in addition to OLIGO and PA, supports the notion that multiple types of glial neoplasms arise from the NG2$^+$/PDGFα-R$^+$ progenitor cell. Gliomas that do not express NG2 and PDGFα-R may have down-regulated these markers because of acquired genetic changes or environmental influences. Alternatively, they may have arisen from a different progenitor.

At least three distinct glial progenitor cells may exist in the adult central nervous system. The NG2$^+$/PDGFα-R$^+$ cells have been studied extensively *in vitro*, where they have been referred to as O-2A progenitor cells because they can differentiate into oligodendrocytes in serum-free medium and type 2 astrocytes in serum-containing medium (13). To date, bipotentiality of NG2$^+$/PDGFα-R$^+$ cells has not been demonstrated in the normal brain *in vivo*; however, as discussed above, neoplastic NG2$^+$/PDGFα-R$^+$ cells may have the potential to display either OLIGO or PA characteristics. A number of gliomas show a mixed OLIGO/fibrillary astrocytoma phenotype (3). Loss of heterozygosity of chromosomes 1p and 19q occurs in both the OLIGO and the astrocytoma areas of the tumors, indicating that some OLIGO and fibrillary astrocytomas have a common origin (43). *In vitro* studies have characterized another astrocyte, termed type 1, which does not

Table 1. NG2 and PDGFα-R immunostain and Western blot analysis of 16 brain tumors

		NG2 immunostain		PDGFα-R	
Tumor no.	Diagnosis	TC*	BV†	TC*	Western blot‡
44	OLIGO	+++	+	−	++
2	OLIGO	+++	−	+++	+
39	OLIGO	+++	+	+++	+
30	OLIGO	+++	+	+++	++
3	AOLIGO	+++	+	+++	+
48	AOLIGO	+++	−	++	++
10§	AOLIGO	+	+	−	++
40	PA	+	−	++	++
58	PA	+++	−	+++	ND
17	PA	++	+	++	++
47	GBM	−	+	−	+
54	GBM	++	+	++	+
45	GBM	−	+	−	++
57	GBM	−	+	−	+
4	GBM	−	−	−	+
24	A	−	+	−	++

ND, not done.
*NG2 or PDGFα-R immunoreactivity in tumor cells (TC). −, No detectable immunoreactive TC; +, <5% immunoreactive TC; ++, 5–50% immunoreactive TC; +++, >50% immunoreactive TC.
†NG2 immunoreactivity in blood vessels. Immunoreactive vessels scored as present (+) or absent (−).
‡PDGFα-R band (180 kD). +, detectable band present; ++, intense band present.
§This specimen consisted of mildly hypercellular gray matter infiltrated by immunoreactive TC.

arise from the O-2A progenitor cell (13). Studies on the type 1 astrocyte progenitor have lagged, and phenotypic markers for this progenitor have not been reported. It is possible that this cell also is present in the adult central nervous system and is the source of tumors with more astrocytic characteristics. Other possible progenitors in adult brain include multipotent glial stem cells and multipotent neuroepithelial stem cells (49, 50). Such cells have the capacity to differentiate when injected into neonatal rodent brain (49, 51). Further characterization of these progenitor cells *in vitro* and *in vivo* is essential for testing their potential role in glial tumorigenesis. In addition, application of progenitor cell culture techniques to primary glioma specimens may provide better *in vitro* models for glioma research (for review see ref. 11).

Studying glial tumors from the viewpoint of progenitor cell biology will help identify markers that may facilitate diagnosis,

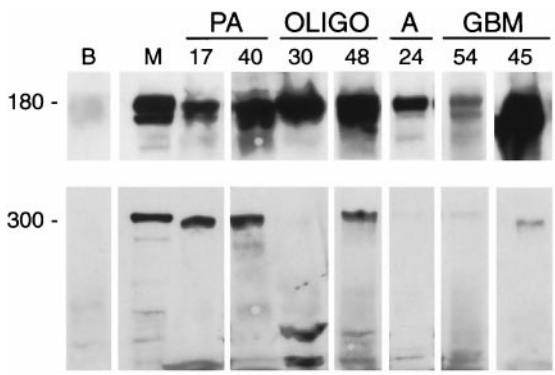

FIG. 4. Western blots detect PDGFα-R and NG2 in gliomas. (*Upper*) Although barely visible in normal brain tissue, PDGFα-R was detectable in extracts from all tumors examined. (*Lower*) A 300-kDa band corresponding to the NG2 core glycoprotein was present in half of the tumors. Lower-molecular-weight bands also were seen (see text). B, normal brain; M, human MG63 osteosarcoma cells.

predict prognosis, and direct therapy. This is particularly important for OLIGO because there is often disagreement among pathologists regarding the criteria for diagnosis of this glioma (52). The most common differential diagnosis is OLIGO vs. fibrillary astrocytoma. The majority of patients with anaplastic OLIGO show a response to chemotherapy (28, 29), but this option might not be offered to a patient given a diagnosis of astrocytoma. Our results also should stimulate research into new treatments. For example, cell surface molecules such as NG2 and PDGFα-R could be targets for cytotoxic therapies. From a broader perspective, investigation of the pathways that regulate the proliferation, survival, or migration of normal progenitor cells may identify additional targets for therapy of gliomas.

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