Immunolocalization of the Oligodendrocyte Transcription Factor 1 (Olig1) in Brain Tumors

Biagio Azzarelli, MD, Leticia Miravalle, PhD, and Ruben Vidal, PhD

Abstract. Recent in situ hybridization studies showed that mRNA levels of OLIG1 and OLIG2 transcription factors are elevated in oligodendrogliomas. We raised polyclonal antibodies against a synthetic peptide homologous to the human transcription factor Olig1 and studied by immunohistochemistry the expression of Olig1 in 84 brain tumors and in non-neoplastic brain tissues. All oligodendrogliomas, oligoastrocytomas, and dysmyeloplastic neuroepithelial tumors showed moderate to strong intranuclear immunoreactivity in cells morphologically identified as oligodendrocytes. In addition, some astrocytomas showed a slight to moderate intranuclear immunoreactivity. None of the other neuroepithelial and non-neuroepithelial tumors showed nuclear immunoreactivity. Double immunostaining of oligodendrogliomas, oligoastrocytomas, and glioblastoma multiforme (GBM) using antibodies against Olig1 and GFAP showed the presence of 3 different cell populations: 1) immunopositive for Olig1 and immunonegative for GFAP, histologically identified as oligodendrocytes; 2) immunopositive only for GFAP, histologically identified as astrocytes; and 3) immunonegative for both antibodies (“null cells”), histologically observed as a population of cells usually with round nuclei and a small amount of cytoplasm. The use of double immunostaining facilitated the distinction among these 3 different tumors. In summary, the use of immunohistochemistry using Olig1 antibodies alone or in combination with anti-GFAP antibody, which can be performed in the routine diagnostic setting, may help in the diagnosis of neuroepithelial tumors.

Key Words: Astrocytoma; Glioma; Oligodendroglioma; Olig1; Transcription factor.

INTRODUCTION

The differential diagnosis of oligodendrogliomas includes fibrillar astrocytomas, pilocytic astrocytomas, protoplasmic astrocytomas, clear cell ependymomas, central neurocytomas, and dysmyeloplastic neuroepithelial tumors (DNET) among others. The pathological diagnosis of these tumors relies on the recognition of cytological and histological characteristics; however, in some atypical cases it remains problematic. A conclusive diagnosis at this time is imperative because some oligodendrogliomas, in contrast to other gliomas, respond favorably to chemotherapy (1).

Tumor cells can arise from progenitor cells or from mature transformed cells that have adopted a progenitor cell phenotype, and for this reason lineage markers are potential tumor cell markers. Numerous attempts have been made to find markers for neoplastic oligodendrocytes, either by detecting specific proteins (2–8) or molecular genetic changes (9–14). Some lineage markers, such as chondroitin sulfate proteoglycan (NG2) and alpha-receptor of platelet-derived growth factor (PDGFrα), are expressed early in the development of oligodendrocytes as well as in some oligodendrogliomas and pilocytic astrocytomas (15, 16). Loss of heterozygosity (LOH) of 1p and 19q seems to be a specific marker for oligodendrogliomas. Combined loss of 1p and 19q is a statistically significant predictor of prolonged survival in patients with pure oligodendroglioma, independent of tumor grade (17).

The helix-loop-helix (HLH) family of transcription factors includes about 240 members that have been identified in organisms from yeast to man. In metazoa, HLH factors are essential for a variety of developmental processes, including hematopoiesis, pancreatic development, myogenesis, and neurogenesis (18). Recently, 2 oligodendrocyte lineage-specific basic HLH (bHLH) transcription factors, named Olig1 and Olig2, have been identified (19, 20). Olig1 and Olig2 play a critical role in oligodendrocyte specification during development. In rodents, the expression of OLIG1 and OLIG2 genes during embryogenesis is restricted to the central nervous system (CNS) (19, 20). While OLIG1 participates exclusively in oligodendrogenesis, OLIG2 is also expressed in immature neuronal progenitors and in multipotential neuro/glia progenitors as well as in embryonic olfactory neurons (21).

Two studies using in situ hybridization investigated the expression of OLIG1 and OLIG2 transcription factors in non-tumoral brain tissue and in a variety of neuroepithelial brain tumors (22, 23). It was observed that non-neoplastic oligodendrocytes showed minimal expression of OLIG1 and OLIG2 genes, in contrast to the strong expression of these transcription factors in oligodendroglial tumors. In addition, minimal expression was reported in 2 of 8 astrocytomas (22). Very recently, Ohnishi et al (24) reported that the combination of OLIG mRNA expression and immunohistochemistry using an antibody against Olig2 enables the distinction between oligodendrogliomas and astrocytomas.
To determine the value of Olig1 protein expression as a marker for oligodendrocytes, we investigated the expression of Olig1 in different brain tumors and non-neoplastic brain tissues by immunohistochemistry using polyclonal antibodies. Preliminary data obtained in the course of these studies were reported in abstract form (25).

MATERIALS AND METHODS

Patient Population

The study includes tumor samples that were diagnosed at Indiana University School of Medicine from 2000 through 2003. All tumors were classified according to the World Health Organization (WHO) histological typing of tumors of the nervous system (26). The cases studied included low-grade oligodendrogial tumors (grade II; n = 17, age range 18–57 yr, median age, 37 yr), high-grade oligodendrogial tumors (grade III; n = 5, age range 37–46 yr, median age, 43.5 yr), mixed low-grade oligoastrocytomas (grade II; n = 3, age range 5–47 yr, median age, 19 yr), and mixed high-grade oligoastrocytomas (grade III; n = 1, age 36 yr). In addition, we examined DNET (n = 5, age range 4–25 yr, median age, 12 yr), pilocytic astrocytomas (n = 4, age range 5–40 yr, median age, 17 yr), fibrillary astrocytomas (n = 5, age range 4–59 yr, median age, 39 yr), subependymal giant cell astrocytoma (n = 1, age 5 yr), GBM (n = 11, age range 10–82 yr, median age, 46 yr), ependymomas, (n = 6, age range 1–42 yr, median age, 10 yr), choroid plexus papilloma (n = 1, age 1 yr), germinoma, (n = 1, age 16 yr), ganglioglioma, (n = 1, age 3 months), medulloblastomas, (n = 3, age range 3–8 yr, median age, 6 yr), schwannomas, (n = 5, age range 30–70 yr, median age, 47 yr), neurofibroma, (n = 1, age 5 yr), meningiomas, (n = 6, age range 44–72 yr, median age, 60 yr), pituitary adenomas (n = 2, age range 42–48 yr, median age, 45 yr), lymphomas, (n = 2, age range 20–47 yr, median age, 33.5 yr), metastatic carcinomas (n = 3, age range 40–66 yr, median age, 52 yr), and a metastatic melanoma (n = 1, age 60 yr).

Production of Antibodies

Polyclonal antibodies (Oligo-906) were raised in New Zealand White rabbits using as immunogen a 14-amino-acid-long synthetic peptide homologous to amino acids 207–220 (AVGPPDALRPAKYL) of the translated human Olig1 cDNA sequence (http://www.ncbi.nlm.nih.gov:80/entrez/, accession Q8TAK6), coupled to keyhole limpet hemocyanin through a N-terminal cysteine. After collecting preimmune sera, rabbits were immunized by an intradermal injection of 250 μg of antigen in complete Freund’s adjuvant 1:1. Animals were boosted with 100 μg of antigen in incomplete Freund’s adjuvant 1:1 every 2 weeks for 8 weeks. The presence of specific antibodies was tested by ELISA and by immunohistochemistry. Additional booster doses of 50 μg of peptide in incomplete Freund’s adjuvant were administered subcutaneously monthly to maintain a high antibody titer (>50,000 as determined by ELISA). Antibodies were purified by affinity chromatography using the synthetic peptide coupled to cyanogen- bromide activated sepharose 4B (Amersham Biosciences, Piscataway, NJ).

Immunohistochemistry

Immunohistochemistry was performed using tissue samples obtained from 84 patients undergoing resection of brain tumors. In addition, brain tissue from autopsies of a 23-week-gestation premature infant, a term baby, and an adult were used. Tissues studied included optic nerve, corpus callosum, medulla, and spinal cord. In the premature infant, cerebral cortex, centrum semiovale, and matrix tissue (basal ganglia region) were also studied. Tissues were fixed in formalin, embedded in paraffin, and routinely processed for immunohistochemistry according to standard protocols. Microwave antigen retrieval was standard. As a primary antibody we used immunopurified Oligo-906 diluted 1:5,000 in 10 mM phosphate buffer, 137 mM NaCl, 2.7 mM KCl, pH 7.4 (PBS) (Sigma, St. Louis, MO). Incubation time with the primary antibody was 60 min. Secondary antibody incubation followed the instruction of the Universal DAKO Labeled Streptavidin-Biotin peroxidase (DAKO LSAB + System, horseradish peroxidase (DAKO, Santa Barbara, CA).

Double immunostaining was performed using the DAKO EnVision Doublestain System (Dako) following the manufacturer's instructions. As primary antibodies we used Oligo-906 antibody diluted 1:3,000 in PBS and a prediluted monoclonal anti-glial fibrillary acidic protein (GFAP) antibody (Dako). Briefly, tissue specimens were treated with the peroxidase block reagent to quench endogenous peroxidase activity. Specimens were then incubated with Olig1 antibody, followed by incubation with the labeled polymer, HRP. The reaction was completed with incubation with liquid DAB + substrate-chromogen, which results in a brown-colored precipitate. After blocking, specimens were incubated with anti-GFAP, followed by incubation with the labeled polymer, AP. The reaction was completed with incubation with Fast Red substrate-chromogen, which results in a red-colored precipitate. Sections were counterstained with hematoxylin.

The same tumors double stained for Olig1/GFAP were stained with a prediluted antibody against myelin basic protein (MBP) (Biogenex, San Ramon, CA). All DNET were also stained with a prediluted antibody against synaptophysin (Biogenex).

The cytoplasmic and nuclear staining was subjectively evaluated by one of us (BA) on a 4-point scale: 0 = none; 1 + = mild; 2 + = moderate; 3 + = strong. The percentage of immunopositive cells and nuclei was also subjectively estimated. When more than 1 section of the same tumor was evaluated, the percentages represent an average.

Western Blot Analysis

The cases studied included oligodendroglioma (grade II; n = 1 and grade III; n = 1), oligoastrocytoma (grade II; n = 1 and grade III; n = 1), fibrillary astrocytomas (grade II; n = 2), ependymoma, (n = 1), choroid plexus papilloma (n = 1), germinoma, (n = 1), medulloblastoma (n = 1), and metastatic carcinoma (n = 1). Tissue samples were obtained at the time of surgery, snap-frozen in liquid nitrogen, and stored at −70°C. Tissue was minced and repeatedly washed in PBS containing protease inhibitors (Complete, Roche, Indianapolis, IN) until the washings were clear. Tissues were homogenized with a
glass rod and pelleted at 12,000 rev/min for 10 min in an Eppendorf centrifuge. Soluble proteins were separated on a 16% Tris-Tricine SDS-polyacrylamide gel (SDS-PAGE) and electro-transferred for 1 hour at 400 mA at 4°C onto polyvinylidene difluoride membranes (Immobil-ton-P Millipore, Bedford, MA) using 10 M 3-cycloexylaminol-1-propanesulfonic acid (CAPS; Sigma) buffer, pH 11, containing 10% methanol. The membranes were blocked with 5% nonfat dry milk in PBS containing 0.1% Tween-20 (PBS-T) and subsequently incubated for 2 hours at room temperature with Oligo-906 antibody (1:1,000 in PBS-T). Horseradish peroxidase-conjugated goat anti-rabbit (Amersham) was used as the secondary antibody (1:5,000 in PBS-T). Immunoblots were visualized by chemiluminescence (Amersham) according to the manufacturer’s specifications.

RESULTS

Immunohistochemical Studies

No immunoreactivity was observed when sections of a typical low-grade oligodendroglioma (Fig. 1A) were incubated with preimmune serum instead of the Oligo-906 antibodies (Fig. 1B), as well as when the antibodies were preincubated with the synthetic peptide used as immunogen (not shown).

Immunohistochemistry using Oligo-906 antibodies showed that all oligodendroglomas (n = 22), oligoastrocytomas (n = 4), and DNET (n = 5) had a mild to strong nuclear immunoreactivity (Table 1; Fig. 1C–E, H). Grade II oligodendroglomas (n = 17) showed mild (6% of the cases), moderate (47%), and strong (47%) nuclear immunoreactivity. Grade III oligodendroglomas (n = 5) showed mild (20%), moderate (40%), and strong (40%) nuclear immunoreactivity. Grade II oligoastrocytomas (n = 3) showed moderate (33%) and strong (67%) nuclear immunoreactivity, meanwhile grade III oligoastrocytoma (n = 1) showed a strong (100%) nuclear immunoreactivity. DNET (n = 5) showed moderate (60%) and strong (40%) nuclear immunoreactivity (Fig. 1H). The percentage of immunostained nuclei is indicated in Table 1.

Immunohistochemistry using Oligo-906 antibodies showed that pilocytic astrocytomas (n = 4), fibrillary astrocytomas (n = 5), GBM (n = 11), and subependymal giant cell astrocytoma (n = 1) had none to strong nuclear immunoreactivity (Table 1).

Pilocytic astrocytomas showed none (50% of the cases), mild (25%), and moderate (25%) nuclear immunoreactivity, whereas fibrillary astrocytoma showed none (40%), mild (40%) and strong (20%, with only 3% of nuclei stained) nuclear immunoreactivity (Fig. 1F, G). A case of subependymal giant cell astrocytoma showed no nuclear immunoreactivity and GBM showed none (28% of the cases), mild (36%), and moderate (36%) nuclear immunoreactivity. The percentage of immunostained nuclei is indicated in Table 1.

None of the other tumors showed nuclear immunoreactivity for Olig1 (Fig. 2A; Table 1). Some cytoplasmic immunoreactivity was observed in scattered mature neuronal elements in DNET and in neurons of the cerebral cortex adjacent to various tumors. In these cells, the immunoreactivity appeared as perinuclear clumps with a distribution pattern similar to the Nissl substance (not shown).

Double immunostaining using Oligo-906 antibodies and anti-GFAP in oligodendroglomas (n = 14) (Table 2) showed the presence of 3 main cell populations: 1) cells morphologically characterized by a round nucleus and a small amount of cytoplasm recognized as oligodendrocytes, with a mild to strong nuclear immunoreactivity with Oligo-906 antibodies and no cytoplasmic immunoreactivity for GFAP and Olig1 (Fig. 1D, E); 2) “null cells,” characterized by the presence of a round or a slightly irregular nucleus with finely dispersed chromatin,
minimal amounts of cytoplasm, and lack of intracellular immunoreactivity for both Oligo-906 antibodies and anti-GFAP antibodies (Fig. 1E–G); and 3) minigemistocytes, characterized by the presence of a round and eccentric nucleus not immunoreactive with Oligo-906 antibodies and by the presence of a moderate fibrillated cytoplasm strongly immunoreactive for GFAP (Fig. 1D, E). In addition, there were a few scattered reactive astrocytes with a round nucleus not immunoreactive with Oligo-906 antibodies that showed a strong cytoplasmic immunoreactivity for GFAP (not shown).

We observed in 14 oligodendrogliomas double stained with Oligo-906/anti-GFAP the presence of a few scattered cells with Olig1-immunoreactive nuclei and a thin rim of GFAP-immunopositive cytoplasm (Fig. 2B). These cells do not have the typical morphology of oligodendroglial cells and their identity remains to be determined. Occasionally, we observed a close contact between astrocytes and oligodendrocytes (Fig. 2C).

Double immunostaining using Oligo-906 antibodies/anti-GFAP in oligoastrocytomas (n = 2), GBM (n = 6), and fibrillary astrocytomas (n = 3) (Table 2), showed the presence of 3 main cell populations: 1) cells morphologically characterized by a round nucleus and a small amount of cytoplasm recognized as oligodendrocytes, with a mild to strong nuclear immunoreactivity with Oligo-906 antibody and no cytoplasmic immunoreactivity with anti-GFAP and Oligo-906 antibody; 2) cells morphologically recognized as astrocytes, characterized by the presence of pleomorphic nuclei not immunoreactive for Olig1 and by the presence of a cytoplasm strongly immunoreactive for GFAP; and 3) “null cells,” characterized by the presence of round or slightly pleomorphic nuclei, minimal amounts of cytoplasm, and lack of immunoreactivity for GFAP and Olig1 (Fig. 1F, G). We also observed the presence of Oligo-906 antibody-positive staining cells and “null cells” apparently infiltrating the cerebral cortex (Fig. 2E).

DNET were mainly composed of cells with round nuclei that showed a moderate to strong immunoreactivity using Oligo-906 antibody (Fig. 1H). These cells contained a minimal amount of cytoplasm and lacked immunoreactivity for GFAP and synaptophysin. Therefore, they were interpreted as oligodendrocytes. In addition, there were scattered mature neuronal elements with a variable degree of cytoplasmic immunoreactivity for Oligo-906 antibody.

In the cerebral cortex of the adult and the term baby we did not observe immunoreactivity using Oligo-906 antibody. However, in both cases we observed a moderate cytoplasmic immunoreactivity in glial cells in the corpus callosum, optic nerves, and the pyramidal tract in the brainstem (Fig. 2F). The identity of these glial elements remains to be determined and will be the subject of a separate study. In the premature infant we also observed in the cerebral cortex, subcortical white matter, and matrix tissue, the presence of scattered cells with a round nucleus and no visible cytoplasm. They showed a moderate to intense nuclear immunoreactivity for Olig1 and no immunoreactivity for MBP. They were tentatively interpreted as premyelinating oligodendroglial elements (Fig. 2G) (27).

In oligodendrogliomas, immunostaining with anti-MBP showed variable amounts of residual myelinated fibers within the tumor core, however, oligodendroglial cells remained unstained. Under low magnification, few

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Fig. 2. A. Ependymoma. Ependymal rosette. Lack of immunoreactivity after immunostaining with Oligo-906 antibodies. ×1,140 oil immersion. B: Oligodendroglioma (grade II). The cell in the center contains a slightly irregular nucleus with a positive immunoreactivity with Oligo-906 antibodies. In addition, there is a perinuclear rim of GFAP immunopositivity. Double immunostaining using Oligo-906 antibodies (brown)/anti-GFAP (red). ×2,800 oil immersion. C: Oligodendroglioma (grade II). In the center of the field (arrow), there are 2 closely apposed cells. One cell shows an immunoreactive nucleus for Olig1 and the other cell has an immunopositive cytoplasm for GFAP. The cell with the Olig1-immunopositive nucleus may represent an oligodendrocyte, which appears partially surrounded by the cytoplasm of an adjacent astrocyte. Double immunostaining using Oligo-906 antibodies (brown)/anti-GFAP (red). ×2,800 oil immersion. D: Glioblastoma multiforme. The field is mainly composed of small round nuclei lacking immunoreactivity for both, Oligo-906 antibodies, and anti-GFAP antibodies (“null cells”) (thin arrows). There are also a few scattered oligodendrocytes (thick arrows) and few astroglial elements (arrowheads). Double immunostaining using Oligo-906 antibodies (brown)/anti-GFAP (red). ×2,800 oil immersion. E: Glioblastoma multiforme. Neoplastic infiltration with oligodendrocytes (thick arrows), “null cells” (thin arrows), and astrocytic elements (arrowheads). Cerebral cortex (between layers 3 and 5). Neurons (N). Double immunostaining using Oligo-906 antibodies (brown)/anti-GFAP (red). ×1,000 oil immersion. F: Optic nerve (autopsy). The field contains a cluster of cells with round or slightly irregular nuclei lacking immunoreactivity for Olig1. One cell showed a moderate cytoplasmic immunoreactivity for Olig1 (arrow). Term baby. Immunostaining using Oligo-906 antibodies. ×1,000 oil immersion. G: White matter (autopsy). The field contains a few scattered cells with a round nucleus with a moderate nuclear immunoreactivity for Olig1. These cells are interpreted as premyelinating oligodendroglial elements. Twenty-three-week-old gestational infant. Immunostaining using Oligo-906 antibodies. ×1,200 oil immersion. H: Oligodendroglioma (grade II). Cluster of cells with uniform round nuclei and perinuclear halo of clear cytoplasm. There are focal densities of immunoreactivity at the periphery of a few cells suggestive of membrane staining (arrows). Immunostaining for MBP ×2,400 oil immersion.
### TABLE 1

Cases Studied and Immunohistochemistry Results using Oligo-906 Antibodies

<table>
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<th>Diagnosis</th>
<th>n</th>
<th>Range</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>Mean ± SD</th>
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<td>OL grade II</td>
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<td>2.4 ± 0.6</td>
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Abbreviations: n, number of cases; OL, oligodendroglioma; OA, oligoastrocytoma; DNET, dysembryoplastic neuroepithelial tumor; PA, pilocytic astrocytoma; FA, fibrillary astrocytoma; SGCA, subependymal giant cell astrocytoma; GBM, glioblastoma multiforme; CPP, choroid plexus papilloma; PAD, pituitary adenoma; MC, metastatic carcinoma; MM, metastatic melanoma. The intensity reflects the amount of nuclear immunoreactivity on a 4-point scale: 0 = none; 1 = mild; 2 = moderate; 3 = severe. Mean ± standard deviation (SD). N/A = not applicable.

Fig. 3. Western blot analysis of brain tumors using Oligo-906 antibodies. Lanes 1 and 2, oligodendroglioma (grade II). Lane 3, oligoastrocytoma (grade III). Lane 4, oligoastrocytoma (grade II). Lane 5, fibrillary astrocytoma (grade II). Lane 6, ependymoma. No immunoreactivity was seen when the first Ab was omitted in lane 1. Molecular weights are indicated in kDa.
immunoreactive protein with a molecular weight of ~24 kDa (Fig. 3, lane 5), whereas the second case did not show any immunoreactivity (not shown). No immunoreactivity was seen in an ependymoma (Fig. 3, lane 6), a choroid plexus papilloma, a germinoma, a medulloblastoma, and a metastatic carcinoma. We observed a strong correlation between the presence of immunoreactive proteins in Western blot and nuclear staining in immunohistochemistry with Oligo-906 antibodies (Table 1).

**DISCUSSION**

In-situ hybridization studies showed that RNA levels of OLIG1 and OLIG2 transcription factors are elevated in oligodendrogiomas (22, 23), suggesting the potential diagnostic use of Olig markers for CNS tumors. Furthermore, Ohnishi et al described recently the use of quantitative RT-PCR and immunohistochemistry using an antibody against Olig2 for the analysis of glial tumors (24). We raised antibodies against human transcription factor Olig1 and used them to perform an immunohistochemical study of brain tumors and non-neoplastic brain tissues.

We observed in oligodendrogliomas, oligoastrocytomas, and DNET, a strong nuclear immunoreactivity for Olig1 in a large percentage of the cells. These cells were identified as oligodendrogial elements on H&E stain. In addition, we observed in tumors that were classified as astrocytomas (2 pilocytic astrocytoma, 2 fibrillary astrocytoma grade II, and 6 GBM) a small population of cells with nuclei that were moderately immunolabeled for Olig1. The use of antibodies against Olig1 proved to be extremely useful in the neuropathological analysis of the diffuse variant of mixed oligoastrocytomas, for which the criteria for the diagnosis is poorly defined (28–30). The precise characterization of mixed oligoastrocytomas is not only of theoretical interest but it may have therapeutic significance since it has been reported that mixed oligodendrogliomas respond more favorably to chemotherapy than pure astrocytomas (31).

The analysis of tumor samples by Western blot using Olig1 antibodies showed the presence of immunoreactive proteins (~24 kDa and ~27 kDa) only in those cases in which we observed immunohistologically the presence of nuclear immunostaining for Olig 1. The molecular weight of the immunoreactive protein of ~27 kDa is in the range of the predicted molecular weight of the transcription factor Olig1 protein (27.9 kDa; http://ca.expasy.org/tools/tagident.html). We also observed a second immunoreactive band of ~24 kDa, which may represent a proteolytic degradation fragment of Olig1 or a post-translationally modified Olig1 protein. We excluded the possibility that the 24-kDa protein corresponds to an alternatively spliced isoform of Olig1 since the OLIG1 gene, located in chromosome 21, contains a single exon that includes the complete coding sequence of Olig1 (http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?view=graph&val=NT_011512.8&gene=OLIG1). Since the Oligo-906 antibody recognizes an epitope located between amino acids 207 and 220 of Olig1, the 24-kDa protein may represent an N-terminally truncated isoform of Olig1 that can not longer bind to DNA (the DNA binding domain resides in the N-terminus of the molecule). This may in turn transform the Olig1 protein into an ID HLH-like protein, which are proteins known to function as dominant negative regulators of bHLH (32). Further studies are needed to clarify this issue.

In tumors with a high percentage of oligodendroglial cells, the neoplastic nature of these cells is unquestionable. However, in tumors such as fibrillar astrocytomas (Fig. 1F) in which Olig1-immunopositive cells represent a very low percentage of the total number of cells, it is not clear whether the Olig1-immunopositive cells are neoplastic or normal trapped elements overrun by the tumor. These cells show a high degree of nuclear pleomorphism and display a moderate to strong nuclear immunoreactivity for Olig1, which was not seen in normal oligodendrocytes, suggesting that these cells are neoplastic rather than preexisting cells. These findings are in agreement with the in situ hybridization data (19).

Oligodendrogiomas are normally regarded as neoplasms composed of pure oligodendrocytes. In addition to oligodendrocytes, it has been reported that a significant number of oligodendrogiomas contain 2 other cell types, namely minigemistocytes (12) and gliofibrillary oligodendrocytes (2, 28). These cells are considered to be transitional oligodendrogioma-astrocytic elements (12). We observed in double immunohistochemistry for Olig1/GFAP that minigemistocytes were immunolabeled for GFAP and did not show nuclear immunoreactivity for Olig1, suggesting that minigemistocytes are indeed a morphologic variant of astrocytes rather than a transitional cell. In addition, cells that resembled gliofibrillary oligodendrocytes showed nuclear immunoreactivity for Olig1 and a rim of perinuclear GFAP-positive cytoplasm (Fig. 2B). These cells were seen in only 1 of 14 oligodendrogiomas that were double immunostained for Olig1/GFAP. Whether these cells are gliofibrillary oligodendrocytes or represent oligodendrocytes that appear completely surrounded by the cytoplasm and/or processes of an adjacent astrocyte as seen in Figure 2C remains to be determined.

In most of the tumors that were classified by H&E stain as oligodendrogiomas, oligoastrocytomas, and GBM, the use of double immunostaining with Olig1/GFAP revealed a population of cells with round or slightly pleomorphic nuclei that lacked immunoreactivity for Olig1 and GFAP. We called them “null cells.” Although we ignore their nature, we speculate that they may be oligodendrocyte-type 2-astrocytes (O-2A), which were
these tumors may contain a mixed cell population. In J Neuropathol Exp Neurol, Vol 63, February, 2004, previously classiﬁed as typical astrocytomas, suggesting that oligodendrocytic elements in tumors that have been pre-

The use of Olig1 antibodies allowed the identiﬁcation of neoplastic cells and to premyelinating oligodendrocytes. The use of Olig1 antibodies allowed the identiﬁcation of neoplastic cells and to premyelinating oligodendrocytes.

The authors thank Iva Goss and Lee Ann Baldridge for technical assistance and Linda Bailey for secretarial assistance.

Table 2

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n</th>
<th>Range</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>OL grade II</td>
<td>11</td>
<td>15–80</td>
<td>39.5 ± 19.0</td>
<td>10–50</td>
<td>23.2 ± 14.5</td>
<td>10–70</td>
<td>37.3 ± 21.0</td>
</tr>
<tr>
<td>OL grade III</td>
<td>3</td>
<td>5–50</td>
<td>33.3 ± 24.7</td>
<td>25–35</td>
<td>30.0 ± 5.0</td>
<td>20–60</td>
<td>36.6 ± 20.8</td>
</tr>
<tr>
<td>OA grade II</td>
<td>1</td>
<td>50</td>
<td>N/A</td>
<td>20</td>
<td>N/A</td>
<td>30</td>
<td>N/A</td>
</tr>
<tr>
<td>OA grade III</td>
<td>1</td>
<td>10</td>
<td>N/A</td>
<td>40</td>
<td>N/A</td>
<td>50</td>
<td>N/A</td>
</tr>
<tr>
<td>PA</td>
<td>1</td>
<td>5</td>
<td>N/A</td>
<td>80</td>
<td>N/A</td>
<td>15</td>
<td>N/A</td>
</tr>
<tr>
<td>FA</td>
<td>3</td>
<td>5–35</td>
<td>15.0 ± 17.3</td>
<td>30–80</td>
<td>50.0 ± 19.0</td>
<td>15–55</td>
<td>35.0 ± 20.0</td>
</tr>
<tr>
<td>GBM</td>
<td>9</td>
<td>0–60</td>
<td>27.8 ± 22.2</td>
<td>0–40</td>
<td>22.2 ± 26.4</td>
<td>20–80</td>
<td>45.6 ± 21.7</td>
</tr>
</tbody>
</table>

Abbreviations: n, number of cases; OL, oligodendroglioma; OA, oligoastrocytoma; PA, pilocytic astrocytoma; FA, ﬁbrillary astrocytoma; GBM, glioblastoma multiforme; % Olig1, percentage of nuclear staining using Oligo-906 antibodies; % GFAP, percentage of cytoplasmic staining using anti-GFAP antibodies; % Negative, percentage of cells not stained by either antibody. Data represents means ± SD. N/A = not applicable.

initially described in the rat optic nerve (33). These cells were reported to differentiate into ﬁbrous astrocytes if cultured in the presence of fetal calf serum and into oligodendrocytes if cultured in the absence of serum (33), demonstrating developmental plasticity and environmental inﬂuence in the differentiation of CNS glial cells. In 1995, Noble et al (34) isolated precursor cells from a human GBM. Under appropriate conditions, these cells could be differentiated into oligodendrocytes or astrocytes. These precursor cells shared many of the characteristics of the O-A2 cells. The concept of common progenitor cells for both oligodendrocytes and astrocytes is further supported by the observation that oligoastrocytomas show similar genetic aberrations in the oligoden-
drocytic as well in the astrocytic component, indicating that oligoastrocytomas may be clonal tumors originating from a single precursor cell (35). However, recent develop-
ental studies indicated that oligodendroglial and astro-
cytic lineages may arise from different precursor cells located in different regions of the neural tube (36). Other studies on GBM have identiﬁed a subset of GBM with focal histological characteristics of oligodendroglioma showing deletions in chromosome 1p and 19q, which are known to be a marker for oligodendroglialomas, together with a series of genetic aberrations frequently observed in other malignant astrocytomas (37). In these cases, the term “GBM with oligodendroglioma component” has been proposed as a compromised term for these lesions (38).

In summary, Olig1 transcription factor appears to be a very speciﬁc marker for cells that under conventional histology are regarded as oligodendrocytes. Nuclear immuno-
nostaining for Olig1 transcription factor is restricted to neoplastic cells and to premyelinating oligodendrocytes. The use of Olig1 antibodies allowed the identiﬁcation of oligodendrocytic elements in tumors that have been previously classiﬁed as typical astrocytomas, suggesting that these tumors may contain a mixed cell population. In addition, the use of immunostaining for GFAP in combi-
nation with Olig1 led to a better characterization of the cellular elements composing these tumors. Astrocytomas, oligodendrogliomas, and oligoastrocytomas seem to contain a mixed cell population of oligodendrocytes, astro-
cytes, and “null cells,” suggesting a common progenitor cell for these neoplasms. Antibodies against Olig1 could be used as an additional tool to better characterize and diagnose brain tumors. Ultimately, the usefulness of these antibodies needs to be tested against clinical parameters such as response to chemotherapy and survival.

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REFERENCES


