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High-grade Human Brain Tumors Exhibit Increased Expression of Myelin Transcription Factor 1 (MYT1), a Zinc Finger DNA-binding Protein

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Abstract. Detection and characterization of distinct central nervous system (CNS) tumor cell types is clinically important since distinct tumor types are associated with different prognoses and treatments. However, there is currently a lack of markers to identify certain glioma types and insufficient understanding as to which cells give rise to different glioma cell types. In the present study, biopsy specimens from human brain tumors were analyzed for expression of Myelin Transcription Factor 1 (MYT1) to explore the extent to which glioma cells reflect characteristic expression of MYT1 in developing glial progenitor cells. Immunostaining with an antibody against MYT1 revealed widespread immunoreactivity that was most prominent in high-grade oligodendrogliomas, astrocytomas, and mixed oligoastrocytomas as well as in a dysembryoplastic neuroepithelial tumor. MYT1 immunoreactivity in tumor regions generally correlated with the prevalence of cells exhibiting nuclear immunolabeling with an antibody against Ki-67, suggesting an association of MYT1 with cell proliferation that was also observed in normal adult human and rat brain in the germinal subependymal zone. The MYT1 immunoreactivity was frequently nuclear, appearing as dotted or punctate, but in some cases it was localized to the cytoplasm. In combination with histopathological studies and analysis of Ki-67 immunoreactivity, examination of MYT1 immunolabeling may provide additional information to aid in the detection and diagnosis of CNS tumors.

Key Words: Astrocytoma; Dysembryoplastic neuroepithelial tumor; Glioma; Nuclear structure; Oligodendrocyte; Oligodendroglioma; Transcription factor.

INTRODUCTION

The stage of differentiation and specific identity of cells that give rise to various central nervous system (CNS) tumor cell types are poorly understood but clinically significant, as distinct glioma types are typically associated with different prognoses and treatment recommendations. Even classification of glioma types can be difficult because of the heterogeneity of cell types within tumors and inadequate phenotypic or genotypic markers. Cell type identification has been particularly difficult in differentiating between some forms of astrocytoma, oligodendroglioma, and mixed oligoastrocytoma because of the tendency of oligodendrogliomas to lack expression of myelin-specific components while exhibiting an astrocytic marker, glial fibrillary acidic protein (GFAP) (1). Certain rapidly proliferating, high-grade gliomas do not exhibit the distinct histological features of mature oligodendrocytes or astrocytes, but rather appear similar to undifferentiated or immature glial precursor cells. These observations and recent in vitro experiments have led to the hypothesis that a common precursor may be the initial cell in which an oncogenic transformation would occur to give rise to tumors exhibiting oligodendroglial and astrocytic characteristics (2).

Based upon our previous studies of normal developing and adult CNS, we expected that examining the expression of Myelin Transcription Factor 1 (MYT1) would provide further insight into the hypothesis that a common precursor may give rise to certain gliomas. MYT1 is the prototypic member of a new class of DNA-binding proteins among the "zinc-finger" superfamily of transcription factors (3). During normal development in postnatal rat brain, MYT1 protein is present in the nuclei of early precursors of oligodendrocytes and continues to be present in the nucleus of differentiated oligodendrocytes, but then shifts to the cytoplasm and progressively diminishes as the mature oligodendrocytes accumulate myelin-specific proteins (4). MYT1 may also be expressed by astrocyte precursors, but appears to diminish at an earlier point relative to expression in the oligodendrocyte lineage (4). In the adult human brain, a small population of oligodendrocyte precursors, or "pre-oligodendrocytes," persists that displays the antigenic characteristics of immature oligodendrocyte lineage cells and the ability to differentiate into mature oligodendrocytes in vitro in the absence of proliferation (5). Pre-oligodendrocytes have been described in vivo in adult human brain as cells expressing mRNA transcripts for platelet-derived growth factor α receptor and/or MYT1 (6). Cells containing such transcripts were rarely found in human brain parenchyma, but were more prevalent in the subependymal zone that was present in the tissue biopsied in one case. The increased frequency of MYT1-expressing cells associated

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with the subependymal area is interesting in light of studies indicating that a population of neural stem cells persists and proliferates in the subependymal region in adult mammalian forebrain (7).

In the present study, we have examined the cellular expression of MYT1 in diverse glial-derived tumors and in 2 nonglial CNS tumors to determine whether MYT1 is restricted to a particular CNS tumor cell type or more widely expressed, as might be expected of tumors thought to originate from a common precursor. Immunoperoxidase staining was used in combination with hematoxylin or eosin to examine histopathologic features. Immunofluorescence was used to detect MYT1 in some glioma cases to determine the subcellular localization of MYT1 within distinct tumor cell types. Adjacent biopsy sections were analyzed by histopathology and immunostained for Ki-67, a marker of proliferative status, to correlate MYT1 expression with tumor grade and cell proliferation. As a correlation for MYT1 expression relative to a normal population of proliferating CNS cells, in contrast with neoplastic cells, we examined MYT1 and Ki-67 immunoreactivity in neural precursor cells of the subependymal region in normal adult human and rat brain.

MATERIALS AND METHODS

Tissue Preparation

According to an approved protocol for use of human tissues (#089605), archived biopsy material was obtained following diagnostic evaluation. Cases were selected to examine a variety of brain tumor grades and diagnoses, which were determined by one researcher (RBH) according to histopathologic features and clinical information (8–10). These human biopsy tissues were fixed in 10% formalin for 6 to 12 hours (h), paraffin-embedded, and cut as 5-μm sections. A region from a temporal lobe resection for treatment of intractable epilepsy was chosen for comparison of nontumor CNS tissue with identically prepared tumor biopsy tissue. Autopsy tissues were chosen for comparison of adult human brain tissue with no known neurological involvement. Brain slices from 2 autopsy cases were obtained from the Brain and Tissue Bank for Developmental Disorders (Baltimore, MD). One autopsy case was a 36-year-old male who died due to pulmonary embolism, and the postmortem interval was 4 h prior to 10% formalin fixation and storage. The other autopsy case was a 34-year-old male accident victim with multiple injuries, and the postmortem interval was 6 h. For each autopsy case, 4 different CNS regions were examined: the area adjacent to the dorsolateral aspect of the lateral ventricle in both a forebrain slice and a midbrain slice, and the cortex with subcortical white matter in both a forebrain slice and a midbrain slice. Adult rat forebrain was fixed in 4% paraformaldehyde, paraffin-embedded, and cut as 7-μm sections (Novagen; Madison, WI).

Immunohistochemistry

For immunostaining with horseradish peroxidase detection, sections were dewaxed, and the Elite ABC kit (Vector Labs; Burlingame, CA) was used with diaminobenzidine as the substrate. Preliminary tests indicated that there was no difference with or without treatment with 0.3% H_2O_2 in methanol for 30 min to minimize potential endogenous peroxidase activity. The αMYTI-His rabbit polyclonal antibody used (1:50 dilution of affinity purified antibody) to detect MYT1 has been previously described and tested with rodent cells (4). Although αMYTI-His may potentially recognize a newly identified member of the MYT1 family, MYT1 L, the CNS expression of this protein appears to be restricted to neuronal populations (Kim et al, submitted). To confirm the specificity of αMYTI-His immunoreactivity in human tumor tissues, the antibody was absorbed with a 50× excess of antigen overnight at 4°C prior to immunostaining. The Ki-67 rabbit polyclonal antibody (DAKO; Carpinteria, CA) was used in the same ABC Elite protocol at a dilution of 1:100, with inclusion of a microwave treatment prior to incubation with the primary antibody. Ki-67 is a non-histone, nuclear antigen expressed in proliferating and dividing cells during active parts of the cell cycle and absent at Go (11, 12). Sections immunostained for MYT1 or Ki-67 were counterstained with eosin. For immunofluorescence detection, sections were dewaxed and pretreated with a solution containing 25% fetal bovine serum, 0.4% triton-X 100, 1% bovine serum albumin, and 100 mM lysine in phosphate-buffered saline. Antibodies against MYT1 and GFAP (1:4 dilution of monoclonal antibody; Boehringer Mannheim; Indianapolis, IN) were detected with a donkey anti-rabbit IgG conjugated with Cy3 and with a donkey anti-mouse IgG conjugated with FITC, respectively (each from Jackson Immunoresearch, West Grove, PA). To label nuclei with blue fluorescence, sections were stained with 4′,5-diamino-2-phenylindole (DAPI; 100 ug/ml; Sigma, St. Louis, MO). For colocalization of double-label fluorescence, images were merged using Metamorph (Universal Imaging, West Chester, PA) and Adobe Photoshop (Mountain View, CA) software programs.

Analysis of Immunostaining

Qualitative assessment of immunostaining for MYT1 or Ki-67 was performed independently by 2 researchers (RCA and AM) without knowledge of the tumor grade or cellular classification. After the analysis was completed, the findings of the 2 independent assessments were compared and found to be generally in agreement on each of the 40 cases. Quantitative assessment was not attempted because in many cases the heterogeneity of the tissue precluded adequate sampling for accurate comparisons.

RESULTS

Tumor Classification

In total, 40 CNS tumor biopsies were examined (Table 1), as well as 1 nontumor epilepsy temporal lobe resection and 2 autopsy cases without neurological involvement. The diagnoses were determined by histopathological analysis of hematoxylin and eosin stained sections. Astrocytomas of grades II to IV were graded according to the St. Anne's/Mayo system, while all other tumors were graded according to WHO criteria (8–10). In addition to the information provided in Table 1, case 13 was

TABLE 1
Summary of 40 Cases

Case	Diagnosis	Grade	MYT1[1]	Ki-67[2]
1	Oligodendroglioma	2	−	
2,3	Oligodendroglioma	2	±	
4	Oligodendroglioma	2	+	
5	Oligodendroglioma	2	++	
6	Oligodendroglioma	2	++	=
7	Oligodendroglioma	3	++	
8	Oligodendroglioma	3/4	++	=
9	Oligodendroglioma	4	++	
10	Pilocytic astrocytoma	1	+[3]	>
11	Pilocytic astrocytoma	1	±	=
12	Pilocytic astrocytoma	1	±[3]	=
13	PXA	2	+	
14	Astrocytoma	2	+	
35	Astrocytoma	2	+	<
37	Astrocytoma	2	+	>
38, 39, 40	Astrocytoma	2	++	= (n = 3)
15	Astrocytoma	2	++	>
36	Astrocytoma	2	++	<
16	Astrocytoma	2	+++	=
17, 18, 19	Astrocytoma	3	++	
20, 21	Astrocytoma	3	+++	
22	Astrocytoma	3	+++	=
23	Astrocytoma	4	++	
24, 25	Astrocytoma	4	+++	
26, 27, 28, 30	Astrocytoma	4	+++	= (n = 4)
29	Astrocytoma	4	+++	>
31	Oligoastrocytoma	3	+++	=
32	Oligoastrocytoma	4	++	
33	Ependymoma	2	++[4]	
34	DNT	1	+++	<

[1] Qualitative assessment of MYT1 immunoreactivity throughout biopsy section: no notable immunostaining (−), weakly detectable (±), clearly detectable in some areas (+), strong in many areas (++), most intense and widespread (+++).
[2] Assessment of Ki-67 immunoreactivity in 11 biopsies, as >, =, or < areas of MYT1 signal.
[3] No nuclear immunostaining within tissue sampled, only cytoplasmic signal.
[4] Immunostaining was strongly nuclear in only a small area within the biopsy section.

identified as a pleomorphic xanthoastrocytoma (PXA) and case 34 presented as a dysembryoplastic neuroepithelial tumor (DNT; 13).

To aid in the cellular classification of gliomas, several tumors were examined by immunostains to distinguish specific cell types. Oligodendrogliomas cannot be distinguished from astrocytomas by the typical markers of the normal differentiated cell phenotype, as oligodendroglioma cells often do not exhibit immunostaining for the oligodendrocyte- and myelin-specific markers, such as 2′ 3′-cyclic nucleotide 3′-phosphodiesterase, but do exhibit immunostaining for the astrocytic marker GFAP (1 and our data [not shown]). Immunostaining to detect the intermediate filament protein vimentin may help to distinguish astrocytomas from oligodendrogliomas. Astrocytomas will typically exhibit signal for both GFAP and vimentin, while oligodendrogliomas may express GFAP but will infrequently express vimentin (14, 15, and our data [not shown]). However, to our knowledge there is no cellular marker that clearly distinguishes oligodendrogliomas from astrocytomas. Therefore, the cellular classification of the tumors as listed in Table 1 reflects the distinctions based upon histopathological features.

MYT1 Immunoreactivity in Gliomas

In normal adult human brain tissue, MYT1 mRNA transcripts have been shown to be expressed at very low levels relative to developing brain tissue (3) and to be restricted to a small population of cells (6). Our present immunohistochemical analysis demonstrated that MYT1 protein expression in normal areas of adult human white matter and gray matter was very low, with the most prominent immunoreactivity in the nuclei of relatively few small round cells distributed throughout the white matter (Fig. 1A, B). As summarized in Table 1, increased immunoreactivity for MYT1 was found in the overwhelming majority of CNS gliomas examined. Although in early postnatal rodent development, MYT1 immunoreactivity is mainly associated with oligodendrocyte lineage cells, in

CNS tumors, MYT1 immunoreactivity did not distinguish between oligodendroglioma and astrocytoma cells but was evident in cytologically distinct cases of each (Fig. 1D, F). All high-grade gliomas exhibited marked MYT1 immunoreactivity (Table 1). Within a given high-grade tumor, less MYT1 immunoreactivity was typically observed within an area of central necrosis, while adjacent viable tumor cells typically showed strong signal. The only gliomas that did not have marked MYT1 immunoreactivity were low-grade, less aggressive tumors (Fig. 1C, E and Table 1). Although biopsy of small specimens of tumor tissue has been shown to be a useful method for estimating the histological grade even in diffuse astrocytomas (16) in situations where MYT1 was absent or weakly detected, the tumor area may have had a site of stronger MYT1 expression that may not have been included within the biopsy specimen in each case. For example, in case 12 the majority of the area examined was negative for MYT1 (Fig. 1E), but a small area of cells within the biopsy sections was markedly immunolabeled (not shown).

At a subcellular level in some gliomas, MYT1 immunoreactivity was localized in a distinct dotted or punctate pattern within the nucleus (Fig. 2A). However, in many tumor cells the nuclear pattern was not as discrete, and often MYT1 immunoreactivity was localized within both the nucleus and the cytoplasm or almost exclusively within the cytoplasm (Fig. 2B). We could not find a clear determinant that correlated with the observed subcellular variations in MYT1 immunoreactivity. In general, cytoplasmic signal was more frequently found in low-grade astrocytomas as well as in the grade-2 ependymoma. Both the nuclear and cytoplasmic immunoreactivity observed with the αMYTI-His antibody appear to be specific for MYT1 since both are diminished by absorption of the antibody with the MYT1 antigen prior to immunostaining (Fig. 2C, D). Interestingly, cytoplasmic immunoreactivity was also observed in reactive astrocytes in tissue resected for a nontumor case of temporal lobe epilepsy (Fig. 2E, F). In these same sections, MYT1 immunoreactivity was not increased in areas that did not have the increased GFAP expression indicative of reactive astrocytes (Fig. 1B).

The results summarized in Table 1, along with the examples shown in Figures 1 and 2, demonstrate MYT1 expression in oligodendrogliomas, astrocytomas, and oligoastrocytomas, with the most widespread and intense expression corresponding to the higher grades.

Comparison of Glioma Areas Expressing MYT1 with Areas of Ki-67 Immunoreactivity

By immunostaining proximate sections of 21 of the glioma cases for MYT1 and for Ki-67, we found that tumor areas with MYT1 immunoreactivity correlated with areas of Ki-67 immunoreactivity, as did areas that were negative for each antibody (Fig. 3; Table 1). On a cellular level, MYT1 and Ki-67 immunoreactivity may not always overlap—we sometimes observed clusters of Ki-67+ cells without detectable MYT1, while adjacent peripheral cells exhibited strong MYT1 immunoreactivity as well as Ki-67 immunoreactivity. We could not confirm a potential difference between these populations using a double immunostaining protocol since preliminary experiments indicated that MYT1 detection was impaired by the microwave treatments required to detect Ki-67 in paraffin-embedded tissue.

MYT1 Immunoreactivity in DNT

To test the correlation between MYT1 immunoreactivity and cell proliferation, we examined a rare DNT case for both MYT1 and Ki-67 immunoreactivity (Fig. 4A, B). In the area of the DNT, clear nuclear MYT1 immunoreactivity was observed in a large proportion of the cells. In contrast, only a few dispersed cells within this same area exhibited Ki-67 immunoreactivity.

MYT1 Immunoreactivity in Subependymal Zone Cells in Normal Adult CNS

Ki-67 immunoreactivity was clearly present in the nuclei of small round cells within the subependymal zone of the adult rat and human forebrain (Fig. 4C, E). Nuclear immunoreactivity for MYT1 was also noted in cells of this subependymal zone (Fig. 4D, F). Thus, MYT1 immunoreactivity may correlate with a CNS precursor phenotype in areas of cell proliferation in both tumor tissue and normal tissue in the adult CNS.

DISCUSSION

The present findings demonstrate that MYT1, a zinc finger DNA-binding protein, is strongly expressed in many CNS tumors, especially high-grade tumors associated with a poor prognosis (Table 1). MYT1 immunoreactivity was present in both oligodendrogliomas and astrocytomas, as well as a DNT and an ependymoma (Figs. 1D, F; 4A). Tumor areas with marked MYT1 immunoreactivity correlated with areas of Ki-67 immunoreactivity (Fig. 3). Ki-67 immunoreactivity has been demonstrated to be a reliable predictor of poor prognosis due to aggressive brain tumor growth (17, 18). MYT1 immunoreactivity was also observed in areas of cell proliferation in normal adult CNS and in areas of prolonged reactive gliosis in epilepsy tissue (Figs. 2E, F, 4F). Increased MYT1 expression appears to correlate with stimulated transcriptional activity, either associated with cell proliferation or hyperactivity. The lesser MYT1 immunoreactivity in certain lower-grade tumors might reflect the slower growth rate of those tumor cells or that the tumors had developed from more differentiated cells. In combination with Ki-67 and histopathological analysis, the differential cellular and subcellular expression of

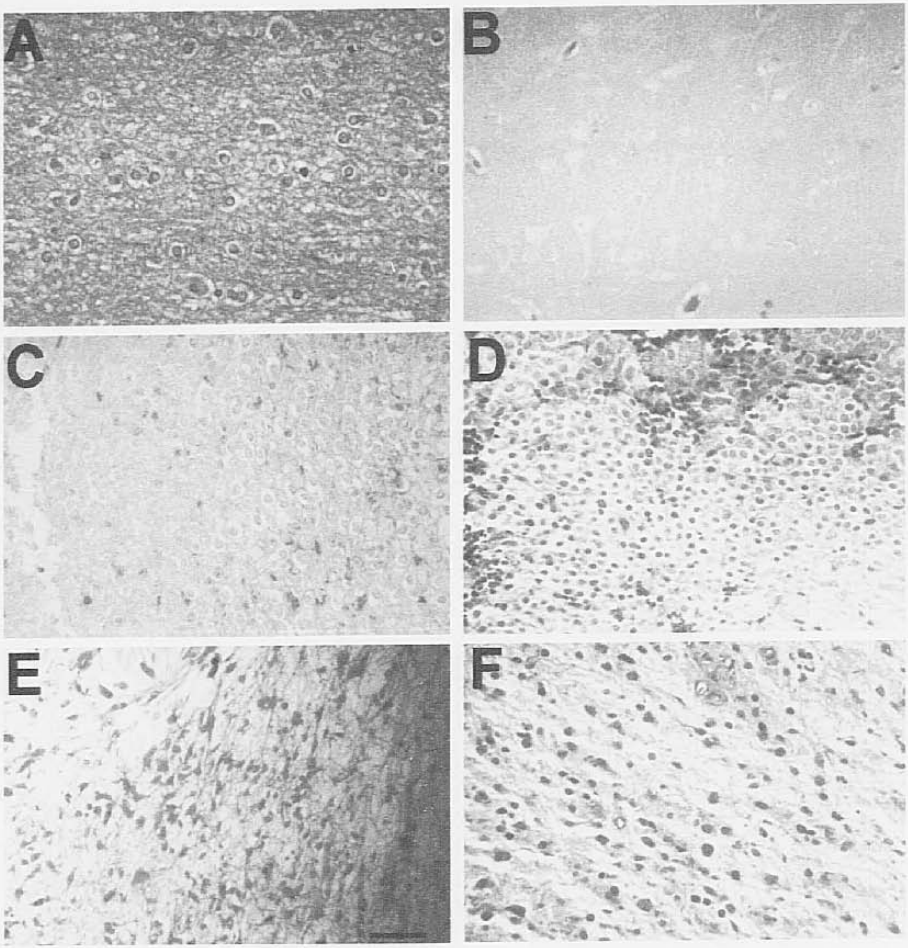

Fig. 1. MYT1 immunoreactivity in gliomas. Immunostaining for MYT1 using the horseradish peroxidase system with the DAB substrate (brown reaction product) and eosin counterstain (pink) in adult human nontumor tissues relative to tumor tissues. In autopsy tissue from normal CNS, MYT1 immunoreactivity is absent in the majority of cells, but is found in the nuclei of a small population of cells in the white matter (A). MYT1 immunoreactivity is not detectable in normal gray matter from autopsy (not shown) or in gray matter areas lacking reactive gliosis in a temporal lobe resection for epilepsy (B). Two different oligodendrogliomas, each classified as grade 2, are shown as examples of strong nuclear MYT1 immunoreactivity (D) and weak scattered MYT1 immunoreactivity (C). A grade 1 pilocytic astrocytoma does not exhibit notable reaction product (E). In contrast, a grade 3 astrocytoma exhibits intense nuclear MYT1 immunoreactivity. For A–F, the scale bar (E) equals 50 μm.

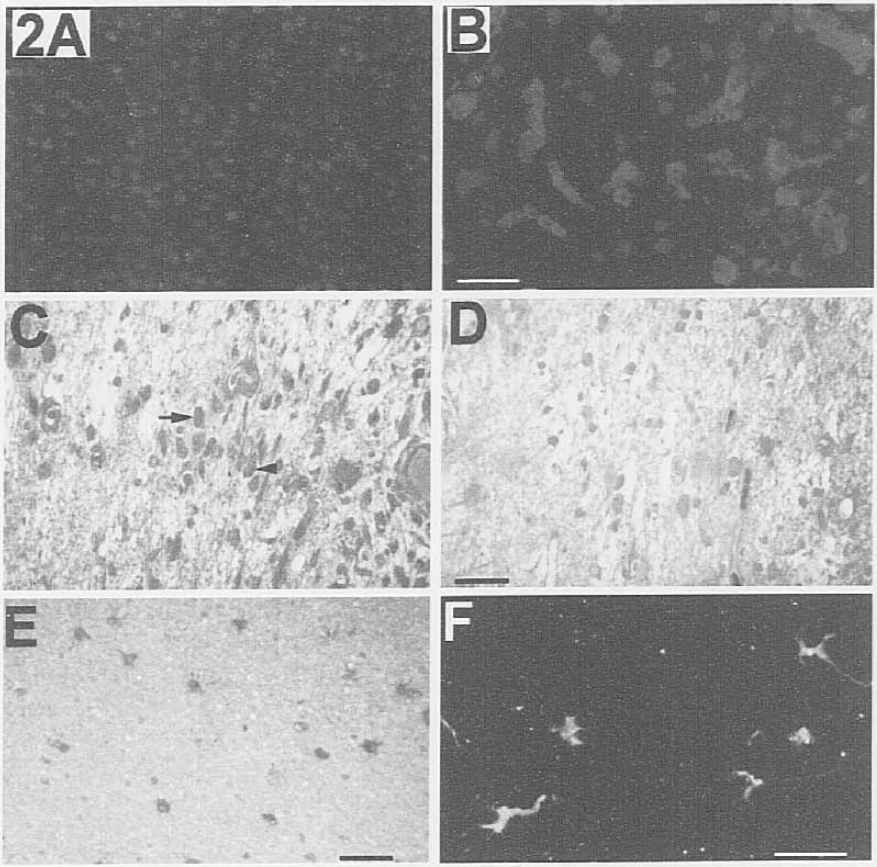

Fig. 2. MYT1 subcellular localization. MYT1 subcellular distribution was variable between tumors as shown by the examples of a dotted nuclear pattern in a grade 2 oligodendroglioma (A) and a mainly cytoplasmic localization in a grade 2 astrocytoma (B). MYT1 immunostaining was detected with a Cy3 (red) -conjugated secondary antibody, and a DAPI (blue) stain was used to label cell nuclei. In C and D, a biopsy with adjacent examples of both nuclear (arrow in C) and cytoplasmic (arrowhead in C) MYT1 localization shows that the immunoperoxidase reaction product in both subcellular compartments is diminished by preincubation of the antibody with the antigen (D) compared with the identical conditions without the antigen absorption (C). In a case of intractable epilepsy, the tissue exhibited an area of cells with cytoplasmic MYT1 immunoreactivity (E) that corresponded with expression in reactive GFAP+ astrocytes (F). Panels C–E show brown immunoperoxidase reaction product with eosin counterstain. Panel F shows double immunofluorescence for MYT1 in red and GFAP in green, with the overlapping signal appearing yellow. All scale bars (A, B; C, D; E; F) equal 50 μm.

MYT1 immunoreactivity may aid in the characterization of CNS tumors.

In normal adult human and rat forebrain, MYT1 was found to be expressed in nuclei of cells of the subependymal zone (Fig. 4F) and in a small proportion of cells within the white matter (Fig. 1A). Expression of MYT1 in each of these regions may correlate with the presence of different types of immature neural cells. In the adult rodent forebrain, the subependymal region continues to serve as a germinal zone. Studies performed using rodents

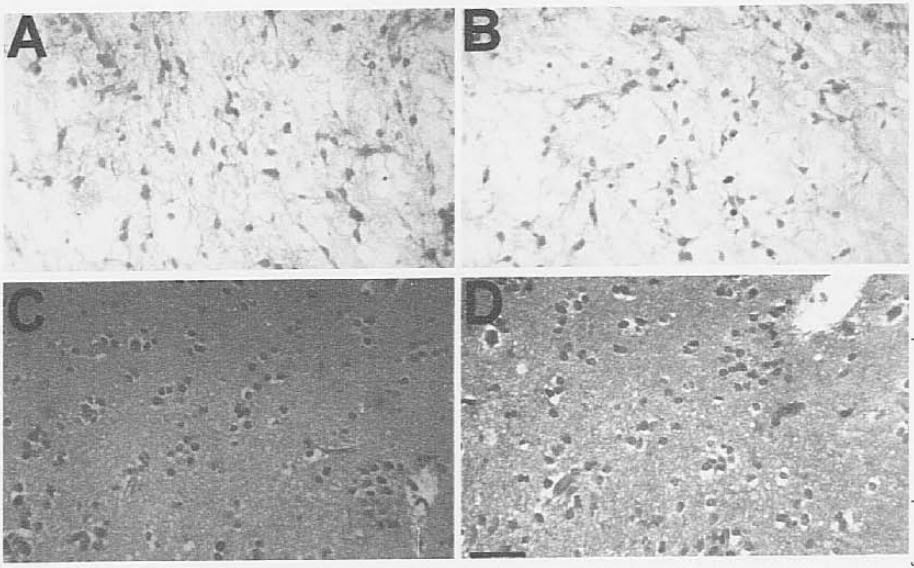

Fig. 3. Correlation of MYT1 and Ki-67 immunoreactivity. Matched areas of a grade 1 pilocytic astrocytoma lack immunoreactivity for MYT1 (A) or for Ki-67 (B), with the exception of occasional scattered cells. An area within a grade 2 astrocytoma exhibits increased MYT1 immunoreactivity (C) with a similar pattern of Ki-67 immunoreactivity (D). A–D show brown immunoperoxidase reaction product with eosin counterstain. For A–D, the scale bar (D) equals 50 μm.

(reviewed in reference 7) have led to the prediction that one subependymal cell population, neural "stem cells," proliferates continuously and also generates a second population, neural "progenitor cells," that can continue to give rise to astrocytes, oligodendrocytes, and/or neurons, depending upon environmental signals. In addition to the immature neural cells of the subependymal region, a recent study has demonstrated a population of proliferating cells in adult rat subcortical white matter (19). Since occasional cells with nuclear MYT1 immunoreactivity were observed in normal adult human subcortical white matter (Fig. 1A) and similar cells were found in Ki-67-immunostained sections (not shown), it may be that MYT1 is expressed in this population in adult rat and human CNS. Also, since MYT1 and platelet-derived growth factor α receptor (PDGFαR) are each expressed in immature rodent oligodendrocyte lineage cells (4, 20, 21), the presence of transcripts for these proteins in the adult human CNS has been suggested to indicate a "pre-oligodendrocyte" phenotype (6). Pre-oligodendrocytes have been characterized as cells that display the antigenic characteristics of immature oligodendrocyte lineage cells and the ability to differentiate in vitro into mature oligodendrocytes in the absence of proliferation (5). Taken together, these studies demonstrate that several populations of immature or precursor cells persist in the adult CNS, possibly reflecting progressive stages of differentiation from neural "stem cells" to neural "progenitor cells" in the subependymal region, and after migration continue to proliferate in subcortical white matter until progressing to the pre-oligodendrocyte stage. Thus, multiple cell types of progressive stages of differentiation that persist in adult CNS and express MYT1 could each serve as a common precursor that has been hypothesized as the initial cell in which an oncogenic transformation would occur to give rise to tumors exhibiting oligodendroglial and astrocytic characteristics (2). Consistent with this possibility is the finding that oligodendroglioma cell lines (2 of 2 tested) display in vitro characteristics akin to oligodendrocyte progenitor cells rather than differentiated oligodendrocytes (2). Further information correlating MYT1 expression with a precursor phenotype may be derived from a recent study indicating that the MYT1 homologue, X-MyT1, is transiently expressed in precursor cells in early Xenopus development, where it plays a role in the differentiation of neurons and is downregulated after terminal differentiation (22).

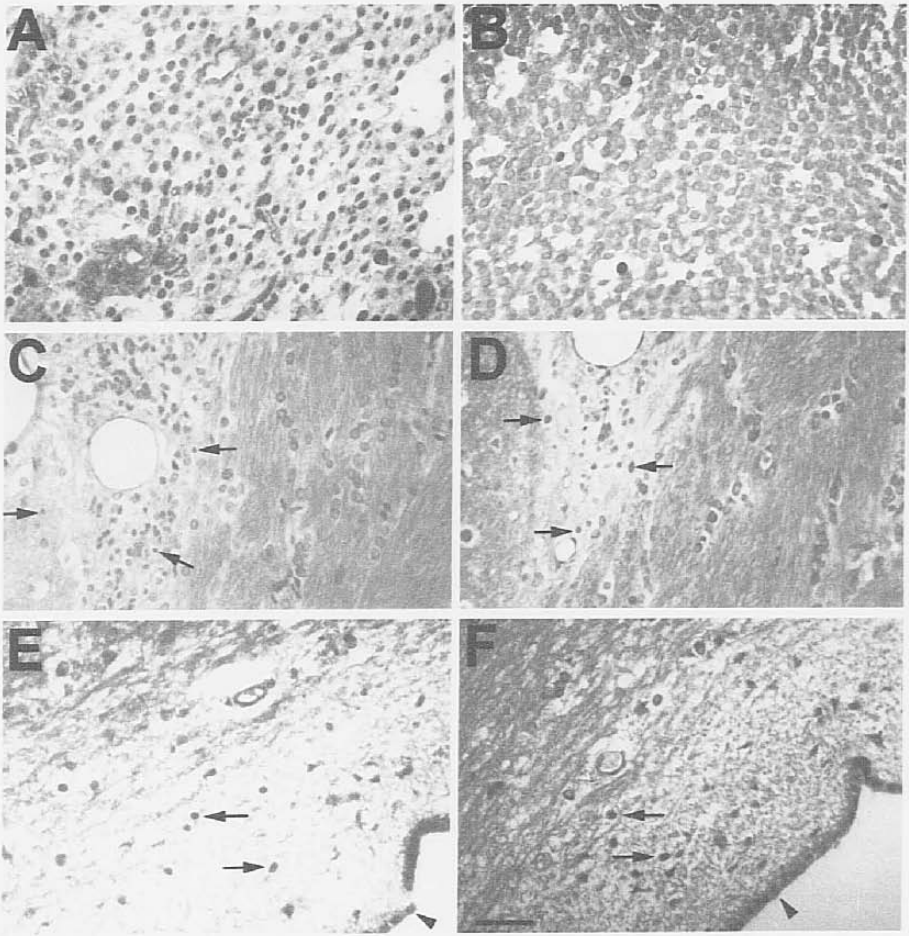

Fig. 4. Expression of MYT1 in a DNT and in the subependymal zone in normal adult CNS. In a DNT, intense nuclear signal for MYT1 immunolabeling was present in the majority of cells (A), although only a few cells exhibited positive nuclear immunostaining for Ki-67 in the same area of a proximate section (B). Panels C–D show the subependymal region adjacent to the dorsolateral aspect of the lateral ventricle with overlying fibers of the corpus callosum. In the subependymal zone in adult rat (C, D) and adult human (E, F), small cells (arrows) exhibit immunoreactivity for Ki-67 (C, E) or for MYT1 (D, F). Cells lining the lateral ventricle also exhibit immunoreactivity (arrowheads). For A–E, the scale bar (F) = 50 μm.

Our finding of strong nuclear MYT1 immunoreactivity in a DNT may relate to the stage of differentiation of the constituent cells that give rise to this pathological region. The nuclear MYT1 immunoreactivity observed in the grade 1 DNT was much more distinct than the MYT1 immunoreactivity observed with grade 1 pilocytic astrocytomas or most of the grade 2 astrocytomas or oligodendrogliomas. DNTs have been reported to be either low-grade tumors or a developmental abnormality resulting from a lack of normal cellular differentiation

(13, 23–25). DNTs have relatively few proliferating cells based upon analysis of Ki-67 immunoreactivity (25; Fig. 4B). The present finding of strong nuclear MYT1 immunoreactivity in a DNT may support the hypothesis that the constituent cells of this lesion are somehow arrested in an early stage of differentiation.

Alternatively, MYT1 immunoreactivity may reflect re-expression of MYT1 in tumors derived from more differentiated cell phenotypes. In this case, MYT1 expression may be sensitive to alterations of the cellular environment. For example, tumor environments can exhibit increased expression of PDGF-AA (26, 27) and bFGF (28). The effect of growth factors on MYT1 expression in cells of adult CNS is not yet known. Oligodendrocyte progenitors cultured from neonatal rodent CNS exhibit nuclear MYT1 immunoreactivity in the presence of PDGF-AA and bFGF (4). However, this observation may relate to the proliferative and developmental cell status in that these growth factors act as mitogens and prevent the differentiation of the oligodendrocyte progenitors (21, 29).

On a subcellular level, the localization of MYT1 immunoreactivity was extremely variable either between tumor types or within a given biopsy (Fig. 2A, B). Nuclear localization was usually associated with oligodendrogliomas and with high-grade astrocytomas. In contrast, cytoplasmic signal was most frequently found in low-grade gliomas, including an ependymoma, and in reactive astrocytes. Further studies are required to identify factors regulating this subcellular localization, which may provide clues to tumor cell origin, growth, and/or migratory behavior. The complexity and heterogeneity of tumor cells and the tumor environment indicate that many possible regulatory mechanisms should be considered, and in fact may differ between tumors. During normal development in postnatal rat brain, MYT1 is present in the nuclei of early progenitors of oligodendrocytes and continues to be present in the nucleus of differentiated oligodendrocytes, but then shifts to the cytoplasm and progressively diminishes as the mature oligodendrocytes accumulate myelin-specific proteins (4). In normal cells, nuclear translocation may be regulated by several different mechanisms, such as hormone binding, dephosphorylation, second messenger binding, and inactivation of nuclear uptake regulatory proteins (reviewed in [30]). Additional considerations are warranted in tumor cells, since nuclear proteins can have a cytoplasmic localization in tumor cells due to mutation, translocation to produce a fusion protein, aberrant expression of a splice variant, or binding to another abnormally expressed protein that controls subcellular localization (31).

The subnuclear distribution of MYT1 in normal cells vs tumor cells is also likely to provide insight into the as yet unknown function of MYT1. MYT1 immunoreactivity in normal developing oligodendrocytes has a distinct dotted or punctate pattern within the nucleus (4). In some tumor cells a dotted MYT1 nuclear pattern (Fig. 2A) was observed, while the nuclear distribution was more diffuse in others. Studies of acute promyelocytic leukemia have shown that alteration of nuclear protein localization may play a role in human oncogenesis. In acute promyelocytic leukemia, at (15:17) translocation leads to production of a promyelocyte-retinoic acid receptor α fusion protein that disrupts promyelocyte oncogenic domain (POD) nuclear structures and correlates with oncogenesis (32, 33). Interestingly, promyelocyte antigen, another POD protein designated Sp100, and MYT1 both contain zinc coordination motifs (3, 34, 35). Also similar to our findings with MYT1, promyelocyte antigen is overexpressed in distinct pathological situations in inflammatory and neoplastic tissues that are associated with stimulated transcription and cellular hyperactivity (36).

Further studies are ongoing to more fully characterize the cellular, subcellular, and subnuclear distribution of MYT1, and should provide important indicators of MYT1 function in the developing and adult normal CNS. The variations of this distribution in tumor cells may be clues to help identify abnormal function of MYT1 with potential insight into cellular mechanisms or effects on oncogenesis. Ongoing functional studies in transgenic mice should reveal critical information for understanding the role of MYT1 in normal and oncogenic conditions.

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