Expression of the Class VI Intermediate Filament Nestin in Human Central Nervous System Tumors

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ABSTRACT

Tumor cells of a particular tissue may show a pattern of gene expression characteristic of the precursor cells of this tissue. To test this proposition for tumors of the central nervous system (CNS) we have used immunohistochemistry to analyze the expression of nestin in primary human CNS tumors and corresponding nonneoplastic brain tissue. Nestin defines a recently discovered sixth class of intermediate filament proteins and in the rat is expressed predominantly in CNS stem cells. In the adult nonneoplastic human brain we have detected only nestin expression in occasional endothelial cells. In contrast, a variety of primary CNS tumors contained substantially elevated nestin levels. The nestin-positive cells in the tumor tissue were tumor cells and/or endothelial cells. Glioblastomas expressed higher nestin levels than less malignant gliomas. This may indicate a correlation between nestin expression and malignancy within the glioma tumor group. In the primitive neuroectodermal class of tumors we observed both nestin-expressing and nonexpressing tumors, suggesting that nestin expression could be used to further characterize this complex and heterogeneous tumor group. Nine metastatic carcinomas were studied, and none showed nestin immunoreactivity in tumor cells. In conclusion, our data support the notion that primary CNS tumors share gene expression patterns with primitive, undifferentiated CNS cells and that nestin, like other intermediate filament proteins, may be useful in tumor diagnosis.

INTRODUCTION

There have been many proposals for the cellular origin of CNS tumors. Bailey and Cushing (1) suggested that they were derived from an undifferentiated blast cell. A specific candidate for the tumor progenitor cells has been identified in the case of medulloblastomas, nests of undifferentiated cells, which normally give rise to the granular neurons of the cerebellum. Such nests have been demonstrated in otherwise normal, differentiated brain tissue (2). It might be expected that CNS tumor cells would show a gene expression pattern similar to that of the cells of the developing CNS from which they arise. Thus, the identification of RNA transcripts and proteins from such specifically expressed genes may be valuable in CNS tumor diagnosis, since they may be produced in CNS tumors only and not in corresponding adult nonneoplastic tissue.

Nestin is a gene which is potentially interesting in this respect. It encodes a newly discovered, sixth class of intermediate filament protein (3) and was originally detected by the monoclonal antibody Rat.401. By immunohistochemistry (4) and double-label fluorescence-activated cell sorting (5) nestin was shown to be expressed in rat CNS stem cells. Molecular characterization of the gene revealed a structural similarity (16-29% amino acid identity) to all previously characterized intermediate filament proteins (3). The similarity resides in an approximately 300-amino acid α-helical region required for filament formation (6, 7). This relatively low degree of amino acid identity and a variant mRNA splice pattern justify the placement of nestin in a novel, sixth class of intermediate filaments (3, 8). The recent cloning of the human nestin gene reveals that it is distinctly similar to the rat gene, most notably in the α-helical region (9).

During CNS development the intermediate filament component of the cytoskeleton undergoes substantial remodeling. First, nestin is expressed in the CNS stem cells (3), and after its down-regulation, GFAP and neurofilaments are expressed in differentiated astrocytes and neurons, respectively (for a review, see Refs. 8 and 10). In addition, peripherin is expressed in subsets of neurons primarily in the peripheral nervous system (11), and internexin is transiently expressed in developing CNS (12).

Because of the strict temporal and spatial control of intermediate filament expression during development, they have been widely used to identify different cell types, both during normal development and in various tumors. Thus, identification of GFAP and neurofilaments have been used in the diagnosis of CNS tumors, and desmin and the cytokeratins in the diagnosis of rhabdomyosarcomas and carcinomas, respectively (see Ref. 13 for a review).

Based on the frequent use of intermediate filaments in tumor pathology, the evolutionary conservation of nestin between rat and humans (9), and the recent observation that neuroepithelium in developing human CNS transiently expresses nestin (14), we decided to analyze nestin expression in human CNS tumors and in adult nonneoplastic tissue.

MATERIALS AND METHODS

Antibodies. The two anti-nestin antisera 129 and 130 were produced in two separate rabbits immunized with a bacterially produced fusion protein containing the 4000 carboxy-terminal base pairs of the rat nestin gene (15, 16). Monoclonal antibodies against GFAP (TPG-FAP3) and the phosphorylated form of the M, 200,000 (NFH) neurofilament (TnPFPIA3) have been described previously (17, 18).

Western Blot Analysis. Frozen tumor tissue was homogenized directly into sample buffer (19), and total protein was extracted. After shearing of high-molecular-weight DNA, separation of cellular debris by centrifugation, and boiling, approximately 2 μg of total protein were loaded in each lane of a 6% sodium dodecyl sulfate-polyacrylamide gel (19). The electrophoretically separated proteins were transferred to Immobilon P membranes (Millipore) using a BioRad transfer apparatus for 2 h at 90 V. After blocking in 3% bovine serum albumin for 1 h the filter strips were incubated overnight with the two polyclonal anti-nestin antiserum or Coomassie blue stained to visualize the protein pattern. Antiserum 129 was used at a dilution of 1:600 and antiserum 130 at 1:2000 in phosphate-buffered saline (140 mM NaCl, 2.7 mM KCl, 10
Tumors were classified according to the WHO (20), using the criteria of
detected by a biotinylated anti-rabbit antibody and avidin-conjugated
mM Na2HPO4, 1.7 IHMKH2PO4. pH 7.2). The immunoreactivity was
fixed according to the B5 method (22). Sections (4 urn) were cut and
mounted on microscope slides. The tissue sections were dried at 37°C
for 4 h and then stored at –20°C until further use.

Immunohistochemical Staining Techniques. The mounted tissue
sections were deparaffinized in xylene and then rehydrated through
rinses in 99% and 70% ethanol. At this point, the slides were submerged
into 70% ethanol with 5% iodine for 10 min, since some tumors had
been fixed with B5 (22). After another rinse in 70% ethanol the slides
were rinsed in water, and endogenous peroxidase was blocked by an
incubation in 0.7% hydrogen peroxide in methanol, followed by a rinse
in TBS and blocking for 30 min in 3% bovine serum albumin in phos-
phate-buffered saline. The slides were then incubated with first antibody
for at least 12 h at room temperature. First antibodies were used at the
following dilutions in phosphate-buffered saline: 129, 1:600; 130,
1:2000; TpGFAP3, 1:100; and TpNFPIA3, 1:50. After three rinses in
TBS the second antibody was added and incubated for 60 min (bioti-
nylated swine anti-rabbit Ig, Dakopatts E 353 at 1:500 for 129 and 130;
biotinylated rabbit anti-mouse Ig Dakopatts E 354 at 1:250 for TpGF-
FAP3 and TpNFPIA3). Following three rinses in TBS a premixed
complex of horseradish peroxidase-conjugated avidin and biotin was
added in 50 HIM Tris pH 7.6 (Dakopatts K 355) and incubated for 30
min. After three rinses in TBS, diaminobenzedine at 0.6 mg/ml in TBS
was added in 50 HIM Tris pH 7.6 (Dakopatts K 355) and incubated for 30
min. After three rinses in TBS, diaminobenzedine at 0.6 mg/ml in TBS
was added for 10 min, and the reaction was terminated by a quick rinse
in TBS, followed by rinses in water and 70% and 90% ethanol. Some
sections were counterstained with haematoxylin. Finally, coverslips
were mounted with Mountex (Histolab AB). The sections were in-
spected under a Leitz light microscope and photographed at x100
magnification.

Double Immunofluorescence Experiments. Tumor sections were
treated as above for hydration and blocking. Sections were then incu-
bated overnight with anti-nestin antiserum 130 at 1:500, rinsed three
times in TBS as above, and incubated with fluorescein-conjugated sheep
anti-rabbit IgG antiserum (Boehringer 1238 833, diluted 1:80) for 60
min. After three rinses in TBS the sections were incubated overnight
with the monoclonal TpGFAP3 antibody (1:250). Following three
rinses in TBS the sections were finally incubated with rhodamin-con-
jugated sheep anti-mouse IgG antiserum (Boehringer 1214 608, diluted
1:80) for 60 min. The sections were analyzed and photographed for
fluorescence and rhodamin fluorescence under a Nikon microscope
using a Leitz trinocular microscope at x100 and x200 magnification. Control experiments
were performed in which the anti-nestin and the anti-GFAP antibodies were
omitted in separate experiments. No cross-binding between the two antibodies
could be detected.

Northern Blots. Polyadenylated RNA (1.25 mg) from human adult
brain (a 21-year-old, disease-free male) was denaturated and electro-
phoresed in a 1% agarose gel containing 2.2 m formaldehyde in 1X
4-morpholinepropanesulfonic acid buffer for 4 h (23). The RNA was
then transferred to Nylon membrane (Hybond-N; Amersham). The
filter was prehybridized and hybridized (5X standard saline citrate, 5X
Denhard’ts, 250 mg/ml salmon sperm DNA, 50% formamide, 50 mm
sodium phosphate [pH 6.5], 0.5% sodium dodecyl sulfate at 42°C for 16
h) with 2 x 106cpm/ml of a mixture of two different 32P-labeled probes
(24) of the human nestin gene (9): the first derived from 900 base pairs
from the carboxy-terminal region (corresponding to amino acids 375 to 1453). After
hybridization the filter was washed (final wash, twice for 30 min each at
65°C in 0.2 X standard saline citrate, 0.2% sodium dodecyl sulfate) and
exposed to X-ray film at –70°C for 14 days with intensifying screens.

Results

Characterization of the Two Anti-Nestin Antisera

Sections from a variety of CNS tumors and nonneoplastic adult brain tissue were analyzed by immunohistochemistry with antibodies to the intermediate filaments GFAP, neurofilament, and nestin. The two antibodies against nestin, 129 and 130, are specific for nestin in rat (16) and recognize a molecule of similar size in human material (14, 16). To confirm that the antibodies truly recognized the equivalent human protein in our tumor material we performed Western blot analysis of an immunohis-
tochemically nestin-positive tumor. The antisera identified a protein in the expected molecular weight range (Fig. 1a). Fig. 1b shows the intermediate filament-like staining pattern, observed for all nestin-positive cells in the various tumors. Anti-
sera 129 and 130 gave similar staining patterns in the tumors, while the incidence of positive cells could vary somewhat. This may be a consequence of differences between different sections or slight differences between the two antisera (Table 1). Preim-
mune serum from the same rabbits, used as a control under identical conditions, did not give any reaction (data not shown).

Staining of Nonneoplastic Adult Human Brain Tissue

Sections from brain tissue removed in the treatment of epi-
lepsy from six patients were analyzed for nestin, GFAP, and
neurofilament expression. None of the cases were tumor
related. The expected neurofilament and GFAP staining was easily
identified in neuronal fibers and astrocytes, respectively (Fig.
2, a and b). High levels of GFAP expression in reactive astro-
cytes were seen in some cases and were probably related to the
epileptic focus.

In contrast, only a very small number of cells reacted with the
anti-nestin antibodies, and these were endothelial cells (Table
1). In the positive cells the intensity of the immunoreactivity
was very low (Fig. 2c). Nestin-positive cells were identified in
only three of the specimens, one each from the hippocampus, fronal lobe, and lateral neocortex. In the other three speci-
mens, taken from temporal lobe, temporal cortex, and lateral
neocortex, no nestin immunoreactivity was detected. Even in
regions that contained nestin-positive endothelial cells, the vast
majority of endothelial cells were negative for nestin expres-
sion. Neither preimmune serum nor the monoclonal antibodies

Fig. 1. Identification of nestin in Western blots and in tumor sections. In a, 2 
pg of total protein from a glioblastoma tumor (Table 1, tumor 12) were Western
blotted with the anti-nestin antisera 129 (Lane 2) and 130 (Lane 3). Lane 1, a strip
from the same filter stained with Coomassie blue. Indicated molecular weights are
in kilodaltons. b, immunofluorescence microscopy of a glioblastoma tumor sec-
tion (Table 1, tumor 7) with anti-nestin antiserum 130. Cytoplasmic staining with
an intermediate filament-like pattern can be observed. Bar, 10 gm.
Table 1—Continued

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Abbreviations for adult control brain: l.n., lateral neocortex; f.r.l., frontal lobe; r.t.l., right temporal lobe; r.t.c., right temporal cortex; h. (hippocampus). Nestin staining in endothelial (endo) and tumor cells (tumor) is denoted separately. *, presence of occasional nestin-expressing cells in adult tissue (nest.) or astrocytes expressing GFAP. Data are from immunohistochemical analysis (avidin-biotin-peroxidase complex) of 67 tumors of CNS origin, 10 tumors that have metastasized to the brain, and one retroperitoneal neuroblastoma. * Staining patterns: 0, no staining; 1, staining in less than 5% of cells in the tumor; 2, staining in 5–50%; 3, staining in more than 50%. For nestin the staining results from the two antibodies (129 and 130) in tumor cells (tumor) and endothelial cells (endo) are denoted separately. GFAP staining (GFAP turn.) is only denoted when it occurs in tumor cells, not in astrocytes.

Nestin expression in adult brain was analyzed also at the RNA level. When a Northern blot filter of polyadenylated RNA from adult human brain was hybridized with a probe from the cloned human nestin gene, we observed a weakly hybridizing band, corresponding to a 6-kilobase mRNA species (Fig. 3).

Nestin Staining in Tumors

Sectioned material from the surgical specimens of 78 formalin-fixed tumors was analyzed with the antibodies to nestin, GFAP, and neurofilament protein. The specimens consisted of 67 primary tumors, 10 metastatic tumors to the CNS (nine low differentiated carcinomas of known origin and a single metastatic rhabdomyosarcoma), and one primary retroperitoneal neuroblastoma. In total, nestin expression was detected in the tissue of 62 of the tumors (Table 1). The nestin immunoreactivity was detected only in two cell types: endothelial cells and tumor cells.

Endothelial Cells. Forty-seven tumors (60%), of both primary and metastatic origin, contained nestin-positive endothelial cells (Fig. 4a). Nestin immunoreactivity was most intense in proliferating endothelium, particularly in the malignant gliomas, but was detected in all types of primary CNS tumors analyzed. The single neuroblastoma and the single plexus papilloma showed no immunoreactivity for nestin (Table 1). In some tumors nestin expression was confined to endothelial cells, whereas other tumors contained both nestin-positive endothelial and tumor cells (see below).
NESTIN EXPRESSION IN CNS TUMORS

Fig. 2. Immunostaining of adult, nonneoplastic human brain. A section from an adult epileptic neocortex was immunostained (avidin-biotin-peroxidase complex) with antisera against M, 200,000 neurofilament protein (a), GFAP (b), and nestin 130 (c). Immunoreactivity is noted in nerve fibers (neurofilament), reactive astrocytes (GFAP), and endothelial cells (nestin). Bar, 100 μm.

Fig. 3. Expression of the human nestin gene in adult brain. Northern blot analysis of 1.25 μg polyadenylated RNA from adult human brain. The blot was probed with a portion of the cloned human nestin gene and exposed with an intensifying screen for 14 days.

Patterns of Nestin Expression in the Various Tumor Types

Gliomas. Tumors from nine types of gliomas were analyzed, and nestin immunoreactivity could be detected in all classes, although to varying degrees (Table 1). Glioblastomas (malignancy grade IV) expressed the highest incidence of nestin-positive cells and in general the highest levels of nestin staining. Of the 15 cases analyzed all expressed nestin in tumor cells and nine in endothelial cells, and in most cases the immunostaining was observed in a large number of cells in the tumor (Table 1). Five cases of anaplastic astrocytoma (malignancy grade III) were analyzed. Two expressed nestin only in tumor cells, and two others only in endothelial cells. In the single case of anaplastic mixed glioma (malignancy grade III) only a few positive tumor cells were found. Astrocytomas (malignancy grade II) contained nestin-positive tumor cells in two and endothelial cells in six of seven tumors. Anaplastic oligodendrogliomas (malignancy grade III) expressed nestin in tumor cells in three of five tumors, whereas in all five cases the endothelial cells were positive. In the five cases of oligodendrogliomas (malignancy grades I-II) we found nestin-immunoreactive tumor cells in three tumors and endothelial cells in four cases. The single case of anaplastic ependymoma (malignancy grade III) contained both nestin-immunoreactive tumor cells and endothelial cells. Three of the six cases of ependymomas (malignancy grade II) were nestin-positive in tumor cells and three in endothelial cells. No nestin-positive tumor cells were detected in the six cases of pilocytic astrocytomas (malignancy grade I), while two of these tumors contained nestin-positive endothelial cells.

PNE Tumors. Fifteen cases of PNE tumors were analyzed. Five of these showed nestin immunoreactivity in tumor cells and nine in endothelial cells. Four of the latter were also positive in tumor cells (Table 1). Analysis of GFAP immunoreactivity revealed that 4 of the 15 tumors showed GFAP-positive tumor cells. Eight tumors contained neurofilament-positive cells (data not shown).

Other Primary Tumors. No detectable levels of nestin were expressed in the single cases of neuroblastoma (retroperitoneal tumor) and plexus papilloma tumors analyzed.

Metastases. We analyzed ten cases of metastases to the brain: nine carcinomas of known origin and one rhabdomyosarcoma (Table 1). The rhabdomyosarcoma tumor tissue showed expression of nestin in tumor cells but not in endothelial cells.
NESTIN EXPRESSION IN CNS TUMORS

Fig. 4. Expression of nestin in endothelial and tumor cells. Immunofluorescence microscopy of tumor sections with anti-nestin antiserum 130. a, immunopositive endothelial cells in a metastasizing tumor (Table 1, tumor 75). Note that only endothelial cells stain with the anti-nestin antiserum. b, immunopositive tumor cells in a glioblastoma tumor (Table 1, tumor 9). Multinucleated tumor cells are positive. In c, a lower magnification of b is included to demonstrate the variation of immunopositivity for nestin in the tumor. Bar, 100 µm (a and b), 1000 µm (c).

Of the other nine metastases none contained nestin-positive tumor cells, whereas six contained immunoreactive endothelial cells.

Nestin Staining in Tissue Adjacent to CNS Tumors

We analyzed the expression of nestin, GFAP, and neurofilament in sections taken from regions in close proximity to the tumor tissue of four astrocytomas. The overall morphology was nontumoral, as determined from adjacent hematoxylin-eosin sections (data not shown). This was evident also from the patterns of GFAP and neurofilament immunoreactivity: reactive astrocytes with increased GFAP levels and ordered neurofilament-positive nerve fibers were seen (Fig. 6, a and b). In these sections we found low levels of nestin staining only in a proportion of the endothelial cells (Fig. 6c). This was, however, somewhat more intense than that found in control brain but less intense than in tumor tissue.

Double-Label Immunofluorescence with Nestin and GFAP

The peroxidase staining suggested that nestin and GFAP in certain tumors had very similar expression patterns. To investigate in more detail the possible coexpression of nestin and GFAP, we analyzed their intracellular distribution by double immunofluorescence. In some tumors the distributions of nestin and GFAP were quite distinct, e.g., where nestin was mainly expressed in endothelial cells and GFAP only in astrocytes in the same tumor (Fig. 5, a and b). This demonstrates the specificity of each antibody for its target intermediate filament protein. In certain tumors, however, many cells coexpressed nestin and GFAP, in particular tumor cells surrounding small blood vessels, whereas in adjacent endothelial cells nestin alone was expressed (Fig. 5, c and d). At high magnification it was apparent that the intracellular distribution in coexpressing cells is very similar (Fig. 5, e and f).

DISCUSSION

The rat nestin gene encodes a recently discovered intermediate filament protein expressed predominantly in CNS stem cells (3-5). Stimulated by the long-standing interest in the relationship between undifferentiated cells of the developing CNS and CNS tumor cells (1, 25) and the fact that other intermediate filaments have significantly contributed to the diagnosis of various tumors (13), we have analyzed nestin expression in human CNS tumors and control nonneoplastic brain tissue.

In the adult, nonneoplastic human brain nestin immunoreactivity was detected only in occasional endothelial cells in the frontal lobe, hippocampus, and lateral neocortex. A small number of cells expressing nestin at a low level is supported by our observation of very low nestin mRNA levels in RNA from adult brain. Our findings extend a recent analysis of nestin expression during human embryonic CNS development (14). Tobyama et al. (14) observed a transient nestin expression in human neuroepithelium but noted in addition an endothelial expression pattern that was maintained until at least 40 weeks after gestation. Taking into account our finding of sporadic adult expression in endothelial but not in the neuroectodermally derived CNS cells in the adult brain, the available data suggest that human brain nestin expression occurs (a) as a transient phase in embryonic neuroectodermal development and (b) probably throughout life in the endothelial component. Nestin expression in the endothelial cell may be related to proliferation, as discussed below.

Increased Nestin Expression in CNS Tumors. In contrast to the rare findings of nestin immunoreactivity in endothelial cells of adult nonneoplastic brain, we detected considerably elevated nestin immunoreactivity in a variety of CNS tumors. Both endothelial cells and tumor cells expressed nestin. Nestin expression in endothelial and tumor cells is presumably independently regulated, since tumors containing positive cells of both, one, or neither cell type were found.

Our data indicate that the nestin expression in endothelial cells may be correlated with the degree of cellular proliferation. First, the most intensely nestin-immunoreactive cells were found in the proliferating endothelium of tumors, frequently of the glioblastomas. Second, in nonneoplastic brain tissue, where proliferation occurs at very low rates, only occasional endothelial cells expressed nestin, and then at low levels. Third, in
Fig. 5. Double-immunofluorescence studies of tumors with anti-GFAP and anti-nestin antisera. Sections from an anaplastic astrocytoma (a and b) (Table 1, tumor 16), a glioblastoma (c and d) (Table 1, tumor 11), and a PNE tumor (e and f) (Table 1, tumor 53) were double-stained with antisera to nestin 130 (a, c, and e) and GFAP (b, d, and f). The staining of proliferating endothelial cells for nestin (a) and probably reactive astrocytes for GFAP (b) can be observed. In e the endothelial cells are stained only by nestin, and the tumor cells by both nestin (c) and GFAP (d). The similarities of the intracellular nestin (e) and GFAP (f) staining patterns are shown at higher magnification. Occasional cells stain with only one of the antisera. Bar, 200 μm (a and b), 50 μm (c and d), and 20 μm (e and f).
Fig. 6. Expression of nestin in tissue adjacent to a tumor. M, 200,000 neurofilament protein (a), GFAP (b), and nestin 130 (c) immunostaining (avidin-biotin-peroxidase complex) of part of a section taken from the periphery of an astrocytoma (malignancy grade II). a, neurites; b, probable reactive astrocytes. Nestin staining is noted only in some endothelial cells (c). Bar, 100 μm.

reactive nonneoplastic tissue from the immediate vicinity of tumors, where increased endothelial proliferation may occur, endothelial cells also expressed nestin, the percentage of positive cells being intermediate between nonneoplastic control brain and the highly proliferating endothelium of tumors. In addition, in some carcinoma metastases nestin immunoreactivity was readily detected in endothelial cells. The endothelial cells are not themselves neoplastic, but their degree of proliferation may be subject to control by angiogenic factors produced by adjacent tumor cells. It has been shown that CNS cells grown in primary culture respond to the angiogenic factor basic fibroblast growth factor, in synergy with nerve growth factor, by maintaining high levels of nestin protein (26). However, the absence of nestin-positive cells in the endothelium of some of the very malignant tumors, where angiogenesis would be expected to occur, suggests that the correlation between nestin expression and endothelial proliferation is not absolute.

In contrast to the endothelial expression pattern, where nestin apparently can be directly or indirectly induced by adjacent tumor cells, the neuroectodermally derived cells seem to be more selective in their nestin expression. Our data suggest that glial cells do not express nestin except when transformed. No nestin expression could be detected in the reactive glioses surrounding the tumors or in relation to some of the epilepsy foci. This implies that exposure to growth and other factors secreted by the tumor cells does not induce nestin expression in adult glial cells, while expression in endothelial cells appears to be affected by such factors. Furthermore, nestin expression was detected in tumor cells of various types of primary CNS tumors, which are of neuroectodermal origin, but not in metastasizing carcinoma cells. Tumor cells of the rhabdomyosarcoma, a tumor of muscle origin, expressed nestin. This is to be expected, since it has been established that developing muscle cells normally express nestin (3). Immortalization of primary CNS and non-CNS cells with the oncogene SV40T results in nestin expression only in cell lines derived from neuroectodermal cells (15). It thus appears that in neuroectodermally derived cells, nestin, in addition to the transient normal embryonic expression pattern, specifically reappears following transformation, an observation which supports the proposed relationship between gene expression patterns in CNS tumor cells and cells of the developing CNS.

Nestin and Diagnosis of CNS Tumors. There are several aspects to the use of nestin in CNS tumor diagnosis. First, the immunostaining is distinct and localized to the cytoplasm, which facilitates identification of positive cells. Second, nestin immunoreactivity in the nonneoplastic brain is very low, occurring only in endothelial cells. Third, the absence of nestin-positive tumor cells in the metastases and its frequent presence in anaplastic gliomas may assist in the differentiation of primary from metastatic tumors, particularly when small stereotactic biopsies are being used for diagnosis. The fact that a large proportion of highly malignant gliomas express nestin, as compared to the less malignant forms (e.g., glioblastomas versus pilocytic astrocytomas), also suggests that nestin expression may relate to the degree of malignancy within the glioma group. High levels of nestin expression, in particular in glioblastomas, have also been reported by Tohyama et al. (14). Fourth, among the PNE tumors we observed nestin-expressing as well as non-expressing tumors. Expression in PNE tumors has also been reported by Valtz et al. (16) and by Tohyama et al. (14). The different levels of nestin expression may be useful in the characterization of this tumor class, which is currently considered a heterogeneous and complex group (2, 27, 28). Currently, PNE tumors are primarily classified simply on the basis of their undifferentiated and variable morphology (29) (see Ref. 25 for an overview). GFAP, neurofilament, synaptophysin, and desmin immunoreactivity have been used to characterize PNE tumors and to indicate their differentiation potential (see Ref. 25). Considering that nestin expression during normal CNS development precedes GFAP and neurofilament expression (3, 8), it is possible, at least in a schematic model, that nestin expression in PNE tumors may be correlated with a more undifferentiated state, whereas coexpression of nestin and GFAP or neurofilament may define tumors in an intermediate differentiated state, while the expression of GFAP or neurofilament alone would define the most differentiated forms.

To improve the diagnosis of CNS tumors it is important to rely on objective criteria such as expression patterns of RNA or proteins from characterized genes. By improving our knowledge of such gene expression patterns we may be able to correlate this information with the biological aggressiveness of the tumor or its sensitivity to various forms of therapy. With its predominantly early CNS-specific expression in normal tissue and expression particularly in malignant CNS tumors, nestin is one candidate for this type of analysis. The data show that CNS tumor cells can reexpress a gene normally active during CNS development. This supports the notion that the mechanisms controlling gene expression patterns are likely to be shared between developing and transformed cells.
Further investigations of larger numbers of tumors will reveal more precisely the role nestin will play in the clinical diagnosis of CNS tumors, but the results presented here demonstrate that nestin has a potential for joining the ranks of the other intermediate filaments, like neurofilament and GFAP, in providing useful information in tumor diagnosis.

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