α-Internexin in the Diagnosis of Oligodendroglial Tumors and Association With 1p/19q Status

Sabina Eigenbrod, MD, Sigrun Roeber, MD, Niklas Thon, MD, Armin Giese, MD, Anna Krieger, Eva Grasbon-Frodl, MD, Rupert Egensperger, MD, Jörg-Christian Tonn, MD, Friedrich-Wilhelm Kreth, MD, and Hans A. Kretzschmar, MD, FRCPath

INTRODUCTION

In World Health Organization (WHO) grade II to IV gliomas, the distinction between astrocytoma (A), oligodendroglioma (O), and mixed oligoastrocytoma (OA) has critical impact on prognostic evaluation and treatment considerations but can be difficult because of the somewhat subjective histopathologic criteria (1). Analysis of molecular markers, specifically loss of heterozygosity on chromosomes 1p and 19q (LOH 1p/19q), has considerably reduced diagnostic uncertainties because 1p/19q codeletions frequently occur in O and OA and correlate with favorable outcome scores (2–5). To date, however, the molecular/genetic effects of LOH 1p/19q have not been fully determined, and the clinical impact of isolated or partial deletions of 1p or 19q remains poorly defined.

A recent study suggests that α-internexin (INA), an intermediate neurofilament that has been associated with developmental, degenerative, and inflammatory processes of the central nervous system (CNS), is a surrogate marker for the combined 1p/19q deletion in WHO grade II to IV glioma (6). However, whether INA expression correlates with isolated and/or partial deletions on 1p and 19q has not been clarified. This study was conducted to evaluate INA expression in WHO grade II to IV oligodendrogial and astrocytic gliomas and to determine the extent to which it correlates with 1p/19q codeletions and isolated and/or partial deletions on chromosomes 1p and 19q. In addition, we analyzed INA expression in CNS tumors that can mimic oligodendrogiomas to assess its role for the differential diagnosis of these challenging tumors.

MATERIALS AND METHODS

Tumor Samples

We studied 83 untreated gliomas, including A, OA, O, glioblastoma without oligodendroglial component (GBM), and glioblastoma with oligodendroglial component (GBMO) and 21 oligodendroglial phenotype-mimicking tumors (OMTs); the latter group included dysembryoplastic neuroepithelial tumor (DNT), central neurocytoma (CNC), clear cell ependymoma (CCE), pilocytic astrocytoma (PA), and metastasis of renal cell cancer (MRCC). The cases had been collected between 2006 and 2009 at the Center for Neuropathology and Prion Research at the Ludwig Maximilians-University Munich, Germany. An overview of the cases is provided in Tables 1 and 2. The study protocol was reviewed and approved by the institutional review board of the Ludwig-Maximilians-University, Munich, Germany (AZ 216/14).

Histology and Immunohistochemistry

For histopathologic evaluation, samples were fixed with 4% buffered formalin (Fisher Scientific GmbH, Schwerte, Germany) and embedded in paraffin. Tumor morphology in
2-μm paraffin sections was visualized using hematoxylin and eosin staining. For immunohistochemistry (IHC), sections were subjected to standardized staining on a benchmark staining machine with a 3,3′-diaminobenzidine detection system according to the manufacturer’s instructions (Ventrica Medical Systems, Tucson, AZ). Antibodies used were anti–human glial fibrillary acidic protein (monoclonal mouse, clone 6F2; Dako, Glostrup, Denmark) and anti–microtubule-associated protein 2 (clone HM-2; Sigma, St. Louis, MO), and mouse anti–α-internexin monoclonal antibody (clone 2E3; Invitrogen, Darmstadt, Germany). The histologic diagnosis was made according to the 2007 WHO classification of tumors of the CNS (1). α-Internexin expression was evaluated by 2 observers semiquantitatively as described previously (6) and scored as negative (no staining), weakly positive (<10% stained cells), or strongly positive (>10% stained cells). This classification is based on the overall percentage of tumor cells independent of their distribution within the tumor.

In cases of a biphasic INA expression pattern, proliferative activity in the respective areas was determined using anti-human Ki67 antibody (mouse monoclonal, clone MIB-1; Dako). For quantification of Ki67-positive cells, INA strong and weak or negative areas were selected in the anti-INA–stained sections and the corresponding areas were identified in consecutive Ki67-stained sections. For all cases, representative pictures of the Ki67 labeling from both an area with strong INA staining and an area with weak/negative staining (400× magnification; area, 35.525 μm² for each) were taken with a BX50 Olympus microscope (Olympus Deutschland GmbH, Hamburg, Germany). Cell count was performed using Cell D Software (Olympus Deutschland GmbH) according to the manufacturer’s instructions; 236 ± 14 cells were counted per representative high-power field.

**Polymerase Chain Reaction Amplification of Microsatellite Markers**

Loss of heterozygosity on the short arm of chromosome 1 (1p) and on the long arm of chromosome 19 (19q) was detected using 5 tetranucleotide markers for each chromosomal region (D1S1608, D1S548, D1S1592, D1S1184, and D1S1161 for chromosome 1p; D19S433, D19S431, D19S718, D19S559, and D19S601 for chromosome 19q), as previously described (7, 8). The distribution of the markers throughout the entire chromosomal arms also allowed the detection of partial deletions (Table 3). Special care was taken to avoid cross-contamination. To this end, the samples of each patient were analyzed in separate experimental rounds including a no template control for each microsatellite primer pair.

**Microsatellite Analysis**

Polymerase chain reaction products were analyzed on highly resolving Spreadex gels using the Elchrom submerged gel electrophoresis system (both from Elchrom Scientific, Cham, Switzerland) and subsequently visualized by SYBR Gold. For LOH analysis, allele signal intensity of the tumor sample was compared with the corresponding allele band of the blood control sample taken from the same patient. Loss of heterozygosity was diagnosed when at least 3 of 5 tetranucleotide markers per chromosome were informative showing a clear signal loss as determined by 2 independent observers. Partial deletions were diagnosed when at least 1 marker showed a clear signal loss and at least 1 other did not.

**Statistics**

Graphs and statistics were done with Excel (Microsoft, Redmond, WA), SigmaPlot, and SigmaStat (Systat Software).
The correlation between INA expression and 1p/19q status was assessed by the Fisher exact probability test (www.graphpad.com/quickcalcs/contingency1.cfm). Continuously scaled variables (e.g., Ki67 labeling index) were analyzed with the Mann-Whitney rank sum test. p < 0.05 was considered significant. All data are expressed as mean ± SEM.

**RESULTS**

Histologic classification, 1p/19q status, and INA expression of the 83 glioma cases examined are summarized in Table 1, and the 21 OMT cases are summarized in Table 2.

**INA Expression Within WHO Grade II to IV Gliomas**

α-Internexin expression analysis by IHC gave reproducible results in all patients. α-Internexin expression was observed within the cytoplasm of tumor cells with accentuation around their nuclei. In 44 of 83 tumors, INA-positive cells were diffusely scattered and closely intermingled with INA-negative cells. In 10 gliomas, however, there was a “biphasic” distribution pattern with areas of INA-positive cells that were clearly separated from areas with weak (2 O II, 2 O III, 1 OA II, 1 OA III, and 2 GBMO) or no expression (1 O II and 1 O III) (Figs. 1 and 2). Biphasic tumors were defined as those that had areas with strong INA staining and had sharp borders with areas with weak/negative staining, that is, at least a 5-fold difference in labeling ratio. These distinct patterns could be identified easily at both low and high magnifications (Fig. 1).

**Correlation of INA Expression and Histology**

α-Internexin expression (weakly and strongly positive) was detected in 65.1% (54/83) of WHO grade II to IV gliomas. In these cases, INA staining was observed in 77.6% (38/49) of oligodendroglial and in 47.1% (16/34) of astrocytic tumors; the difference between oligodendroglial and astrocytic tumors was significant (p < 0.01). When only a strong expression of INA was considered, the association with oligodendroglial phenotype (O, OA, and GBMO) was even more pronounced, that is, it was present in 53% (26/49) of cases versus 5.9% (2/34) of astrocytic tumors (p < 0.0001).

In 22.4% (11/49) of oligodendroglial tumors and in 52.9% (18/34) of astrocytomas, there was no INA staining.

**Correlation of INA Expression and 1p/19q Status**

Overall, 44.6% (37/83) of gliomas exhibited 1p/19q codeletion. Partial deletion of 19q was seen in 19.3% (16/83) of cases. Of the 83 gliomas, 5 (6.0%) had a partial deletion on 1p. Chromosomal aberrations on chromosomes 1p and/or 19q were not unequivocally associated with an oligodendroglial phenotype (Tables 1 and 3). Expression of INA (weak and strong) was observed not only in 92% (34/37) of 1p/19q

| Table 3. Overview on Cases Harboring Isolated/Partial Deletions of 1p or 19q |
|------------------|------------------|------------------|------------------|------------------|------------------|
|                  | INA D1S1608 D1S548 D1S1592 D1S1161 D1S1184 C D19S433 D19S431 D19S718 D19S559 D19S601 |
| A II             | w + NI NI – – C NI NI – – – |
| A II             | w – NI – – – – C + NI + + NI |
| A II             | w NI – – NI – + NI C + NI + + + |
| A III            | n – – + NI – – – C NI – – – |
| A III            | n NI NI – – NI – – – C – – – + + |
| A III            | w – – – – – – C – – – + NI |
| A III            | w NI NI NI – – – C – – – + + |
| GBM IV           | n NI + + + – – – C – NI – – – |
| GBM IV           | n – + ND – – – C – – – – NI |
| GBM IV           | n + + + ND NI – – – C – – – – NI |
| GBM IV           | n – NI – – – – C – NI + + + |
| GBM IV           | n – – NI – + NI – – – C – + + ND |
| GBM IV           | w – NI – – – – C – NI + + + |
| OA II            | s – – NI – – – C – NI + + + |
| OA II            | s – NI – – – C – – + – – |
| OA III           | w – – NI – – – C – – – + + |
| OA III           | w NI – – – ND – + ND + + + C |
| OA III           | w – – – – – NI – – – C – – – + + |
| GBMO IV          | s NI – – – – – C – – – + + |
| O III            | n NI – – – – – C – – + – |
| O III            | n NI – – – – – C – – – + + |

Each row shows an individual tumor; the corresponding columns show the status of the respective microsatellite markers on 1p and 19q arranged in the same order as on the chromosomal arms relative to the centromere region (C).

Latin numbers indicate WHO grade (II–IV).

INA expression was as follows: negative (n), weak positive (w), strong positive (s).

A, astrocytoma; GBM, glioblastoma; GBMO, glioblastoma with oligodendrogial component; O, oligodendroglioma; OA, oligoastrocytoma.

+, LOH; -, no LOH; NI, not informative; ND, not determined.
codelleted tumors (3/5 A II, 2/2 A III, 7/7 OA II, 4/5 OA III, 3/3 GBMO, 9/9 O II, and 6/6 O III) but also in 36% (9/25) of tumors without any deletion (3/5 A II, 1/5 A III, 1/4 GBM, 1/2 OA II, 2/3 OA III, 1/3 GBMO, 0/1 O II, and 0/2 O III). The difference was highly significant (p < 0.0001). Moreover, strong INA expression was highly associated with the 1p/19q codeletion (23/37 vs 2/25 tumors without any deletion; p < 0.0001). Furthermore, expression of INA (weak and strong) was also detected in 63% (10/16) of tumors with an isolated/partial 19q deletion (2/2 A II, 2/3 A III, 1/3 GBM, 2/2 OA II, 2/3 OA III, 1/1 GBMO, 0/0 O II, and 0/2 O III), as well as in 20% (1/5) of tumors with an isolated/partial 1p deletion (1/1 A II, 0/1 A III, and 0/3 GBM). Although the difference regarding percentage of INA-positive cases versus tumors without any 1p and/or 19q deletion did not reach statistical significance (p > 0.05), there was a trend toward INA positivity in tumors with an isolated/partial 19q deletion (Fig. 3). The overall positive predictive value (PPV) of INA expression (weak and strong) for the 1p/19q codeletion was 63%, the negative predictive value (NPV) was 90%, sensitivity (SEN) was 92%, and specificity (SPEC) was 57%. The corresponding values for strong INA expression were PPV 82%, NPV 75%, SEN 62%, and SPEC 89%, respectively. The respective values for strong INA expression within the subpopulation of gliomas harboring an oligodendrogial phenotype (including GBMO) were PPV 85%, NPV 65%, SEN 73%, and SPEC 79%. The specificity of INA expression for detecting a 1p/19q codeletion was higher in high-grade glioma (HGG, 67%) than in low-grade glioma (LGG, 31%). Also, the NPV increased from 67% in LGG to 96% in HGG. The other parameters were PPV (LGG, 68%; HGG, 58%) and SEN (LGG, 90%; HGG, 94%).

An association of INA expression and 1p/19q codeletion was also observed when the data were stratified according to histologic subgroups (Table 1). For example, within the group of pure oligodendroglialoma (O II, O III), 100% (15/15) of tumors with the 1p/19q codeletion showed INA expression, whereas none of those without the codeletion (5/5) were positive for INA (p < 0.0001). Also, within the group of tumors with astrocytic differentiation (A II, A III, GBM, OA II, OA III, GBMO), there was a significant association of the 1p/19q codeletion with INA expression; 86.4% (19/22) of tumors with the 1p/19q codeletion showed INA expression, whereas 48.8% (20/41) of tumors without the codeletion exhibited INA positivity (p < 0.01). In the group of mixed oligodendroglial/astrocytic tumors (OA II, OA III, GBMO) and the group of pure astrocytic tumors (A II, A III, GBM), there was a clear trend toward an association of 1p/19q codeletion with INA expression. In mixed tumors, 93.3% (14/15) of tumors with 1p/19q codeletion showed INA expression; INA expression was observed in 64.3% (9/14) of tumors without the 1p/19q codeletion (p = 0.08). For pure astrocytic tumors, INA expression was found in 71.4% (5/7) of tumors with 1p/19q codeletion and 40.7% (11/27) of tumors without this deletion (p = 0.21). All statistically significant associations of INA expression with 1p/19q loss were within subgroups that contain tumors with at least some degree of oligodendroglial differentiation. Therefore, we cannot exclude the possibility that the significant differences in these subgroups are essentially due to oligodendroglial differentiation.

### Correlation of Biphasic INA Expression Pattern and Intratumoral 1p/19q Status

Because INA expression has been proposed as a surrogate marker for LOH 1p/19q, it is of special interest to have identified several cases with a biphasic expression pattern of INA. We performed a separate LOH analysis of the differentially INA-expressing areas of biphasic tumors. The 2 tumors with the most prominent biphasic INA expression (strong positive, i.e. presumably 1p/19q codelleted areas vs negative areas, i.e. potentially without deletion) were analyzed. Interestingly, both INA-positive and -negative areas clearly harbored the 1p/19q codeletion (Fig. 4).

### Correlation of INA Expression and Ki67 Labeling Index

Within biphasic tumors, comparative analysis of Ki67 labeling revealed a significantly higher labeling index in INA-high-expressing areas (n = 10; 8.4% ± 1.9%) versus the areas with weak (n = 8; 1.4% ± 0.4%) or no INA expression (n = 2; 1%) (Mann-Whitney test, p < 0.01; Fig. 2).

### INA Expression in Tumors Mimicking an Oligodendroglial Phenotype

To investigate the usefulness of INA staining in the differential diagnosis of entities mimicking the histomorphology of oligodendrogial tumors, 15 primary brain tumors (4 DNT, 1 CCE, 4 CNC, and 6 PA) and 6 intracranial MRCC were examined for INA expression. Weak INA expression was found in 13% of primary brain tumors (1/4 CNC and 1/6 PA). Notably, none of these tumors exhibited strong INA staining. In MRCC, there was weak INA expression in 66.7% (4/6); again, no case displayed strong INA positivity. 1p/19q status was available for all of the PA, and none showed any loss of chromosomal material.

### DISCUSSION

α-Internexin expression has recently been proposed as a surrogate marker for prognostic/predictive favorable 1p/19q codeletions in WHO grade II to IV glioma with oligodendroglial differentiation, but its relationship to isolated/partial deletions on chromosomes 1p or 19q has not been addressed (6). The issue is of clinical relevance as the prognostic/predictive value of partial deletions considerably differs from 1p/19q codeletions. Isolated/partial deletions of 1p are rare events predominantly found in astrocytoma and data regarding prognostic relevance remain controversial (4, 9–15). In contrast, isolated deletions of 19q can be found in up to 19% of oligodendrogial tumors and may precede 1p deletions in them (4); however, they do not correlate with favorable outcome scores (4, 14–16). Therefore, only 1p/19q codeletions are considered to be clinically relevant (5). Thus, to determine the utility of INA IHC as surrogate marker for the favorable 1p/19q codeletion in routine diagnosis, it is important to characterize INA expression levels in cases with isolated/partial 1p or 19q deletions. Polymerase chain reaction–based microsatellite...
analysis for 1p/19q LOH is well suited for routine clinical use and enables reliable and reproducible mapping of LOH on both chromosomes; the use of multiple microsatellite markers allows the determination of isolated/partial deletions at different sites on both chromosomes (Table 3) (8). Moreover, this method allows differential analysis of small areas with different morphologic phenotypes within a single tumor (7).

INA Expression in WHO Grade II to IV Gliomas
Histological examination of all 83 gliomas studied revealed that INA was highly associated with an oligodendrogial phenotype. Strong INA positivity was found only in 5.9% (2/34) of astroglial tumors. Interestingly, one of the latter groups presented with pure astrocytic morphology (A II) but harbored a 1p/19q codeletion. This case was diagnosed by stereotactic biopsy procedures, so it is possible that an oligodendrogial tumor component was missed. In contrast to the study of Ducray et al (6), who did not find any INA expression within WHO grade II astrocytomas, approximately two thirds of A II in the present study had at least weak INA expression. This finding, however, is in accordance with recently published data from a tissue microarray-based study (17): in that study low INA expression (0%–5% positive cells) was detected in 93.4% of astrocytoma and a medium/strong (5%–25% or ≥25% positive cells) expression in 6.6% of cases; the respective values for oligodendrogial tumors were 45.8% and 54.2%.

Notably, a so-far-unknown subset of 10 cases had a biphasic INA expression pattern exhibiting distinct areas of strong expression alternating with areas if weak or no INA expression. This pattern was independent of differential astroglial versus oligodendrogial tumor components, which again indicates that INA expression is not exclusively related to oligodendrogial morphology but probably influenced by other unknown factors. Biphasic tumors were additionally assessed for possible relationships between INA expression and Ki67 labeling index. Quantitative analysis of proliferation activity detected a significantly higher number of Ki67-positive cells in areas of strong INA versus weak or no INA expression. At first view, this seems to contradict the fact that INA was found to be a prognostic marker associated with prolonged survival (6), whereas high Ki67 values generally are associated with higher tumor grade and worse prognosis (18). However, in the study of Ducray et al (6), the hazard ratio associated with INA expression was no longer significant when adjusted for 1p/19q codeletion. Thus, INA expression may provide a surrogate marker for the overall molecular effects of 1p/19q codeletion with respect to prognosis, whereas at the molecular level, INA itself may be associated with as-yet-undefined molecular/genetic processes that accompany malignant tumorigenesis.

INA Expression Correlates With 1p/19q Status
In line with the study of Ducray et al, our data highlight a positive correlation between INA and favorable 1p/19q codeletion; 92% of codeleted tumors expressed INA. On the other hand, 36% of nondeleted tumors and 52% of tumors with isolated/partial deletions on chromosomes 1p or 19q had INA expression. Thus, the specificity of INA expression for 1p/19q codeletions was considerably lower (57%) versus the finding of 86% by Ducray et al. This, in turn, critically limits the utility of INA expression as a reliable surrogate marker for 1p/19q codeletions. A higher specificity (89%) could be achieved when only strong INA expression was considered, but this lowered the SEN to 62%. Moreover, biphasic intratumoral INA expression patterns (despite homogeneous 1p/19q status) indicated that INA expression is regulated by additional factors. In summary, INA expression was associated both with an oligodendroglial phenotype and with a 1p/19q deletion but not exclusively linked to the clinically relevant codeletion. Interestingly, a trend toward INA expression was also observed in cases with isolated/partial deletions of 19q, whereas cases with isolated/partial expression of 1p resembled cases without any deletion (Fig. 3). Moreover, INA expression was more specific for the 1p/19q codeletion in HGG compared with LGG.

Biologic Relevance of INA Expression
The finding that INA may also be expressed in cases of isolated/partial deletions of 1p or 19q is important from a biologic point of view. The frequent loss of 1p/19q in oligodendrogial tumors suggests that these chromosomal arms carry as-yet-undefined tumor suppressor genes, the absence of which is a key event in the pathogenesis of oligodendrogliomas. However, the deletion of tumor suppressor genes does not provide an explanation for the favorable clinical behavior in 1p/19q codeleted oligodendrogial tumors, indicating that other genes within the relatively large region of LOH on 1p and 19q are involved. This hypothesis is compatible with data on small interstitial deletions on 1p (11), which have been associated with worse clinical outcome instead of longer survival. Unfortunately, little is known about the functional role of INA and potential interacting signaling molecules in primary brain tumors. Some studies indicate

**FIGURE 1.** Spectrum of immunohistochemical α-internexin (INA) expression. **(A)** Low-magnification overview showing an INA-stained section of a grade II oligodendroglioma (O) with 1p/19q codeletion and bipheric INA expression. **(B-E)** Corresponding histologic fields from representative areas indicated in **A** with strong **(B, C)** and no **(D, E)** INA expression, respectively. **(F-H)** A grade IV glioblastoma with oligodendrogial component and 1p/19q codeletion with bipheric INA expression. The border between areas with low and high INA expression is shown at low magnification **(F)**; histologic details of both areas are shown at higher magnification **(G, H)**. **(I, J)** Typical grade III oligoastrocytoma (OA) with 1p/19q codeletion and strong INA expression in a scattered distribution. **(K, L)** Typical grade II oligodendroglioma with 1p/19q codeletion displaying strong INA expression in a scattered distribution. The underlying reticular background is due to staining of preexisting neuronal tissue. **(M)** A grade III anaplastic astrocytoma with isolated 19q deletion showing scattered INA-positive tumor cells corresponding to weak INA expression. **(N)** A grade III anaplastic OA with 1p/19q codeletion but without INA expression. Scale bars = 200 μm **(F, I, K, M, N)**; 50 μm **(B-E, G, H, J, L)**.
 Eigenbrod et al

J Neuropathol Exp Neurrol • Volume 70, Number 11, November 2011

Copyright © 2011 by the American Association of Neuropathologists, Inc. Unauthorized reproduction of this article is prohibited.
functional roles for INA in neuronal differentiation (19, 20), neurite outgrowth (21), and axonal transport processes (22). Therefore, INA has been introduced as a marker for developing and mature neurons during neurogenesis. Expression of INA in oligodendroglial tumors might thus originate from bipotential precursor cells capable of differentiating in both neurons and oligodendrocytes. Indeed, this fate has been described for Olig-2 positive precursor cells in the ventral ventricular zone of the spinal cord (23). Furthermore, Olig-2 expression seems to be more intense and uniform in high-grade oligodendroglial tumors (24). Analogous results were found in our study regarding INA, that is, the expression showed a higher specificity in HGG versus LGG. Furthermore, it was accompanied by a higher proliferation activity in all 10 biphasic tumors analyzed. These observations fit well with the model of tumor cells frequently reexpressing earlier developmental markers on dedifferentiation.

**INA Expression in OMT**

We also investigated the applicability of INA IHC in the differential diagnosis of tumors mimicking an oligodendroglial phenotype. Although primary brain tumors only occasionally showed weak INA staining, renal cell carcinoma metastases frequently exhibited weak INA expression. This is consistent with previous studies reporting coexpression of neuronal intermediate filaments in nonneuronal tumors (25). In contrast to oligodendroglial tumors, no OMT case displayed strong INA expression. Thus, strong INA expression would militate against the diagnosis of an OMT.

**FIGURE 2.** Histologic and immunohistochemical analysis of a World Health Organization grade III oligodendroglioma with biphasic α-internexin (INA) expression. Left column: representative area of strong (s) INA expression, accompanied by high proliferation activity (Ki67) and an obvious oligodendroglial phenotype with typical honeycomb pattern with hematoxylin and eosin staining, faint immunopositivity for glial fibrillary acidic protein (GFAP) and strong perinuclear positivity for microtubule-associated protein 2 (MAP2). Right column: representative area without (n) INA expression, accompanied by lower proliferation activity (Ki67), and less prominent oligodendroglial phenotype demonstrated by hematoxylin and eosin, GFAP, and MAP2 staining. Scale bar = 50 μm.

**FIGURE 3.** Correlation of α-internexin (INA) expression with 1p/19q status and histologic subgroups. (A) INA expression is associated with glioma showing an oligodendrogial and mixed oligoastrocytic differentiation but not with pure astrocytic differentiation. (B) INA expression is associated with the 1p/19q codeletion. A trend toward INA expression is also observed in cases with isolated/partial deletions of 19q. Cases with isolated/partial expression of 1p display a distribution similar to that of cases without any deletion. The data shown refer to all gliomas (n = 83) listed in Table 1. A indicates astrocytoma; GBM, glioblastoma without oligodendrogial component; GBMO, glioblastoma with oligodendrogial component; O, oligodendroglioma; OA, oligoastrocytoma.

**FIGURE 4.** Microsatellite analysis of a case with biphasic α-internexin (INA) expression. Upper gel markers: 1, D1S1608; 2, D1S1592; 3, D1S458; 4, D1S1161; 5, D1S1184. Lower gel markers: 6, D19S718; 7, D19S433; 8, D19S601; 9, D19S559; 10, D19S431, 100-bp ladder. For each marker, polymerase chain reactions derived from peripheral blood leukocytes were loaded on the left lane (C); polymerase chain reactions from INA-strongly positive areas in the middle lane (S), and polymerase chain reactions from INA-negative areas in the right lane (N). Eight of 10 markers (nos. 1, 2, 4, 5, 6, 7, 8, and 10) were informative and showed loss of chromosomal material in areas with strong INA expression, as well as in areas without INA expression.

Taken together, our data confirm a strong association between the 1p/19q codeletion characteristic for oligodendrogial tumors, and INA, especially in cases of strong INA expression.
or high-grade tumors. Therefore, INA is a useful marker in the differential diagnosis of oligodendroglial versus astrocytic tumors. This is of particular interest in small stereotactic biopsy specimens in which the typical “honeycomb” pattern of oligodendroglial tumors is often less obvious. Furthermore, INA is a useful marker in the differential diagnosis of OMT because its expression in these tumors is weak, if at all. Our data also provide a note of caution regarding the use of INA IHC as a replacement for classic molecular-genetic analysis; it has limited diagnostic specificity because it can also be found in cases of isolated/partial deletions of 19q or 1p or cases without deletion. In addition, it can be heterogeneously expressed even in tumors with 1p/19q codeletion. Therefore, INA staining cannot replace the molecular genetic analysis of the 1p/19q status. Finally, our data on INA expression might enhance understanding of the biology of 1p/19q codeleted tumors.

ACKNOWLEDGMENTS

The authors thank Angelika Henn and Brigitte Kraft, Elzbieta Staniszewski, and Michael Ruiter for expert technical assistance.

REFERENCES