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Review Article

Alfa Internexin Expression in a Series of 137 Gliomas and its Correlation with Oligodentroglial Morphology IDH1, P53 SYN and EGFR Expression

Healthcare

Keywords: Glial tumuors, Alpha-internexin, IDH1, P53, Synaptophysine, EGFR protein.

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Abstract

Background: Distinguishing glial subtypesbased on nuclear and cellular morphology alone is subjective,with significant interobserver variability, even among highlyexperienced neuropathologists. Geneticsubtyping of a given histological phenotype and robust biomarkershas improved the diagnostic and prognostic assessment. Recently, IDH1 (more rarely IDH2) mutations have been found in nearly 40% of gliomas and strongly predict lower grade in histology and better outcomes. Aim: To evaluate if expression of alpha-internexin (INA) can be used a reliable diagnostic, prognostic and cost-effectivemarker, a proneural gene-coding neurofilament interacting protein significantly correlated with oligodendroglial phenotype, 1p/19q codeletion as well as higher chemosensitivity and better prognosis to our study population. Matherial: We studied INA expression in 137gliomasand correlated it with pure oligodentroglialhistology, IDH1, p53, EGFR and SYN expression by immunohystochemestry.Results: INA was expressed in 72.2% of grade II oligodendrogliomas (n = 22), 62.5% of grade III oligodendrogliomas (n = 16), 57.2% of grade II oligoastrocytomas (n = 7), 66.7% of grade III oligoastrocytomas (n = 6), 66.7% of glioblastomas with oligodendroglial component (n = 12), 0% of grade I astrocytomas (n = 13), 0% of grade II astrocytomas (n = 4), 0% of grade III astrocytomas (n = 12) and 2.5% of glioblastomas and gliosarcomas (n = 40).INA was expressed by 27(71.1%) of pure oligodentrogliomas(n=38) versus 17(17.2%) of non pureoligodentrogliomas(n=99),Chi square was $p < 10\text{-}4$; Cramer's V was 0.517; $p < 10\text{-}4$, which show a very strong relationship.INA was expressed by 32(45.1%) of gliomas with IDH1 mutation (n=71) versus 12(18.2%) of gliomas without IDH1 mutation (n=66), Chi square was $p < 0.001$; Cramer's V was 0.288; $p < 0.001$, which show a very strong relationship. INA was expressed by 26(27.4%) of gliomas with P53 mutation (n=95) versus 18(42.9%) of gliomas without P53 mutation (n=42), Chi square wasp=0.05 which show they were negatively correlated. INA was expressed by 30(50.0%) of gliomas with SYN expression (n=60) versus 14(18.2%) of gliomas without SYN expression (n=77), Chi square was $p < 10\text{-}4$; Cramer's V was 0.338; $p < 10\text{-}4$, which show a very strong relationship. INA was expressed by 12(27.3%) of gliomas with EGFR expression (n = 44) versus 32(34.%) of gliomas without EGFR expression (n=44), Chi square was p=0.05 which show they were negatively correlated. Conclusion: INA expression is a fast, cheap and reliable diagnostic and prognostic marker, which helpsindentify patients of different prognostic groups in diffuse gliomas and should be used routinely in the pathologic diagnosis of glial tumours.

Introduction

Glial tumors, which include astrocytomas, oligodendrogliomas,mixedgliomas, and ependymomas, are the mostcommon primary malignancy of the central nervous system(CNS) and account for 78% of cases globally in neurosurgery (1). From a therapeutic and especially prognostic point of view, the differentialdiagnosis among these entities is of clinical importanceto predict biological behavior and to determine the optimaltreatment protocol. Distinguishing glial subtypesbased only on morphologic critheria of the WHO system of 2007 as nuclear and cellular changes(5), is very subjective,with significant inter /intra observer variability, even among highlyexperienced neuropathologists(3, 4). Furthermore, even in a same histological entity, tumors harboring different molecular profiles (IDH1/2 mutations and 1p/19q codeletion) (7,8), show different survival pattern. (6).

It has been well demonstrated that 1p/19q codeletion, P53 and epidermal growth-factor-receptor (EGFR) gene amplification are mutually exclusive in gliomas (9-12). Internexin alpha (INA), a neuronal intermediate filament is found in most gliomas especially those with oligodendroglial features and 1p19q codeletion and seems to represent a valuable diagnostic and prognostic marker in clinical routine (13-14). The aim of this study was to evaluate the possible relationship of INA with pure oligodendroglial phenotype, P53 expression, IDH1 mutation, SYN and EGFR immunoreactivity which can further help identifying and stratifying patients according to their clinical, pathological and survival characteristics.

Materials and Methods

Histological cases were selected from pathologically proven low grade and high grade gliomas operated at the University Hospital Center "Mother Teresa ". From this database, only cases with thorough information on immunohistochemical expression of IDH1, P53, SYN, INA and EGFR were selected. A total of 137 patients who underwent a neurosurgical operation from 2010-2014, were included when complete clinical information and tissue paraffin blocks were available. Tumor histology was classified according to the 2007 WHO classification.

Each tumor tissue sample was fixed with formalin and embeddedin paraffin. Representative paraffin blocks were selectedand mounted on slides with hematoxylin and eosin (H&E) stainingbefore they were prepared for the tissue microarray. Coresfrom representative areas of each tumor were marked on both anH&E stained tissue section and an original donor block. 4-mm diameter tissue cores were extracted from the marked areaof each donor block and placed in tissue cores. Five 4-μm thicksections were cut from each array block.

Immunohistochemistry was performed on these sections. The immunolabelling technique was performed by a Bench Mark XT automated tissue staining system. The markers used, their clones, manufacturers and dilutions are shown in Table1.

Antibody	Clone	Manufacturer	Dilution
P53	318-6-11	DAKO	1:50
IDH1	H09	DIANOVA	1:50
INA	ID2	ACRIS	1:1000
SYN	SP11	VENTANA	ready to use
EGFR	3C6	VENTANA	ready to use

Table 1. The markers used in this study, their clones, manufacturers and dilutions.

Immunoreactivity of p53 was expressed in the percentage of immunostained nuclei. Immunoreactivity of INA, IDH1, SYN and EGFR was classified as positive if >10% cells were positive, and negative if <10% cells were positive.Immunoreactivity for INA was considered positiveif intracytoplasmic crescents or paranuclear dots were present.

Presentation of data is done through tables and diagrams. Data processing was done with the statistical program SPSS 20. Statistical techniques selectedwere method of X2 (chi -square test), correlation methods by Pearson, Spearman and Cramer's.

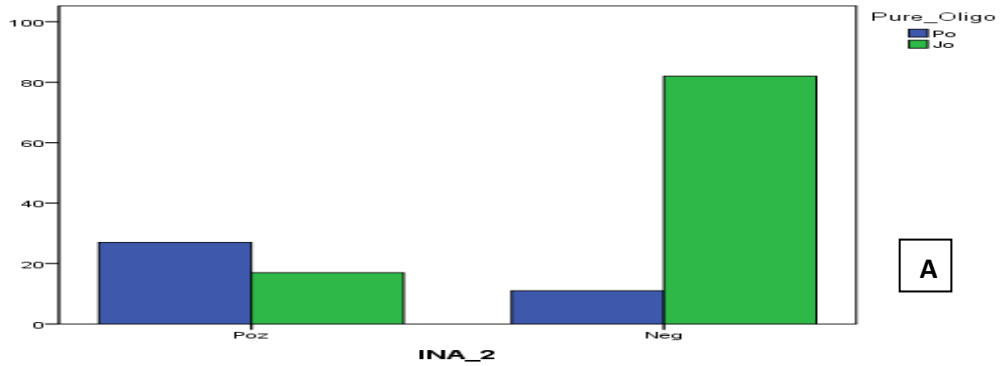
Results

The histology, INA, IDH1, p53, SYN, EGFR expression of the 137 gliomasare reported in Table 2. INA was expressed in 72.2% of grade II oligodendrogliomas (n = 22), 62.5% of grade III oligodendrogliomas (n = 16), 57.2% of grade II oligoastrocytomas (n = 7), 66.7% of grade III oligoastrocytomas (n = 6), 66.7 % of glioblastomas with oligodendroglial component (n = 12), 0% of grade I astrocytomas (n = 13), 0% of grade II astrocytomas (n = 4), 0% of grade III astrocytomas (n = 12) and 2.5% of glioblastomas and gliosarcomas (n = 40).

	INA	**IDH1**	**P53**	**SYN**	**EGFR**
Pilocytic astrocytoma (n=13)	0/13 (0%)	0/13 (0%)	2/13 (15.4 %)	1/13 (7.7%)	0/13 (0%)
Grade2 astrocytoma (n=4)	0/4 (0%)	3/4 (75%)	3/4 (75%)	1/4 (25%)	1/4 (25%)
Grade 3 astrocytoma (n =12)	0/12 (0%)	10/12 (83.3%)	12/12 (100%)	2/12 (16.7%)	2/10 (16.6%)
Glioblastoma (n =40)	1/40 (2.5%)	14/40 (35%)	40/40 (100%)	11/40 (19%)	22/40 (55%)
Glioblastoma with oligo component (n =12)	8/12 (66.7%)	5/12 (41.7%)	12/12 (100%)	8/12 (66.7%)	6/12 (50%)
Grade2 oligodendroglioma (n = 22)	17/22 (77.2%)	16/22 (72.7%)	2/22 (18.2%)	16/22 (72.7%)	2/22 (9.1%)
Grade3 oligodendroglioma (n =16)	10/16 (62.5%)	10/16 (62.5%)	6/16 (37.5%)	11/16 (68.8%)	9/16 (56.3%)
Grade2 oligoastrocytoma (n =7)	4/7 (57.2%)	5/7 (71.4%)	7/7 (100%)	4/7 (57.2%)	0/7 (0%)
Grade3 oligoastrocytoma (n = 6)	4/6 (66.7%)	5/6 (83.3%)	6/6 (100%)	3/6 (50%)	1/6 (16.7%)

Table 2. The histology, INA, IDH1, p53, SYN, EGFR expression of the 137 gliomas.

INA was expressed by 27 (71.1%) of pure oligodentrogliomas (n = 38) versus 17 (17.2%) of non pureoligodentrogliomas (n = 99) .INA was expressed by 32 (45.1%) of gliomas with IDH1 mutation (n = 71) versus 12 (18.2%) of gliomas without IDH1 mutation (n = 66) .INA was expressed by 26 (27.4%) of gliomas with P53 mutation (n = 95) versus 18 (42.9%) of gliomas without P53 mutation (n = 42). INA was expressed by 30 (50.0%) of gliomas with SYN expression (n = 60) versus 14 (18.2%) of gliomas without SYN expression (n = 77). INA was expressed by 12 (27.3%) of gliomas with EGFR expression (n = 44) versus 32 (34.%) of gliomas without EGFR expression (n = 44).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Jo
100
80
60
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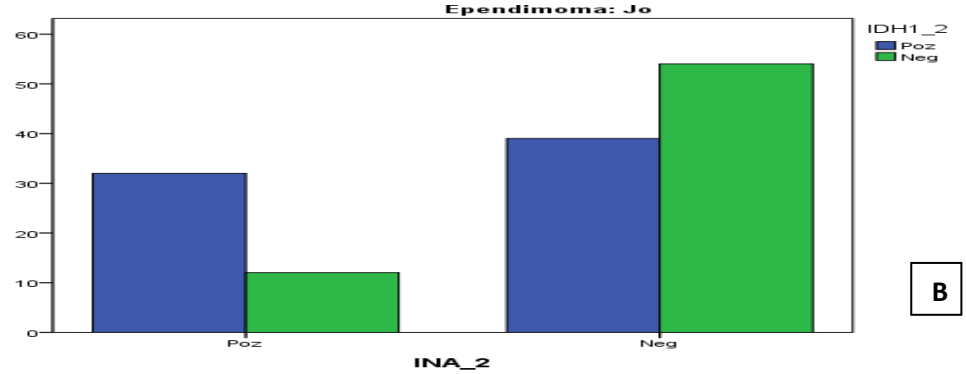
Neg
INA_2

A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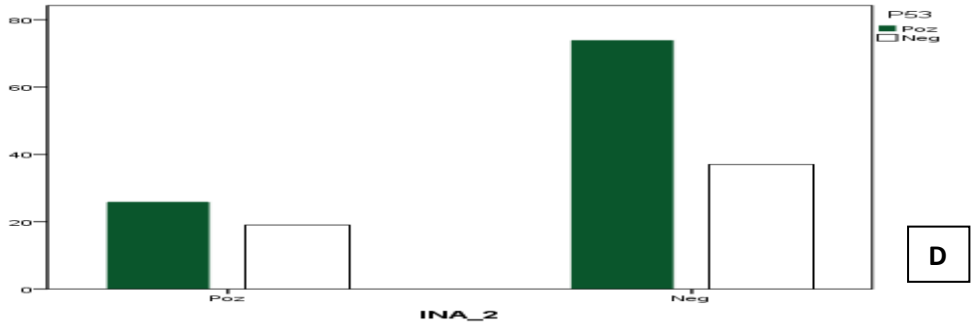
INA_2

B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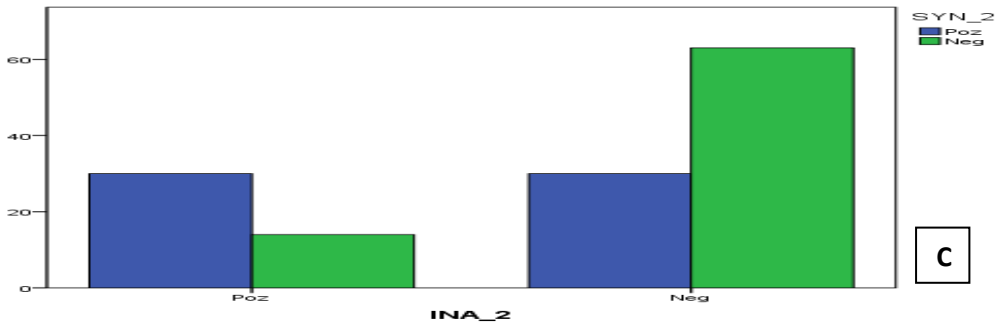
60
40
20
0
Poz
Neg
INA_2

D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INA_2

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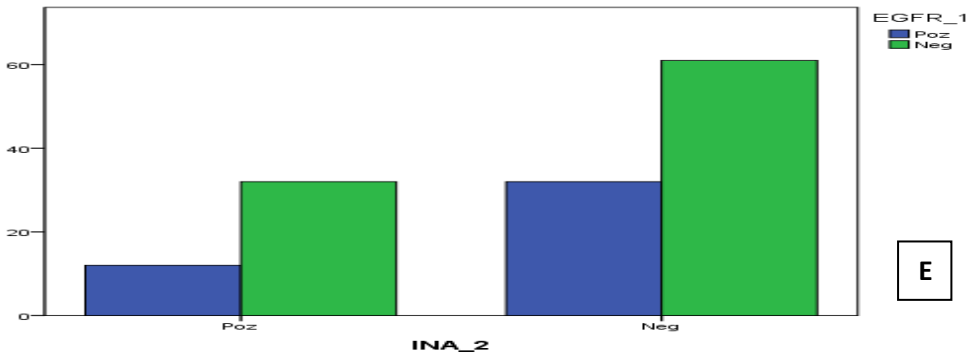

Figure1: A) Correlations with alpha-internexin (INA) and pure oligodendrogliomas.B) Correlations with alpha-internexin (INA) and IDH1 mutation C) Correlations with alpha-internexin (INA) and P53 expression D) Correlations with alpha-internexin (INA) and SYN immunoreactivity E) Correlations with alpha-internexin (INA) and EGFR immunoreactivity

INA expression was tightly related to pure oligodendroglial phenotype (Chi square was $p < 10^{-4}$; Cramer's V was 0.517; $p < 10^{-4}$), to IDH1 mutation, (Chi square was $p < 0.001$; Cramer's V was 0.288; $p < 0.001$), whereas it wasnegatively correlated with p53 expression ($p = 0.05$). In the diagrams below are shown theimmunoreactivityprophiles of INA, IDH1 and P53 in oligodentrogliomas, astrocytomas and mixed gliomas in our study. Combining INA, DH1, P53 we identified the likelihood of pure oligodendrogliomas with the presence of 1p/19q co-deletion.

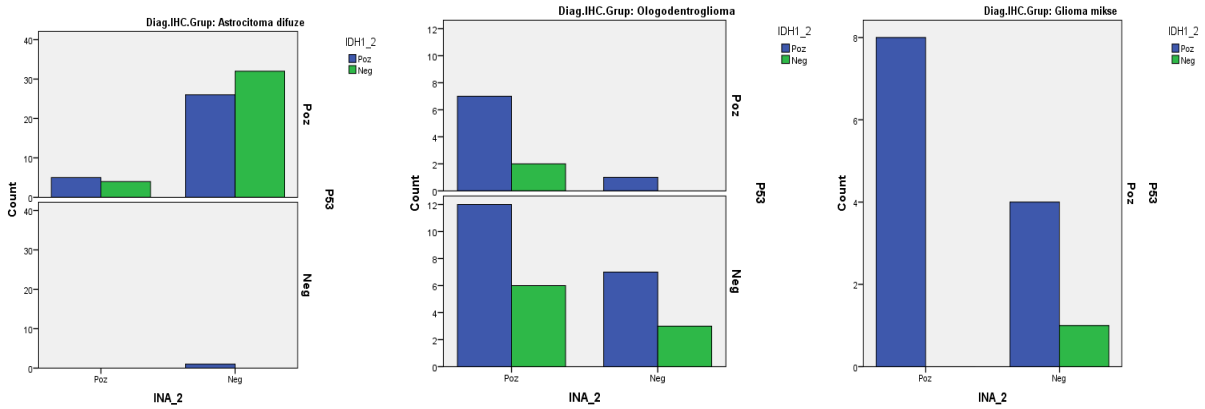

Figure 2. Distribution of immunoprophiles IDH +/p53-/INA-; IDH +/p53 +/INA-; IDH +/p53-/INA+; andIDH -/p53-/INA- in difusegliomas.

Discussion

For the prognostic and predictive values of the adult gliomas, oligodendroglial phenotype is sufficient to determine a treatment option (13). The oligodendroglial phenotype indicates a better prognosis and more chemosensitivity than astrocytic tumors, but the histological diagnosis is subjective and suffers from interobserver variability and discrepancies (2, 14). In the other hand, the 1p19q codeletion, the MGMT promoter methylation and the IDH1 mutation are currently the most important prognostic biomarkers in adult gliomas. However, their assessmentrequires molecular biology techniques that in contrast to immunohistochemistry are not available worldwide and not always feasible. 1p/19q codeletion status, which is related to an unbalanced

t(1;19) (q10;p10) translocation, is generally mutually exclusive with P53 mutation and EGFR gene amplification (15) and is a diagnostic, prognostic, and predictive marker for ODGs.

Comparative genomic hybridization array analysis (14, 18), loss of heterogeneity analysis, multiplex ligation-dependent probe amplification (19, 20), and FISH are available to identify a 1p/19q deletion (2, 6), but they have been rarely performed in clinical practice. Moreover, these are complex, sophisticated and expensive techniques, and all have limitations, such as contamination with normal cells or poor sensitivity and specificity (21,22). Therefore, diagnostic, prognostic, and predictive markers are needed that can replace 1p/19q deletion. INA, which is mainly studied in further studies leads to the accumulation of neurofilamentsand is tightly related to oligodendroglial histology and to 1p19q codeletion. INA expression can be assessed quickly from a simple biopsy, is reliable and inexpensive and does not need any special equipment.(13).

In our study we demonstrate that INA expression is overrepresented in tumours with IDH1 mutation (45.1%, $p<0.001$) and underrepresentedin tumours with p53 expression (27.4%, $p> 0.05$) and EGFR amplification (27.3%, $p> 0.05$). We also noted that INA expression was overrepresented intumours with Syn expression (50.0%, $p < 10^{-4}$) The absence of INA expression in an oligodendroglialtumour makesthe 1p19q codeletion very unlikely particularly if the tumour is p53 positive. In contrast, a tumour expressing INA has a 70%chance to be 1p19q codeleted and an 80% chance if p53 is negative (13). In our study 11 cases of ODG were negative for INA 29.9 % and 8of 38 ODG were positive for P53 that is 21.05 % of them. In our study, INA overexpression cases were also present among the EGFR amplificated cases. INA overexpression is common in ODGs (62.5%-77.2%)but not inastrocytomas and GBMs, which have a lower frequency of INA overexpression (2.5%). INA was overexpressed in Glioblastomas with oligodendroglial component(66.7%).In contrast, EGFR overexpression is common in GBMs, which have a lower frequency of INA overexpression (27.3%), and is low in grade III ODGs (16.7%).

Conclusions

We know that our study have some limitations such as a small study group, a high percentage of oligodendroglialvsastrocytic tumors (bias selection or intra-observer bias), unavailable data about 1p/19q co-deletion and subsequent lack of evidence of this interesting correlation (INA and 1p/19q), but beside this we can confirm that INA expression is tightly related to oligodendroglial histology. We demonstrate that INA expression is overrepresented in tumors with IDH1 mutation and SYN expression (complementary information**)**,INA expression is underrepresented in tumors with p53 expression and EGFR amplification. We also show that combining INA, IDH1, P53may help identify the likelihood of oligodendrogliomas with 1p/19q co-deletion with a higher sensitivity and specificity.

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