Use of molecular markers immunoreactivity can help stratify glioma's patients into groups within the same grade and histological entity

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Abstract

Aim: Glial tumors are evaluated pathologically, based on the World Health Organization (WHO) or the St Anne - Mayo system. In recent years, some molecular markers are used to stratify patients into groups within the same grade and histological entity, in order to determine in the best possible way, the prognostic factors for each patient and to give them a targeted treatment. The most used markers are: protein 53 (p53), Isocitrate dehydrogenase-1 (IDH1), Internexin-Alpha (INA), Synaptophysin-syn, and Her-1 (EGFR).
Methods: We studied INA expression in 137 gliomas and correlated it with histology, IDH1, p53, EGFR and SYN expression by immunohystochemestry.
Results: INA was expressed by 71.1% of pure oligodentrogliomas versus 17.2% of non-pure oligodentrogliomas. In addition, INA was expressed by 45.1% of gliomas with IDH1 mutation versus 18.2% of gliomas without IDH1 mutation. Furthermore, INA was expressed by 27.4% of gliomas with P53 mutation versus 42.9% of gliomas without P53 mutation. Also, INA was expressed by 50.0% of gliomas with SYN expression versus 18.2% of gliomas without SYN expression. Finally, INA was expressed by 27.3% of gliomas with EGFR expression versus 34.0% of gliomas without EGFR expression. Our study has demonstrated that molecular markers used are very useful for determining the entity in glial tumor (P=0.0001) and even for determining the grade in these tumors (P=0.0001).
Conclusion: Use of molecular markers is a fast, cheap and reliable diagnostic and prognostic method, which helps identify patients of different prognostic groups in diffuse gliomas and should be used routinely in the pathologic diagnosis of glial tumors.

Keywords: alpha-internexin, EGFR protein, glial tumors, IDH1, P53, synaptophysin.

Introduction

Glial tumors, which include astrocytomas, oligodendrogliomas, mixed gliomas, and ependymomas, are the most common 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 differential diagnosis among these entities and their histologic grades is of clinical importance to predict biological behavior and to determine the optimal treatment protocol. Distinguishing glial subtypes based only on morphologic criteria of the WHO system of 2007 as nuclear and cellular changes (2), is very subjective, with significant inter/intra observer variability, even among highly experienced neuropathologists (3,4). Furthermore, even in a same histological entity and tumor grade there are tumors harboring different molecular profiles (IDH1/2 mutations and 1p/19q codeletion) (5,6), which show different survival patterns. (7). It has been well demonstrated that 1p/19q codeletion, P53 and epidermal growth-factor-receptor (EGFR) gene amplification are mutually exclusive in gliomas (8-10). So are the IDH1 mutation and the EGFR amplification. Isocitrate dehidrogenase-1 mutation (IDH1) is founded in the major part of diffuse gliomas, but infantile gliomas and primary glioblastoma and is related with elevated overall survival and secondary glioblastoma (8,9). 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, prognostic and predictive marker in clinical routine (12-13). EGFR is present in 30-40% of primary glioblastomas, is related with a worst prognosis and seems to predict e better reaction to avastine treatment (15). 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. Another aim of this study is to show the value of these markers in optimizing the pathological diagnosis for glial tumors in daily routine.

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 embedded in paraffin. Representative paraffin blocks were selected and mounted on slides with hematoxylin and eosin (H&E) staining before they were prepared for the tissue microarray. Cores from representative areas of each tumor were marked on both an H&E stained tissue section and an original donor block. 4-mm diameter tissue cores were extracted from the marked area of each donor block and placed in tissue cores. Five 4-μm thick sections 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 Table 1.

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

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

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 positive if 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 selected were 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 gliomas are 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 oligo-

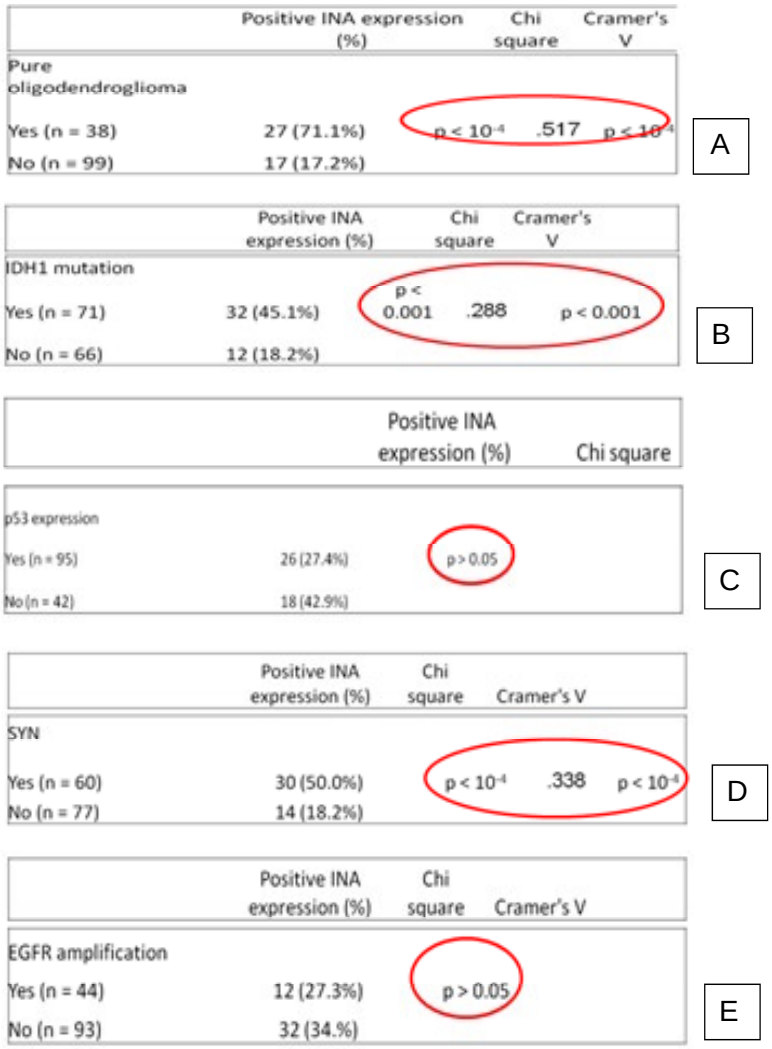
Table 2. The histology, INA, IDH1, p53, SYN, EGFR expression of the 137 gliomas

	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%)

astrocytomas (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).

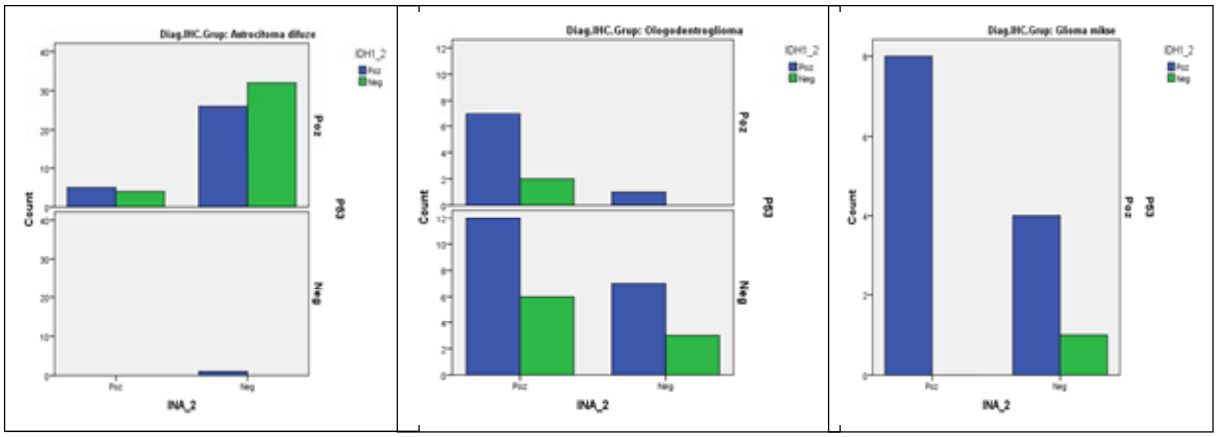
INA was expressed by 27 (71.1%) of pure oligodentrogliomas (n=38) versus 17 (17.2%) of non pure oligodentrogliomas (n=99). INA was expressed by 32 (45.1%) of gliomas with IDH1 mutation (n=71) versus 12 (18.2%) of gliomas without IDH1 mutation (n=66). INA was expressed by 26 (27.4%) of gliomas with P53 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).

Figure 1. Correlations of alpha-internexin (INA) with oligodendrogliomas (A), IDH1 mutation (B), P53 expression (C), SYN immunoreactivity (D) and EGFR immunoreactivity (E)



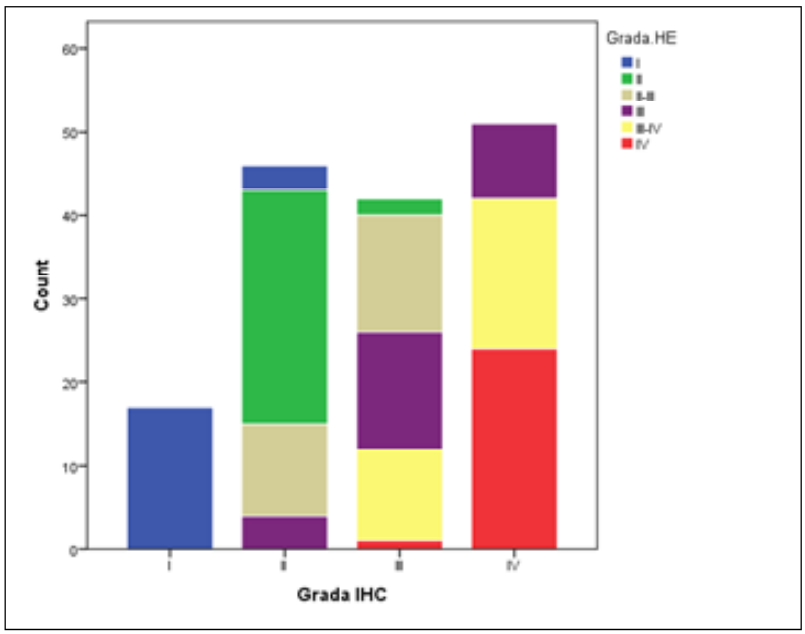
INA expression was tightly related to pure oligodendroglial phenotype (Chi square was $p < 10^{-4}$; Cramer's V was 0.517; $p < 10^{-4}$), to IDH1 mutation, (Chi square was $p < 0.001$; Cramer's V was 0.288; $p < 0.001$), whereas it was negatively correlated with p53 expression ($p = 0.05$). In the diagrams below are shown the immunoreactivity profiles 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 and showed which profile predominates in each entity.

Figure 2. Distribution of immunoprofiles IDH +/p53-/INA-; IDH +/p53 +/INA-; IDH +/p53-/INA+; and IDH -/p53-/INA- in diffuse gliomas



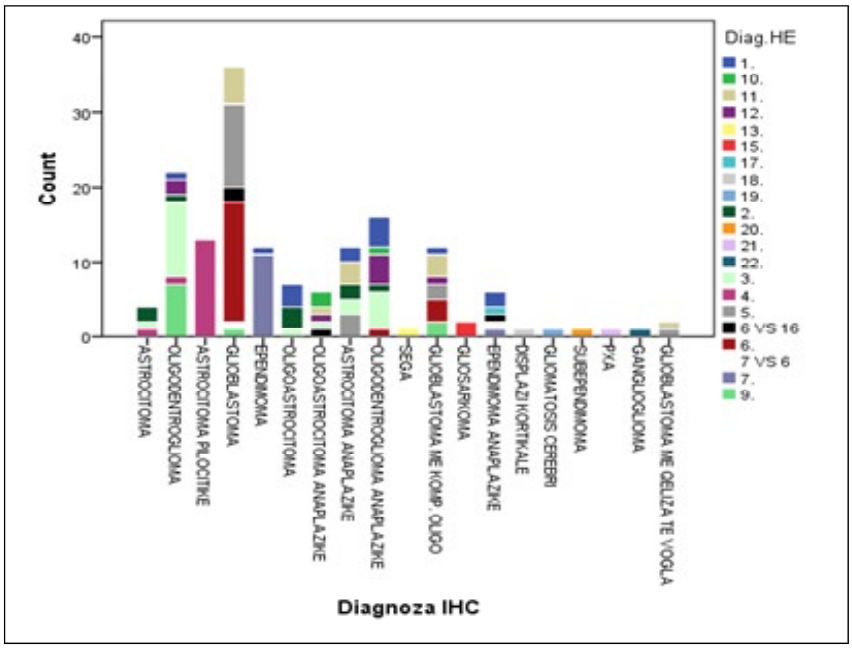
In conclusion, the following charts will show how the tumors entity is changed and the histological grade of tumors in the cases included in the study, after application of molecular markers, in comparison with the conventional method with H-E.

Figure 3. The distribution of tumors by grade in H-E compared with distribution of tumors by grade in IHC after the use of molecular markers



The graph shows that the tumor markers, used in the study through IHC method, are very useful for determining the tumor grade in glial tumors, with a Cramer's V = 0761 and P = 0.0001.

Figure 4. The distribution of tumors by entities in H-E, compared with the distribution by entities in IHC



The figure shows that tumor markers used in the study are very useful for determining the entity in glial tumors, with a Cramer's V = 0761 and P = 0.0001.

Discussion

For the prognostic and predictive values of the adult gliomas, oligodendroglial phenotype is sufficient to determine a treatment option (11). 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 (12,13). 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 assessment requires 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 (14) and is a diagnostic, prognostic, and predictive marker for ODGs. Comparative genomic hybridization array analysis (15), loss of heterogeneity analysis, multiplex ligation-dependent probe amplification (16,17), and FISH are available to identify a 1p/19q deletion (13,18), 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 (10,19). 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 neurofilaments and 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 (11). In our study we demonstrate that INA expression is overrepresented in tumors with IDH1 mutation (45.1%, $p< 0.001$) and underrepresented in tumors with p53 expression (27.4%, $p> 0.05$) and EGFR amplification (27.3%, $p> 0.05$). We also noted that INA expression was overrepresented in tumors with Syn expression (50.0%, $p < 10^{-4}$). The absence of INA expression in an oligodendroglial tumor makes the 1p19q codeletion very unlikely particularly if the tumor is p53 positive. In contrast, a tumor expressing INA has a 70% chance to be 1p19q codeleted and an 80% chance if p53 is negative (11). In our study 11 cases of ODG were negative for INA 29.9 % and 8 of 38 ODG were positive for P53 that is 21.05 % of them. In our study, INA overexpression cases were also present among the EGFR amplification cases. INA overexpression is common in ODGs (62.5-77.2%) but not in astrocytomas 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%). Our study has demonstrated that molecular markers used are very useful for determining the entity in glial tumor with a Cramer's V = 0761 and P = 0.0001 and even for determining the tumor grade in these tumors, with a Cramer's V = 0761 and P = 0.0001.

Conflicts of interest: None declared

Conclusion

Our study has some limitations including the relatively small group, a high percentage of oligodendroglial vs. astrocytic 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, P53 may help identify the likelihood of oligodendrogliomas with 1p/19q co-deletion with a higher sensitivity and specificity. We demonstrated that in astrocytomas predominates the group with P53+/INA-, in oligodentrogliomas predominates the group P53+/INA- and in mixed gliomas predominates the group P53+/INA+. We also demonstrated that molecular markers are very useful in optimizing the diagnosis of glial tumors in daily routine, in terms of tumor entity and tumor grade, with a Cramer's V = 0761 and P = 0.0001.

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