Original article

Folia Neuropathologica

Expression of mutated isocitrate dehydrogenase-1 in gliomas is associated with p53 and EGFR expression

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Folia Neuropathol 2011; 49 (2): 88-93

Abstract

Gliomas are the most common primary brain tumours. Several independent studies have shown that isocitrate dehydrogenase 1 (IDH1) mutation in diffuse gliomas is associated with a more favourable patient outcome. The aim of this study was to evaluate the prognostic relevance of an antibody specifically detecting the R132H point mutation of IDH1 in tissue sections in a large series of human gliomas. Surgical specimens of 220 consecutive patients with infiltrative low and high-grade gliomas were included in this retrospective study. In multivariate analysis, IDH expression did not reach significance ($p = 0.122$) in regard to prognosis, in contrast to WHO grade and age at time of surgery ($p < 0.001$, Cox regression). A significant correlation of p53 expression to mutated IDH1 and histological grading and an inverse correlation to truncated EGFR expression were observed ($p < 0.001$, Mann-Whitney test). In sum, our results indicate that IDH^{R132H} mutation correlates significantly with p53 and inversely with EGFR mutations. Further studies should investigate whether these correlations reflect involvement of these three molecules in a common signalling pathway.

Key words: *gliomas, isocitrate dehydrogenase 1 mutation, p53 expression, EGFR expression.*

Introduction

Gliomas are the most common primary brain tumours [11]. Recent genome-wide mutational analysis has demonstrated that 12% of glioblastomas (GBM) harbour mutations of the cytosolic $NADP^+$-dependent isocitrate dehydrogenase (IDH) gene [15], an enzyme involved in isocitrate oxidative decarboxylation. Most GBM mutations replace arginine in position 132 with histidine (R132H), thus producing a dominant-negative form of the protein [15,19]. IDH1 mutations were more frequent in secondary GBM [1,8,9,15,16], anaplastic astrocytomas, and grade II gliomas [1,8,9,16,17]. By contrast, IDH1 mutations have only been found in a few types of other CNS tumours.

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IDH1 and IDH2 mutations are found in both astrocytomas and oligodendrogliomas, but accompanying genetic alterations differ. Most low-grade diffuse astrocytomas have both p53 and IDH1 mutations, whereas most oligodendrogliomas show both IDH1 mutations and chromosome 1p/19q co-deletion [17].

In several studies it has been demonstrated that an IDH1 mutation is associated with good prognosis in patients with gliomas [15,16,18]. Median overall survival in GBM patients with IDH mutations was significantly longer than that in GBM patients with wild-type IDH1 and IDH2 [15]. Mutations of IDH have also been shown to be associated with improved prognosis in patients with anaplastic astrocytomas [15,16]. Furthermore, immunohistochemical detection of IDH expression is considered as a very useful tumour cell marker in gliomas with an *IDH* mutation [5].

Recently, an antibody specifically detecting the R132H point mutation of IDH1 in tissue sections has become available [7]. The aim of this study was to evaluate the prognostic relevance of this *IDH* mutation assessed by this antibody in a large consecutive series of low- and high-grade human gliomas.

Material and methods

Surgical specimens of 220 patients used previously [3,12] with low- and high-grade infiltrative gliomas were included in this retrospective study. These patients comprised all glioma cases treated in the period from July 2004 to February 2008 at St. Ivan Rilski University Hospital. In all patients with glioblastoma, there was no clinical history of a preceding glioma with higher differentiation. Standard postoperative adjuvant radiotherapy (50-60 Gy) was applied to all patients with grade III and grade IV glioma, representing the standard adjuvant therapy in Bulgaria in the given period.

One hundred and five patients (47.7%) were female, 115 (52.3%) were male. Mean age of patients was 52.9 ± 13.8 years. One hundred and forty-five (65.9%) patients were classified as WHO grade IV, 32 patients (14.5%) as WHO grade III, and 43 (19.6%) as grade II.

In addition, from 6 patients (4 with grade II glioma, 2 with grade III) tissue from subsequent secondary glioblastomas was available.

Mean observation time was 978 ± 59 days (standard error). During this period 9 patients (20.9%) with initial grade II glioma, 21 (65.6%) with initial grade III glioma and 127 (87.6%) with glioblastoma died of their brain tumour.

Immunohistochemistry

Expression of IDH, p53 and EGFR was determined immunohistochemically in paraffin-embedded, paraformaldehyde-fixed specimens. Tissue microarrays (TMA) with fourteen 5 mm diameter samples per slide were used in this study. Histological slides, 4 µm in thickness, were deparaffinized in xylol. Endogenous peroxidase was blocked with methanol containing 0.3% hydrogen peroxide for 30 min.

The following antibodies were used: mouse monoclonal anti-human IDH1 R132H antibody (Dianova, clone H09, 1 : 10, 1 h at room temperature [RT]), mouse monoclonal anti-human p53 (Dako, clone DO-7, 1 : 50, 25 min at RT), mouse monoclonal binding to the type III truncated form of epidermal growth factor receptor (EGFR) without cross-reacting with the wild-type form (Novocastra, clone DH8.3, 1 : 100, 4°C overnight). The affinity-purified anti-IDH antibody reacts specifically with the IDH1 R132H point mutation in tissue sections from formalin-fixed brain tumour specimens [4,6,8].

A specimen was considered as positive for mutated IDH expression if the vast majority of glioma cells (> 80%) showed strong cytoplasmic and nuclear (Figs. 1A and B) staining, positive for p53 expression if strong nuclear staining was present (Figs. 1C and D), and positive for EGFR expression if strong cytoplasmic immunostaining (Figs. 1E and F) was observed.

Statistics

Chi-square test and Mann-Whitney test were used as appropriate. Overall survival was defined as the period from primary surgery until the death of the patient. Deaths from a cause other than glioma and survival until the end of the observation period were considered censored observations. Univariate analysis of overall survival was performed by Kaplan and Meier analysis or univariate Cox regression. Multivariate analysis of survival was performed by the Cox proportional hazards model. SPSS 17.0 (SPSS Inc., Chicago, IL) was used for all calculations. For all tests, a two-tailed $p \leq 0.05$ was considered as significant. Numbers given are mean values ± standard deviation, if not stated otherwise.

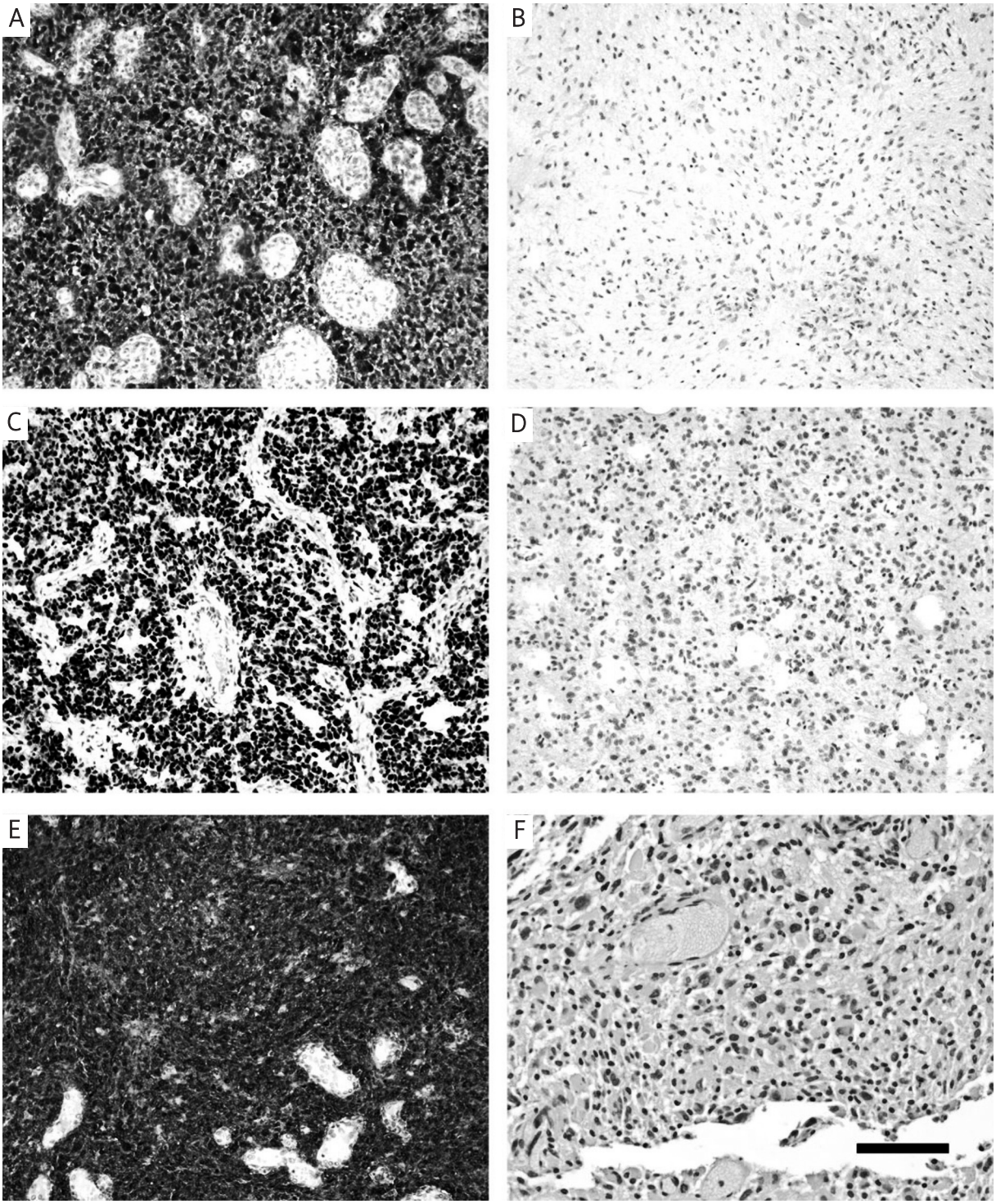

Fig. 1. Glioma specimens considered as positive (**A**) and negative (**B**) for IDH1 expression. Glioma specimens considered as positive (**C**) and negative (**D**) for p53 expression. Glioma specimens considered as positive (**E**) and negative (**F**) for EGFR expression. Immunoperoxidase. Scale bar = 100 μm.

Results

IDH

The results of immunohistochemistry are summarised in Table I. Fifty-one cases (23.72%) were positive for IDH^{R132H} (Fig. 1). Half of the histologically proven secondary glioblastomas were IDH^{R132H} positive (n = 3 out of 6 cases). Patients with mutated IDH positive tumours were significantly younger than those with mutated IDH negative ones ($p < 0.001$, Mann-Whitney test, mean age 45.3 ± 13.8 vs. 55.2 ± 13 years). Mean survival of wild type IDH (IDH^{wt}) GBM cases was 342 days and 326 in IDH^{R132H} cases. Mean survival of IDH^{R132H} glioma grade III cases was 773 days while IDH^{wt} cases survived 597 days. None of the above differences showed statistical significance.

In univariate analysis, IDH^{R132H}-positive tumours had a significantly better survival ($p = 0.023$, log rank test) (Fig. 2). In multivariate analysis, IDH expression did not reach significance ($p = 0.122$) in regard to prognosis, in contrast to WHO grade and age at time of surgery ($p < 0.001$, Cox regression). No significant influence of IDH status on survival was also seen when investigating grade II, III and IV gliomas separately ($p > 0.05$, Cox regression).

p53

Also patients with p53 positive tumours were younger than those with negative ones ($p = 0.013$, Mann-Whitney test, 48.8 ± 15.2 vs. 54 ± 13.2), while patients with EGFR expression were significantly older ($p < 0.001$, Mann-Whitney test, 59.8 ± 9.2 vs. 40.9 ± 14.3). No association of p53 expression with histological grading ($p > 0.05$, Mann-Whitney test) and EGFR expression ($p > 0.05$, Chi square test) was observed. A significant correlation to IDH status and histological grading was observed ($p < 0.001$, Mann-Whitney test): Median histological grading was III in IDH^{R132H} positive cases, while it was grade IV in IDH negative gliomas. A significant association of IDH and p53 expression was found ($p < 0.001$, Chi square test): In the subgroup of 169 IDH^{wt} cases, p53 positivity was seen in 25 cases (14.8%), while in the subgroup of 51 cases expressing IDH^{R132H}, p53 positivity was seen in 21 (41.2%) cases. Interestingly, in p53 positive tumours, all IDH positive cases (n = 21) were negative for EGFR expression.

EGFR

Although the median histological grading was IV in both EGFR negative and positive tumours, there was a significant association of EGFR positivity with higher grade gliomas ($p = 0.027$, Mann-Whitney test). A reverse association of IDH and EGFR expression was observed ($p < 0.001$, Chi square test). In the subgroup of 51 IDH^{R132H} positive cases only 2 cases (3.9%) expressed EGFR, while in the subgroup of 168 IDH^{wt} cases 46 (27.4%) showed EGFR expression. (Only 219 cases were investigated for EGFR expression, since no material was left in one case.)

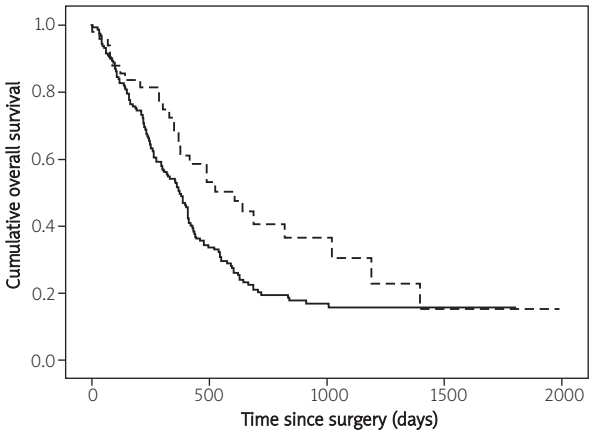

Fig. 2. Cumulative overall survival of patients (n = 220). Dotted line shows survival of patients with expression of IDH^{R132H}, full line survival of patients without IDH expression.

Table I. Results of immunostaining

All cases (n = 220)	IDH+	p53+	EGFR+*
WHO grade II (n = 43)	24 (55.8%)	11 (25.6%)	3 (7%)
WHO grade III (n = 32)	7 (21.9%)	8 (25%)	8 (25%)
WHO grade IV (n = 145)	20 (13.8%)	27 (18.6%)	37 (25.5%)*

**No tissue left for EGFR staining in one case*

Discussion

Several studies have shown that IDH1 mutation is an independent favourable prognostic marker in GBM and anaplastic gliomas after adjustment for other genomic profiles and treatment modality [13,15,19]. In contrast to those reports, the results in our consecutive series of glioma patients show that expression of mutant IDH is not an independent prognostic factor.

There are several possible explanations for this discrepancy. Most other studies have used DNA sequencing to detect IDH mutations. In our study an immunohistochemical method was used to detect mutant IDH^{R132H}, which may result in a different rate of detection of IDH mutations compared to sequencing. This explanation seems unlikely since the immunohistochemical approach has been shown to perfectly match the results of direct sequencing of IDH1 codon R132H with a sensitivity of 94% and a specificity of 100% [6]. Another possible explanation is the fact that most studies have investigated the prognostic sum effect of several mutations of the IDH gene R132H, R132C, R132S, R132L, and R132G. Our study however investigated only R132H, which is the most common mutation. Thus in our study all other mutations, R132C, R132S, R132L, and R132G, comprising 12% of all IDH mutations [13], were not identified and so were included in the IDH^{wt} group. The presence of other IDH mutations in the control group might distort the significance.

Furthermore, our study shows that IDH^{R132H} mutation correlates significantly with p53 accumulation and is in a reverse correlation to EGFR expression. Increased expression of p53 most likely reflects the presence of loss of function mutations in the protein [10]. Mutations of the p53 gene are most commonly found in low-grade gliomas and younger patients [14]. Previous reports of our group have shown that glioblastoma patients with immunohistochemically detectable p53 protein expression had significantly better overall survival [2], but our current study investigating a different set of patients was not able to confirm these findings. The discrepancy could be explained by the different treatment modalities of the patient groups, since most of our patients did not receive chemotherapy, contrary to the patient group in the previous report [2].

To our knowledge, no data indicating a functional relationship between IDH and p53 or EGFR exist. Since our study revealed strong correlations between IDH and p53 as well as EGFR protein expression levels, potential interactions between these genes in human gliomas deserve further investigations.

In sum, our results indicate that IDH^{R132H} mutation correlates significantly with p53 and inversely with EGFR mutations. Further studies should investigate whether these correlations reflect involvement of these three molecules in a common signalling pathway.

Acknowledgments

The authors are grateful to Dr. Johannes Hainfellner, Vienna, for advice and critical discussions. This work was supported by European Union Social Fund Program BG051PO001/07/3.3-02 grant number 80/17.06.2008 and BSF grant number L01-840/16.10.2007.

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