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***IDH1* mutations are common in malignant gliomas arising in adolescents: a report from the Children's Oncology Group**

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Abstract

Purpose—Recent studies have demonstrated a high frequency of *IDH* mutations in adult “secondary” malignant gliomas arising from preexisting lower grade lesions, often in young adults, but not in “primary” gliomas. Because pediatric malignant gliomas share some molecular

features with adult secondary gliomas, we questioned whether a subset of these tumors also exhibited *IDH* mutations.

Experimental design—We examined the frequency of *IDH* mutations, using real-time polymerase chain reaction and sequencing analysis, in a cohort of 43 pediatric primary malignant gliomas treated on the Children's Oncology Group ACNS0423 study. The relationship between *IDH* mutations and other molecular and clinical factors, and outcome, was evaluated.

Results—*IDH1* mutations were observed in 7 of 43 (16.3%) tumors; no *IDH2* mutations were observed. A striking age association was apparent in that mutations were noted in 7 of 20 tumors (35%) from children ≥ 14 years, but in 0 of 23 (0%) younger children ($p=0.0024$). No association was observed with clinical factors other than age. One-year event-free survival was $86\pm 15\%$ in the *IDH*-mutated group versus $64\pm 8\%$ in the non-mutated group ($p=0.03$, one-sided logrank test). One-year overall survival was 100% in patients with mutations versus $81\pm 6.7\%$ in those without mutations ($p=0.035$, one-sided logrank test).

Conclusions—*IDH1* mutations are common in malignant gliomas in older children, suggesting that a subset of these lesions may be biologically similar to malignant gliomas arising in younger adults and may be associated with a more favorable prognosis.

Keywords

Anaplastic glioma; Childhood; Glioblastoma; *IDH*

Introduction

High-grade gliomas are among the most therapeutically challenging central nervous system tumors of childhood [1]. Although these neoplasms are histologically comparable to malignant gliomas in adults, they have several molecular features that distinguish them from their adult counterparts. For example, childhood primary malignant gliomas usually lack *EGFR* amplification or *PTEN* deletion [2-9], which are characteristic features of adult primary glioblastoma, but often exhibit *TP53* mutations, particularly in tumors that arise in older children. Such molecular alterations resemble those observed in so-called secondary adult glioblastomas, which arise in the setting of a prior lower grade lesion [10-14], often in adults younger than 40 years of age, although a similar pattern of histological progression is uncommonly observed in childhood lesions.

Recent studies have demonstrated a high frequency of mutations of the *IDH1* and *IDH2* genes, which encode the isocitrate dehydrogenase (*IDH*) enzymes, in adult secondary malignant gliomas [14-24]. These alterations inhibit the normal function of the *IDH* enzyme in converting isocitrate to α -ketoglutarate [25], and instead drive the conversion of α -ketoglutarate to R(-)-s-hydroxyglutarate [26], a metabolite that may contribute to tumor development. *IDH* mutations likely represent an early step in tumorigenesis because such alterations are also observed commonly in grade II (diffuse) astrocytomas, oligodendrogliomas, and oligoastrocytomas, in some instances preceding the acquisition of other characteristic molecular features, such as *TP53* mutations in astrocytomas and 1p/19q deletions in oligodendroglial neoplasms [15-17]. In contrast, *IDH* mutations are uncommon in primary adult malignant gliomas [14-17], supporting the existence of mechanistically distinct pathways of tumorigenesis.

Although several large surveys of *IDH* mutations that have included childhood brain tumors have reported that such alterations are uncommon [15,19,22,24,27], many of these studies have predominantly incorporated pilocytic astrocytomas and medulloblastomas, which are distinct histologically from gliomas that arise in adults. Given the molecular similarities that

have been noted between primary pediatric malignant gliomas that arise in older children and secondary malignant gliomas that occur in adults, we questioned whether pediatric tumors exhibited a high frequency of *IDH* mutations, and if so, whether there were age-related differences in mutation frequency. Our results indicate the *IDH* mutations are common in malignant gliomas that occur in older children, suggesting that a subset of such lesions may be comparable on a molecular basis to lesions that arise in young adults. In contrast, such alterations are rare in tumors arising in younger children, supporting the existence of age-related pathways of tumorigenesis in childhood.

Methods

Tumor samples

Tumor samples were obtained from children enrolled on the Children's Oncology Group ACNS0423 study, which involved post-surgical administration of alkylator-based chemotherapy and irradiation. Institutional IRB approval was required for protocol enrollment. All patients were previously untreated and between 3 and 21 years of age at the time of diagnosis. Maximal surgical resection was encouraged, but was limited in some cases based on the location of the tumor. Adjuvant therapy consisted of daily temozolomide during irradiation followed by post-irradiation temozolomide and lomustine.

Eligibility required an institutional histopathologic diagnosis of anaplastic astrocytoma (AA), glioblastoma (GBM), or gliosarcoma and validation of the diagnosis by central pathology review to exclude patients with discordant histologies, such as low-grade glioma, which has been a concern on previous studies [28,29]. Tissue accrual for the current study was coordinated by the Pediatric Branch of the Cooperative Human Tissue Network (CHTN). Specimens were de-identified by the CHTN to mask clinical and outcome data from investigators.

DNA isolation

Formalin-fixed paraffin-embedded (FFPE) tissue specimens were used for all analyses. Slides were reviewed by a neuropathologist (RLH) to confirm that tumor tissue of sufficient quantity was available for the planned studies. Tumor targets were manually microdissected from 4- μ m unstained histologic sections under the guidance of a corresponding hematoxylin- and eosin-stained slide using an Olympus SZ61 stereo microscope (Olympus, Hamburg, Germany). DNA was isolated from each target with the DNeasy Blood and Tissue kit on the automated QIAcube (Qiagen, Valencia, CA) instrument according to the manufacturer's instructions. The quantity of isolated DNA was assessed using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE).

Detection of *IDH1/2* mutations by real-time polymerase chain reaction and fluorescence melting curve analysis

Detection of *IDH1* and *IDH2* mutations was performed using real-time polymerase chain reaction (PCR) and post-PCR fluorescence melting curve analysis (FMCA) on the LightCycler (Roche Applied Science, Indianapolis, IN). A pair of primers flanking each mutation site and two fluorescent probes were designed using LightCycler Design Software 2.6 (Roche, Indianapolis, IN; Table 1). All primers and probes were obtained from TIB Molbiol (Berlin, Germany). Amplification was performed in a glass capillary tube using 5–50 ng of DNA in a 20- μ l reaction volume. In detail, 2 μ l of 10 $\times$ LightCycler Master Mix from the LightCycler FastStart DNA Master HybProbe Kit (Roche, Indianapolis, IN, USA) containing PCR buffer, deoxynucleotide triphosphates, 10 mM MgCl₂, and Taq polymerase, 1.6 μ l of 25 mM MgCl₂, 40 pmol of each forward primer, 10 pmol of each reverse primer, and 5 pmol of each hybridization probe was used. The reaction mixture was subjected to 40

cycles of rapid PCR consisting of denaturation at 95°C for 5 s, annealing at 54°C for 20 s, and extension at 72°C for 12 s. Post-amplification FMCA was performed by gradual heating of samples at a rate of 0.1°C/s from 40°C to 95°C. Fluorescence melting peaks were built by plotting the negative derivative of fluorescent signal corresponding to the temperature ($-dF/dT$). All positive cases were directly sequenced.

Detection of *IDH1/2* mutations by sequencing analysis

Sanger (dideoxy) sequencing analysis was performed as previously described [30]. In detail, *IDH1* and *IDH2* primers (Table 1) were used for PCR amplification and sequencing analysis. Each PCR reaction was performed using 5–50 ng of DNA, 0.2 μ mol of each primer, and AmpliTaq Gold PCR Master Mix (Applied Biosystems, Inc., Foster City, CA) in a total volume of 50 μ l. The reaction mixture was subjected to an initial denaturation of 95°C for 10 min, followed by 40 cycles of amplification consisting of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension 72°C for 60 s. The PCR products were sequenced in both sense and antisense directions using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions and were analyzed on ABI 3130 (Applied Biosystems, Foster City, CA). The sequence electropherograms were analyzed using Mutation Surveyor software (SoftGenetics, LLC., State College, PA). Each case was classified as either positive or negative for the *IDH* mutation based on the sequencing results.

Immunohistochemistry

FFPE specimens were used for all analyses. Slides were reviewed by a neuropathologist (RLH) to confirm that tumor tissue of sufficient quantity was available for the planned studies. Tumor-containing sections were baked at 60°C for 30 min, deparaffinized in xylene, and rehydrated in graded concentrations of ethanol. Endogenous peroxidase activity was quenched by incubation in 0.3% hydrogen peroxide solution. Antigen retrieval [31] was performed by heating the slides in 10 mmol citrate buffer (pH 6.0) for 20 min. Nonspecific antibody binding was blocked by incubation in Protein Blocking Reagent (Thermo Corp, Pittsburgh, PA) for 20 min. Sections were then incubated with primary antibodies against Ki-67 (Immunotech, Westbrook, ME; USA 1:100), p53 (DO-7, Dako Corporation, Carpinteria, CA, 1:300), and pAkt (Se⁴⁷³) [Cell Signaling Technology, Inc., Danvers, MA; 1:100], in Common Antibody Diluent (BioGenex, San Ramon, CA, USA) at room temperature for 2 h. The p53 antibody recognizes a denaturation-stable determinant of wild-type and mutant p53 [32]. Negative control sections were treated with diluent and mouse IgG (5 μ g/ml, Dako Corporation, Carpinteria, CA) alone. Slides were then rinsed twice with PBS and antibody binding was localized using a Universal Labeled Streptavidin-Biotin 2 System (LSAB 2-HRP, Dako). Slides were incubated for 30 min in Biotinylated Link reagent at room temperature, followed by a 10-min PBS wash. Slides were then incubated in Streptavidin-HRP solution for 30 min at room temperature. Antibody binding was visualized using 3,3'-diaminobenzidine [33]. The slides were counterstained with Mayer's hematoxylin, dehydrated through graded concentrations of ethanol, cleared in xylene, and mounted and examined using a light microscope. Positive controls were included with each batch of sections to confirm the consistency of the analysis.

Central pathology review

Because previous studies involving pediatric malignant gliomas have often included a high percentage of cases that were reclassified as low-grade gliomas on blinded central neuropathology review [28,29], the current study incorporated a panel of neuropathology reviewers (PCB, MKR, DJB) to review the tumor specimens based on contemporary classification criteria [34,35], restrict the eligible cohort to those cases confirmed to be high-grade gliomas, and establish a central review diagnosis to be used for outcome analyses. The

IDH and other correlative analyses were performed independently of this review, so the review diagnosis of each specimen was not known during the molecular analyses.

Statistical analysis

For outcome analysis, tumors were classified according to the presence or absence of *IDH* mutations. The endpoints for analysis were overall survival (OS) and event-free survival (EFS). Overall survival was defined as the time from study entry to death from any cause. Event-free survival was defined as the time from study enrollment to the first occurrence of disease progression, relapse, second malignancy, or death from any cause. Nonparametric EFS and OS curves were computed using the product-limit estimates, with standard error via the Greenwood formula [36]. Comparisons of outcome were based on one-sided logrank test. Comparisons of the distribution of *IDH* mutations between different patient subgroups defined by age, gender, histology, p53 expression, extent of resection, and MIB1 labeling were based on two-sided Fisher exact test [37].

Results

Frequency of *IDH* mutations in children with newly diagnosed malignant gliomas

Of 106 eligible patients enrolled on ACNS0423, 43 patients had specimens that were available for *IDH* mutation analysis in the current study. Seven of these tumors (16.3%) had mutations of *IDH1*; none had mutations of *IDH2*. As a group, the 43 patients who had specimens had a more favorable outcome than the cohort without specimens. None of these patients had a known prior lower grade lesion. Figure 1 shows an illustrative example of a tumor with an *IDH1* mutation as contrasted against another case lacking this feature. Four tumors were positive for R132H mutations, two for R132S, and one R132C.

The distribution of *IDH* mutations as a function of clinical and other molecular factors is shown in Table 2. When the cohort was subdivided as a function of age, it was determined that *IDH1* mutations were seen in 7 of 20 (35%) children older than 14 years versus 0 of 23 (0%) children younger than 14 years ($p=0.0024$). No association between *IDH* mutations and pAkt immunoreactivity or MIB1 labeling was apparent. Among the seven tumors with *IDH* mutations, p53 overexpression was seen in 1 (14.3%) versus 20 of the 36 tumors lacking mutations (55.6%; $p=0.09$). Average MIB labeling in the *IDH1* mutated subset was 34.6 ± 11.4 versus 32.0 ± 3.18 in those without *IDH* mutations ($p=0.83$). The frequency of particularly high MIB1 indices (>36), which has been previously associated with an adverse prognosis, did not differ significantly between the two groups (3 of 7 versus 10 of 35, $p=0.87$).

We also examined whether *IDH* mutations might be more common in grade IV tumors compared to grade III lesions. Contrary to this assumption, *IDH* mutations were seen in 2 of 24 grade IV lesions (8%) versus 5 of 19 AAs (26%; $p=0.21$).

IDH mutations and outcome

To assess the prognostic significance of *IDH* mutations in pediatric malignant gliomas, overall survival and event-free survival were compared between the subgroup of tumors showing *IDH* mutations and those lacking this feature. One-year event-free survival was $83 \pm 15\%$ in patients with *IDH* mutations versus $64 \pm 8\%$ in those without mutations (Fig. 2; $p=0.03$, logrank test). One-year overall survival was 100% in patients with mutations versus $81 \pm 6.7\%$ in those without mutations (Fig. 3; $p=0.035$, logrank test). These results show that there is a significant difference in early outcome as a function of *IDH* mutation status. The median follow-up for non-failures was 1.5 years, and more extended follow-up data will be needed to determine whether *IDH* mutation status is associated with long-term outcome.

Because extent of resection is a known prognostic factor in patients with high-grade glioma, stratified logrank tests were performed based on this factor. After stratifying the data based upon extent of resection (less than 90% versus greater than or equal to 90% resection), the early difference in outcome was still evident (stratified logrank test, $p=0.04$ for event-free survival and $p=0.05$ for overall survival).

Discussion

Recent studies have demonstrated frequent mutations of the *IDH1* and *IDH2* genes in adult secondary malignant gliomas that arise from preexisting lower grade lesions [14-24]. Alteration in a single *IDH1* allele significantly inhibits the normal function of IDH in promoting the oxidative decarboxylation of isocitrate to α -ketoglutarate, with resultant conversion of NADP⁺ to NADPH [25]. Because α -ketoglutarate enhances the degradation of hypoxia-inducible factor subunit HIF-1 α , inhibition of α -ketoglutarate formation increases the levels of HIF-1 α , which favors tumor growth under hypoxic conditions. *IDH1* mutations also drive the conversion of α -ketoglutarate to R(-)-s-hydroxyglutarate, a metabolite that may contribute to tumor development [26]. The potentially contributory role of this alteration to tumor development is highlighted by the fact that *IDH* mutations are observed not only in secondary glioblastomas, but also in diffuse gliomas of lower histological grades, in some instances preceding the acquisition of other molecular alterations [15-19]. However, *IDH* mutations are uncommon in primary adult malignant gliomas, suggesting that these lesions arise by a mechanistically distinct pathway of tumorigenesis.

Although several large surveys of *IDH* mutations that have included pediatric brain tumors have reported that such alterations are uncommon [14,15,19,22,24,27], many of these studies have predominantly incorporated pilocytic astrocytomas and medulloblastomas, which are distinct histologically from gliomas that arise in adults, and a comparatively small number of diffuse low-grade gliomas and malignant gliomas of childhood have been reported. The series of Balss et al. [22] included only 14 pediatric GBMs, one of which had an *IDH1* mutation. Likewise, De Carli et al. [24] reported *IDH* mutations in only 4 of 73 children with non-pilocytic gliomas, with an average age of 16 years in the affected subset, but the histological composition of the analyzed tumors was not specified. Yan et al. [15] noted mutations in 0 of 15 primary pediatric glioblastomas (median age 5). Similarly, in a study of 1,010 diffuse gliomas that included a small subgroup of children, Hartmann et al. [18] noted that *IDH1* mutations were rare in the pediatric subset, occurring in only 4 of 32 (12.5%) tumors, and *IDH2* mutations were absent, leading the authors to conclude that *IDH* mutations were rare in childhood tumors, although the frequency of mutations as a function of age and histology was not provided. More recently, Antonelli et al. [27] noted no *IDH1* mutations in a series of 27 pediatric high-grade gliomas, although the age range extended only to 17 years, and the mean age of their overall cohort was 9.4 years, whereas 20 of our 46 patients were 14 to 18 years of age, suggesting that the composition of our analyzed patient groups may have differed somewhat. In this regard, the incidence of *TP53* mutations observed in their patients (18.5%) is also somewhat lower than the frequencies we have previously observed in older children (~40%) [9], suggesting that there may well be several age-related patterns of tumorigenesis within the broad group of childhood malignant gliomas.

Given the molecular similarities that have been noted between primary pediatric malignant gliomas that arise in older children and secondary malignant gliomas that occur in adults, in terms of their high frequency of *TP53* mutations [9] and infrequency of *EGFR* amplification and *PTEN* deletions [2], we questioned whether a more focused analysis of childhood high-grade gliomas, including samples from both older and younger children, might identify an association between *IDH* mutations and age in these tumors. Our results indicate that *IDH*

mutations are relatively common in malignant gliomas that occur in older children, although are less frequent than the reported incidence in secondary adult malignant gliomas, which exceeds 80% in some studies [14–18]. This observation supports the possibility that at least a subgroup of pediatric lesions occurring in older children may be biologically comparable to malignant gliomas that arise in young adults [24], although most lesions in older children appear to arise by a distinct pathway. Our finding that *IDH*-mutated tumors seemed to be associated with a more favorable prognosis than non-mutated tumors is consistent with observations in adults [14,15]. In contrast, *IDH* mutations are rare in tumors arising in younger children, supporting the existence of age-related pathways of tumorigenesis in childhood.

The potential for multiple pathways of tumor development in childhood fits with our previous observations that *TP53* mutations are common in malignant gliomas that occur in older children, but are rare in lesions that arise in younger children [9]. The current results indicate that primary malignant gliomas in young children lack not only *EGFR* amplification, and *PTEN* and *TP53* mutations, but also *IDH* mutations, implying that these lesions incorporate distinct, but so far, uncharacterized pathways of tumorigenesis. However, for tumors arising in older children, the finding of additional similarities to lesions in young adults has important implications for therapeutic stratification. Although strategies specifically directed toward *IDH*-mutated tumors have, to date, not been implemented in adults with malignant gliomas, this alteration or its downstream consequences constitute a logical target, and a similar approach could be considered in appropriately selected patients in the pediatric context.

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References

1. Pollack IF. Current concepts: brain tumors in children. *N Engl J Med* 1994;331:1500–1507. [PubMed: 7969301]
2. Pollack IF, Hamilton RL, James CD, Finkelstein SD, Burnham J, Yates AJ, Holmes EJ, Zhou T, Finlay JL. Rarity of *PTEN* deletions and *EGFR* amplification in malignant gliomas of childhood: results from the Children's Cancer Group 945 cohort. *J Neurosurg Pediatr* 2006;105:3431–3437.
3. Sung T, Miller DC, Hayes RL, Alonso M, Yee H, Newcomb EW. Preferential inactivation of the p53 tumor suppressor pathway and lack of *EGFR* amplification distinguish *de novo* high grade pediatric astrocytomas from *de novo* adult astrocytomas. *Brain Pathol* 2000;10:249–259. [PubMed: 10764044]
4. Bredel M, Pollack IF, Hamilton RL, James CD. Epidermal growth factor receptor (*EGFR*) expression and gene amplification in high-grade non-brainstem gliomas of childhood. *Clin Cancer Res* 1999;5:1786–1792. [PubMed: 10430083]
5. Cheng Y, Ng H-K, Zhang S-F, Ding M, Pang JC-S, Zheng J, Poon W-S. Genetic alterations in pediatric high-grade astrocytomas. *Hum Pathol* 1999;30:1284–1290. [PubMed: 10571506]
6. Raffel C, Frederick L, O'Fallon JR, et al. Analysis of oncogene and tumor suppressor gene alterations in pediatric malignant astrocytomas reveals reduced survival for patients with *PTEN* mutations. *Clin Cancer Res* 1999;5:4085–4090. [PubMed: 10632344]
7. Sure U, Ruedi D, Tachibana O, et al. Determination of p53 mutations, *EGFR* overexpression, and loss of p16 expression in pediatric glioblastomas. *J Neuropathol Exp Neurol* 1997;56:782–789. [PubMed: 9210874]
8. Pollack IF, Finkelstein SD, Woods J, Burnham J, Holmes EJ, Hamilton RL, Yates AJ, Boyett JM, Finlay JL, Spoto R. Expression of p53 and prognosis in malignant gliomas in children. *N Engl J Med* 2002;346:420–427. [PubMed: 11832530]

9. Pollack IF, Finkelstein SD, Burnham J, Holmes EJ, Hamilton RL, Yates AJ, Finlay J, Sposto R. Age and *TP53* mutation frequency in childhood malignant gliomas. Results in a multi-institutional cohort. *Cancer Res* 2001;61:7404–7407. [PubMed: 11606370]
10. Collins VP. Progression as exemplified by human astrocytic tumors. *Semin Cancer Biol* 1999;9:267–276. [PubMed: 10448114]
11. Ichimura K, Bolin MB, Goike HM, Schmidt EE, Moshref A, Collins VP. Deregulation of the p14^{ARF}/MDM2/p53 pathway is a prerequisite for human astrocytic gliomas with G₁-S transition control gene abnormalities. *Cancer Res* 2000;60:417–424. [PubMed: 10667596]
12. von Deimling A, von Ammon K, Schoenfeld D, Wiestler OD, Seizinger BR, Louis DN. Subsets of glioblastoma multiforme defined by molecular genetic analysis. *Brain Pathol* 1993;3:19–26. [PubMed: 8269081]
13. Watanabe K, Tachibana O, Sato K, Yonekawa Y, Kleihues P, Ohgaki H. Overexpression of the EGF receptor and p53 mutations are mutually exclusive in the evolution of primary and secondary glioblastomas. *Brain Pathol* 1996;6:217–224. [PubMed: 8864278]
14. Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, Olivi A, McLendon R, Rasheed BA, Keir S, Nikolskaya T, Nikolsky Y, Busam DA, Tekleab H, Diaz LA Jr, Hartigan J, Smith DR, Strausberg RL, Marie SK, Shinjo SM, Yan H, Riggins GJ, Bigner DD, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW. An integrated genomic analysis of human glioblastoma multiforme. *Science* 2008;321:1807–1812. [PubMed: 18772396]
15. Yan H, Parsons DW, Jin G, et al. IDH1 and IDH2 mutations in gliomas. *N Engl J Med* 2009;360:765–773. [PubMed: 19228619]
16. Ichimura K, Pearson DM, Kocalkowski S, et al. IDH1 mutations are present in the majority of common adult gliomas but are rare in primary glioblastomas. *Neuro-Oncology* 2009;11:341–347. [PubMed: 19435942]
17. Watanabe T, Nobusawa S, Kleihues P, Ohgaki H. IDH1 mutations are early events in the development of astrocytomas and oligodendrogliomas. *Am J Pathol* 2009;174:1149–1153. [PubMed: 19246647]
18. Hartmann C, Meyer J, Balss J, et al. Type and frequency of IDH1 and IDH2 mutations are related to astrocytic and oligodendroglial differentiation and age: a study of 1, 010 diffuse gliomas. *Acta Neuropathol* 2009;118:469–474. [PubMed: 19554337]
19. Korshunov A, Meyer J, Capper D, et al. Combined molecular analysis of BRAF and IDH1 distinguishes pilocytic astrocytomas from diffuse astrocytomas. *Acta Neuropathol* 2009;118:401–405. [PubMed: 19543740]
20. Kang MR, Kim MS, Oh JE, et al. Mutational analysis of IDH1 codon 132 in glioblastomas and other common cancers. *Int J Cancer* 2009;125:353–355. [PubMed: 19378339]
21. Bleeker FE, Lamba S, Leenstra S, et al. IDH1 mutations at residue p.R132(IDH1(R132)) occur frequently in high-grade gliomas, but not in other solid tumors. *Hum Mutat* 2009;30:7–11. [PubMed: 19117336]
22. Balss J, Meyer J, Mueller W, Korshunov A, Hartmann C, von Deimling A. Analysis of the IDH1 codon 132 mutation in brain tumors. *Acta Neuropathol* 2008;116:597–602. [PubMed: 18985363]
23. Yan H, Bigner DD, Velculescu V, Parsons WD. Mutant metabolic enzymes are at the origin of gliomas. *Cancer Res* 2009;69:9157–9159. [PubMed: 19996293]
24. De Carli E, Wang X, Puget S. IDH1 and IDH2 mutations in gliomas. *N Engl J Med* 2009;360:2248. [PubMed: 19458374]
25. Zhao S, Lin Y, Jiang W, et al. Glioma-derived mutations in IDH1 dominantly inhibit IDH1 catalytic activity and induce HIF-1 α . *Science* 2009;324:261–265. [PubMed: 19359588]
26. Dang L, White DW, Gross S, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature* 2009;462:739–744. [PubMed: 19935646]
27. Antonelli M, Buttarelli FR, Arcella A, et al. Prognostic significance of histological grading, p53 status, YKL-40 expression, and *IDH1* mutations in pediatric high-grade gliomas. *J Neurooncol*. 2010 Published Online: 21 February 2010. 10.1007/s11060-010-0129-5

28. Spoto R, Ertel IJ, Jenkin RDT, et al. The effectiveness of chemotherapy for treatment of high grade astrocytoma in children: results of a randomized trial. A report from the Children's Cancer Study Group. *J Neuro-oncol* 1989;7:165–177.
29. Finlay JL, Boyett JM, Yates AJ, et al. Randomized phase III trial in childhood high-grade astrocytoma comparing vincristine, lomustine, and prednisone with the eight-drugs-in-1-day regimen. *J Clin Oncol* 1995;13:112–123. [PubMed: 7799011]
30. Horbinski C, Kofler J, Kelly L, Murdoch GH, Nikiforova MN. The diagnostic utility of IDH1/2 mutation analysis in routine clinical testing of formalin-fixed paraffin-embedded glioma tissues. *J Neuropathol Exp Neurol* 2009;68:1319–1325. [PubMed: 19915484]
31. Shi SR, Key ME, Kalra KL. Antigen retrieval in formalin-fixed, paraffin embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. *J Histochem Cytochem* 1991;39:741–748. [PubMed: 1709656]
32. Vojtesek B, Bartek J, Midgley CA, Lane DP. An immunochemical analysis of the human nuclear phosphoprotein p53. New monoclonal antibodies and epitope mapping using recombinant p53. *J Immunol Methods* 1992;151:237–244. [PubMed: 1378473]
33. Hsu S, Raine L, Fanger H, et al. Use of avidin-biotin peroxidase complex (ABC) in immunoperoxidase techniques. *J Histochem Cytochem* 1981;29:577–580. [PubMed: 6166661]
34. Pollack IF, Boyett JM, Yates AJ, Burger PC, Gilles FH, Davis RL, Finlay JL. The influence of central review on outcome associations in childhood malignant gliomas: results from the CCG-945 experience. *Neuro-Oncology* 2003;5:197–207. [PubMed: 12816726]
35. Kleihues, P.; Burger, PC.; Scheithauer, BW. Histological typing of tumours of the central nervous system. In: Sobin, LH., editor. *International histological classification of tumours*. 2. Vol. 21. Springer-Verlag; Geneva: 1993. p. 11-16.
36. Kalbfleisch, JD.; Prentice, RI. *The statistical analysis of failure time data*. John Wiley and Sons; New York: 1980. p. 163-180.
37. Dixon, WJ.; Massey, FJ. *Introduction to statistical analysis*. 3. McGraw-Hill; New York: 1969.

Fig. 1.

An illustrative example of a tumor with an *IDH* mutation, as contrasted against another case lacking this feature as detected by real-time PCR/FMCA and sequencing. **a** Tumor negative for *IDH1* mutation demonstrates a single wild-type peak on real-time PCR/FMCA (*upper panel*) and a wild-type sequence on sequencing electropherogram (*lower panel*). **b** Tumor positive for *IDH1 R132H* mutation shows mutant peak in addition to the wild-type peak on real-time PCR/FMCA (*upper panel*) and demonstrated G to A substitution at codon 132 on sequencing electropherogram (*lower panel*)

Fig. 2.
Event-free survival in pediatric malignant gliomas exhibiting *IDH* mutations versus those lacking this finding

Fig. 3. Overall survival in pediatric malignant gliomas exhibiting *IDH* mutations versus those lacking this finding

Table 1Primers and characteristics of *IDH1* and *IDH2* loci analyzed

Gene/Gene Bank no.	Primer/Probe ID	Primer/Probe Sequence (5'-3')	Primer/Probe Location
<i>IDH1</i> NC_000002.11	Real-time PCR Forward Primer	ACG GTC TTC AGA GAA GC	6614–6631
	Real-time PCR Reverse Primer	GGT GTA GAT ACC AAA AGA TAA GAA T	6792–6816
	Real-time PCR Anchor Probe	5'-LC640-ATG ATA GGT TTT ACC CAT CCA CTC ACA AGC-P	6656–6685
	Real-time PCR Sensor Probe	ATC CCC ATA AGC ATG ACG ACC TA-FI	6688–6710
	Sequencing Forward Primer	ACC AAA TGG CAC CAT ACG A	6581–6599
	Sequencing Reverse Primer	GCA AAA TCA CAT TAT TGC CAA C	6723–6744
<i>IDH2</i> NC_000015.9	Real-time PCR Forward Primer	TGC AGT GGG ACC ACT ATT ATC	13694–13715
	Real-time PCR Reverse Primer	CTT GAC ACC ACT GCC ATC	14037–14054
	Real-time PCR Anchor Probe	5'-LC640-TGA TGG GCT TGG TCC AGC CAG GG- P	13838–13860
	Real-time PCR Sensor Probe	TGG GCG TGC CTG CCA ATG-FI	13862–13879
	Sequencing Forward Primer	GCT GCA GTG GGA CCA CTA TT	13693–13712
	Sequencing Reverse Primer	TGT GGC CTT GTA CTG CAG AG	13966–13985

Table 2Association between *IDH* mutations and other demographic and molecular features

	<i>IDH</i> Mutations		No <i>IDH</i> Mutations		<i>p</i> value
	<i>N</i>	%	<i>N</i>	%	
Age					0.002
<14 years	0	0	23	63.9	
≥14 years	7	100	13	36.1	
Gender					1.0
Male	4	57.1	22	61.1	
Female	3	42.9	14	38.9	
Histology					0.21
Grade III	5	71.4	14	38.9	
Grade IV	2	28.6	22	61.1	
Extent of Resection					0.68
<90% resection	3	42.86	20	58.8	
≥90% resection	4	57.1	14	41.2	
p53 Overexpression					0.09
Present	1	14.3	20	55.6	
Absent	6	85.7	16	44.4	
pAkt Overexpression					0.57
Present	7	100	29	85.3	
Absent	0	0	5	14.7	
MIB Labeling					0.87
Very High (>36)	3	42.9	10	28.6	
Intermediate (18–36)	2	28.6	11	31.4	
Low (<18)	2	28.6	14	40.0	