ORIGINAL ARTICLE

IDH1 and *IDH2* Mutations in Gliomas

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ABSTRACT

BACKGROUND

A recent genomewide mutational analysis of glioblastomas (World Health Organization [WHO] grade IV glioma) revealed somatic mutations of the isocitrate dehydrogenase 1 gene (*IDH1*) in a fraction of such tumors, most frequently in tumors that were known to have evolved from lower-grade gliomas (secondary glioblastomas).

METHODS

We determined the sequence of the *IDH1* gene and the related *IDH2* gene in 445 central nervous system (CNS) tumors and 494 non-CNS tumors. The enzymatic activity of the proteins that were produced from normal and mutant *IDH1* and *IDH2* genes was determined in cultured glioma cells that were transfected with these genes.

RESULTS

We identified mutations that affected amino acid 132 of *IDH1* in more than 70% of WHO grade II and III astrocytomas and oligodendrogliomas and in glioblastomas that developed from these lower-grade lesions. Tumors without mutations in *IDH1* often had mutations affecting the analogous amino acid (R172) of the *IDH2* gene. Tumors with *IDH1* or *IDH2* mutations had distinctive genetic and clinical characteristics, and patients with such tumors had a better outcome than those with wild-type *IDH* genes. Each of four tested *IDH1* and *IDH2* mutations reduced the enzymatic activity of the encoded protein.

CONCLUSIONS

Mutations of $NADP^+$-dependent isocitrate dehydrogenases encoded by *IDH1* and *IDH2* occur in a majority of several types of malignant gliomas.

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Gliomas, the most common type of primary brain tumors, are classified as grade I to grade IV on the basis of histopathological and clinical criteria established by the World Health Organization (WHO).[1] This group of tumors includes specific histologic subtypes, the most common of which are astrocytomas, oligodendrogliomas, and ependymomas. WHO grade I gliomas, often considered to be benign, are generally curable with complete surgical resection and rarely, if ever, evolve into higher-grade lesions.[2] By contrast, gliomas of WHO grade II or III are invasive, progress to higher-grade lesions, and have a poor outcome. WHO grade IV tumors (glioblastomas), the most invasive form, have a dismal prognosis.[3,4] On the basis of histopathological criteria, it is impossible to distinguish a secondary glioblastoma, defined as a tumor that was previously diagnosed as a lower-grade glioma, from a primary tumor.[5,6]

Several genes, including *TP53*, *PTEN*, *CDKN2A*, and *EGFR*, are altered in gliomas.[7-12] These alterations tend to occur in a defined order during the progression to a high-grade tumor. The *TP53* mutation appears to be a relatively early event during the development of an astrocytoma, whereas the loss or mutation of *PTEN* and amplification of *EGFR* are characteristic of higher-grade tumors.[6,13,14] In oligodendrogliomas, allelic losses of 1p and 19q occur in many WHO grade II tumors, whereas losses of 9p21 are largely confined to WHO grade III tumors.[15]

In a recent genomewide analysis, we identified somatic mutations at codon 132 of the isocitrate dehydrogenase 1 gene (*IDH1*) in approximately 12% of glioblastomas.[16] These mutations were also found in five of six secondary glioblastomas. The results suggested that *IDH1* mutations might occur after formation of a low-grade glioma and drive the progression of the tumor to a glioblastoma. To evaluate this possibility, we analyzed a large number of gliomas of various types.

METHODS

DNA SAMPLES

DNA was extracted from samples of primary brain tumor and xenografts and from patient-matched normal blood lymphocytes obtained from the Tissue Bank at the Preston Robert Tisch Brain Tumor Center at Duke University and collaborating centers, as described previously.[17] All analyzed brain tumors were subjected to consensus review by two neuropathologists. Table 1 lists the types of brain tumors we analyzed. The samples from glioblastomas included 138 primary tumors and 13 secondary tumors. Of the 138 primary tumors, 15 were from patients under the age of 21 years. Secondary glioblastomas were categorized as WHO grade IV on the basis of histologic criteria but had been categorized as WHO grade II or III at least 1 year earlier. Of the 151 tumors, 63 had been analyzed in our previous genomewide mutation analysis of glioblastomas. None of the lower-grade tumors were included in that analysis.[16]

In addition to brain tumors, we analyzed 35 lung cancers, 57 gastric cancers, 27 ovarian cancers, 96 breast cancers, 114 colorectal cancers, 95 pancreatic cancers, and 7 prostate cancers, along with 4 samples from patients with chronic myelogenous leukemia, 7 from patients with chronic lymphocytic leukemia, 7 from patients with acute lymphoblastic leukemia, and 45 from patients with acute myelogenous leukemia. All samples were obtained in accordance with the Health Insurance Portability and Accountability Act. Acquisition of tissue specimens was approved by the institutional review board at the Duke University Health System and at each of the participating institutions.

Exon 4 of the *IDH1* gene was amplified with the use of a polymerase-chain-reaction (PCR) assay and sequenced in DNA from the tumor and lymphocytes from each patient, as described previously.[16] In all gliomas and medulloblastomas without an R132 *IDH1* mutation, exon 4 of the *IDH2* gene (which contains the *IDH2* residue equivalent to R132 of *IDH1*) was sequenced and analyzed for somatic mutations. In addition, we evaluated all astrocytomas and oligodendrogliomas of WHO grade I to grade III, all secondary glioblastomas, and 96 primary glioblastomas without R132 *IDH1* mutations or R172 *IDH2* mutations for alterations in the remaining coding exons of *IDH1* and *IDH2*. All coding exons of *TP53* and *PTEN* were also sequenced in the panel of diffuse astrocytomas, oligodendrogliomas, anaplastic oligodendrogliomas, anaplastic astrocytomas, and glioblastomas. *EGFR* amplification and the *CDKN2A–CDKN2B* deletion were analyzed with the use of quantitative real-time PCR in the same tumors.[18] We evaluated samples of oligodendrogliomas and anaplastic oligodendrogliomas for loss of heterozygosity at 1p and 19q, as described previously.[15,19]

Table 1. Summary of Genetic and Clinical Characteristics of Brain Tumors in the Study.*

Tumor Classification†	No. of Tumors Analyzed	Median Age of Patient‡	Male Sex	Median Survival	Tumors with *IDH* Mutations			Median Age of Patient		Tumors with Other Alterations§				
					IDH1	*IDH2*	Combined	Mutated *IDH*	Wild-Type *IDH*	*TP53*	1p and 19q	*PTEN*	*EGFR*	*CDKN2A* or *CDKN2B*
		yr	%	*mo*	*no.*		%	*yr*				%		
Astrocytic tumors														
Pilocytic astrocytoma (grade I)	21	5	48	ND	0	0	0	ND	5	0	NA	0	0	NA
Subependymal giant-cell astrocytoma (grade I)	2	16	100	ND	0	NA	0	ND	ND	NA	NA	NA	NA	NA
Diffuse astrocytoma (grade II)	30	34	53	132	25	2	90	35	5	74	0	0	0	0
Pleomorphic xanthoastrocytoma (grade II)	7	11	14	44	1	NA	14	20	11	NA	NA	NA	0	NA
Anaplastic astrocytoma (grade III)	52	38	67	51	36	2	73	34	56	65	10	9	2	9
Secondary glioblastoma (grade IV)¶	13	33	70	16	11	0	85	32	62	62	NA	0	0	20
Primary adult glioblastoma (grade IV)	123	59	60	15	6	0	5	32	59	23	4	23	38	42
Primary pediatric glioblastoma (grade IV)	15	5	60	8	0	0	0	ND	5	33	NA	NA	NA	20
Oligodendroglial tumors														
Oligodendroglioma (grade II)	51	37	63	135	41	2	84	37	13.5	16	60	0	0	4
Anaplastic oligodendroglioma (grade III)	36	45	64	84	31	3	94	45	ND	10	84	0	0	14
Oligoastrocytic tumors														
Oligoastrocytoma (grade II)	3	38	67	ND	3	NA	100	38	ND	33	NA	0	0	0
Anaplastic oligoastrocytoma (grade III)	7	30	57	ND	7	NA	100	30	ND	71	50	0	0	0
Ependymoma (grade II)	30	5.5	45	ND	0	0	0	ND	5.5	0	NA	0	NA	NA
Medulloblastoma (grade IV)	55	7	65	27	0	0	0	ND	7	NA	NA	NA	NA	NA

* Of the indicated tumors, 6 secondary and 60 primary glioblastomas were previously described in Parsons et al.[16] Copy-number changes in *EGFR*, *CDKN2A*, and *CDKN2B* were determined by quantitative real-time polymerase chain reaction. For such assays, copy-number levels of more than 6 or less than 0.3 were considered amplifications or losses, respectively. NA denotes not analyzed, and ND not determined because of limited sample size and status of data censoring.

† Tumors were graded according to histopathological and clinical criteria established by the World Health Organization.

‡ Patient age refers to age at which the study sample was obtained.

§ Alterations included mutations in *TP53* and *PTEN*, loss of heterozygosity in 1p and 19q, amplification in *EGFR*, and deletion in *CDKN2A* or *CDKN2B*.

¶ Secondary glioblastoma designates a tumor that was resected more than 1 year after a previous diagnosis of a lower-grade glioma (grade II or grade III).

ENZYMATIC ACTIVITY

To assess the enzymatic activity of wild-type and mutant IDH1 and IDH2 proteins, a human oligodendroglioma line without *IDH1* or *IDH2* mutations was transfected with a vector (pCMV6, Invitrogen) containing the coding sequences of the wild-type *IDH1*, wild-type *IDH2*, or mutant *IDH* genes (corresponding to the most common *IDH1* mutation, R132H, or the *IDH2* mutations R172G, R172K, and R172M). Clones of the wild-type *IDH1* and *IDH2* genes were obtained from Origene, and mutations were introduced by standard methods.

Cells were collected 48 hours after transfection, subjected to centrifugation at 1000×*g* for 10 minutes at 4°C, washed once with cold phosphate-buffered saline, and lysed in buffer containing 0.1% Triton X-100. They were then disrupted by ultrasonication and centrifuged at 12,000×*g* for 30 minutes. The supernatants were used to measure IDH activity. Expression levels of wild-type and mutant IDH proteins were determined by Western blotting with the use of an antibody against FLAG, a polypeptide protein tag. For each enzymatic reaction, a volume of cell lysate containing the same amount of IDH protein was added to 1 ml of assay solution containing 33 mM of Tris buffer, 0.33 mM of EDTA, 0.1 mM of $NADP^+$, 1.33 mM of manganese chloride, and 1.3 mM of isocitrate. The activity of IDH was analyzed through the reduction of $NADP^+$ to NADPH, which was measured at 25°C by spectrophotometry at 340 nm 5 times a second for 300 seconds.[20]

CLINICAL DATA AND SURVIVAL

Clinical information included the date of birth, the date the study sample was obtained, the date of pathological diagnosis, the date and pathology of any preceding diagnosis of a lower-grade glioma, the use or nonuse of radiation therapy or chemotherapy before the date that the study sample was obtained, the date of the last contact with the patient, and the patient's status at the time of the last contact. We calculated overall survival for patients with anaplastic astrocyomas, including 38 patients with mutations in *IDH1* or *IDH2* and 14 with wild-type genes, and for adult patients (≥21 years of age) with glioblastomas, including 14 patients with mutations in *IDH1* or *IDH2* and 115 with wild-type genes, using the date of histologic diagnosis and the date of the last contact with the patient or death. For patients with secondary glioblastomas, survival was calculated from the date of secondary diagnosis. Seven patients with glioblastomas were not included in the statistical analysis because of insufficient survival data.

STUDY DESIGN

The authors designed the study, gathered and analyzed the data, wrote the manuscript, and made the decision to publish the findings. Gene sequencing was performed by Agencourt Bioscience, a subsidiary of Beckman Coulter. The lead academic authors vouch for the completeness and accuracy of the data and the analyses.

STATISTICAL ANALYSIS

We examined the association between the occurrence of mutations in *IDH1* or *IDH2* and other genetic alterations using Fisher's exact test. Kaplan–Meier survival curves were plotted and the survival distributions were compared with the use of the Mantel–Cox log-rank test and the Wilcoxon test. All reported P values are two-sided, and P values of less than 0.01 were considered to indicate statistical significance.

RESULTS

SEQUENCE ANALYSIS

Sequence analysis of *IDH1* in 939 tumor samples revealed 161 somatic mutations at residue R132, including R132H (142 tumors), R132C (7 tumors), R132S (4 tumors), R132L (7 tumors), and R132G (1 tumor) (Fig. 1A; and Fig. 1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). Table 1 and Figure 1B show the tumors with somatic R132 mutations. No other somatic mutations of *IDH1* in the remaining *IDH1* exons of R132-negative tumors were found in all WHO grade I to grade III astrocytomas and oligodendrogliomas, in all secondary glioblastomas, and in 96 primary glioblastomas. No R132 mutations were observed in 21 pilocytic astrocytomas (WHO grade I), 2 subependymal giant-cell astrocytomas (WHO grade I), 30 ependymomas (WHO grade II), 55 medulloblastomas, or any of the 494 non–central nervous system tumor samples.

We also sought alterations in other genes with functions similar to those of *IDH1* in tumors without *IDH1* mutations. For this purpose, we analyzed the *IDH2* gene, which encodes the only human protein homologous to *IDH1* that uses $NADP^+$ as an electron acceptor. Sequence evaluation of all

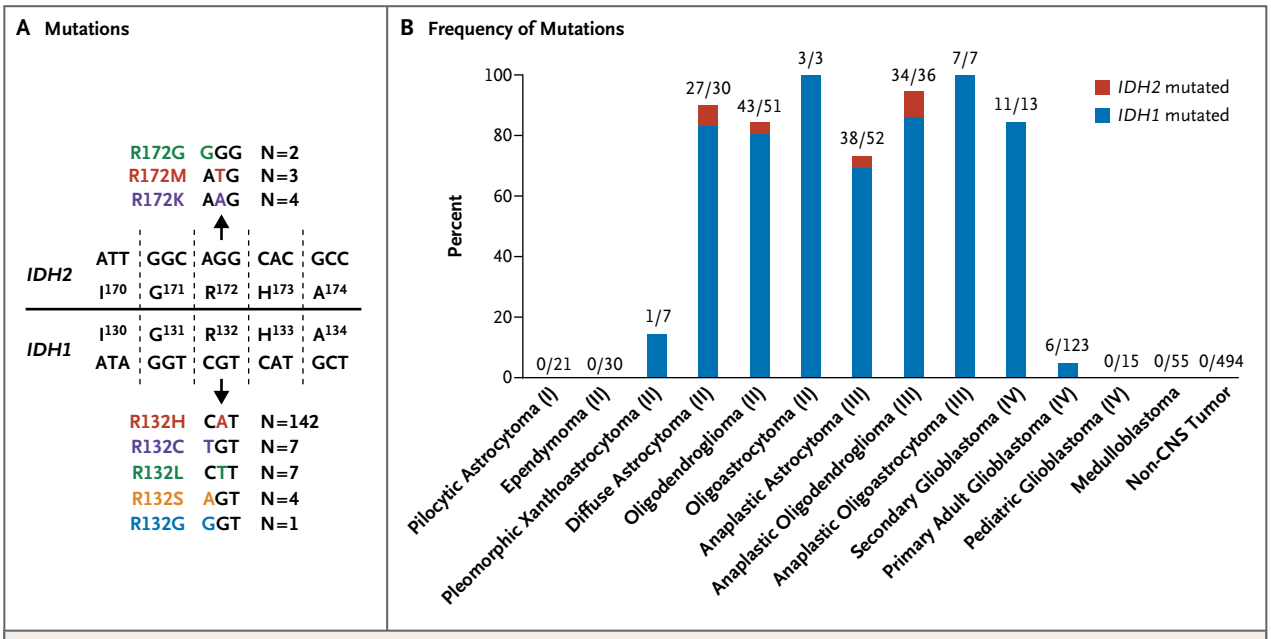

Figure 1. *IDH1* and *IDH2* Mutations in Human Gliomas.

Panel A shows mutations at codon R132 in *IDH1* and R172 in *IDH2* that were identified in human gliomas, along with the number of patients who carried each mutation. Codons 130 to 134 of *IDH1* and 170 to 174 of *IDH2* are shown. Panel B shows the number and frequency of *IDH1* and *IDH2* mutations in gliomas and other types of tumors. The roman numerals in parentheses are the tumor grades, according to histopathological and clinical criteria established by the World Health Organization. CNS denotes central nervous system.

IDH2 exons in these glioma samples revealed nine somatic mutations of *IDH2*, all at residue R172: R172G in two tumors, R172M in three tumors, and R172K in four tumors (Fig. 1A, and Fig. 1 in the Supplementary Appendix). The R172 residue in *IDH2* is the exact analogue of the R132 residue in *IDH1*, which is located in the active site of the enzyme and forms hydrogen bonds with the isocitrate substrate.[21]

To determine whether the mutations in *IDH1* and *IDH2* disturb the function of the corresponding proteins, we measured the enzymatic activity (reduction of $NADP^+$ to NADPH) of IDH1 and IDH2 proteins in an oligodendroglioma line that had been transfected with wild-type or mutant *IDH1* or *IDH2* genes. These mutants represented 88% of the *IDH1* mutations and 100% of the *IDH2* mutations found in patients. Figure 2 shows that exogenous expression of wild-type IDH1 or IDH2 significantly increased the production of NADPH, whereas only endogenous IDH activity was observed in cells that had been transfected with mutant *IDH1* or *IDH2* genes.

To further evaluate *IDH* alterations during glioma progression, we assessed *IDH1* mutations in seven progressive gliomas in which both low-grade and high-grade tumor samples were available. Sequence analysis identified *IDH1* mutations in both the low-grade and high-grade tumors in all seven cases (Table 1, and Fig. 2 in the Supplementary Appendix). These results demonstrate that *IDH1* alterations in high-grade tumors are derived from the earlier lesions.

We also examined diffuse astrocytomas, oligodendrogliomas, anaplastic oligodendrogliomas, anaplastic astrocytomas, and a subgroup of glioblastomas for mutations in *TP53* and *PTEN*, amplification of *EGFR*, deletion of *CDKN2A–CDKN2B*, and allelic losses of 1p and 19q (Table 1). *TP53* mutations were more common in diffuse astrocytomas (74%), anaplastic astrocytomas (65%), and secondary glioblastomas (62%) than in oligodendrogliomas (16%) or anaplastic oligodendrogliomas (9%) ($P<0.001$ for all comparisons by Fisher's exact test). Conversely, deletions of 1p and 19q were found more often in oligodendrocytic than in astrocytic tumors, as expected.[15]

Most (80%) of the anaplastic astrocytomas and glioblastomas with mutated *IDH1* or *IDH2* genes also had a mutation of *TP53*, but only 3% had al-

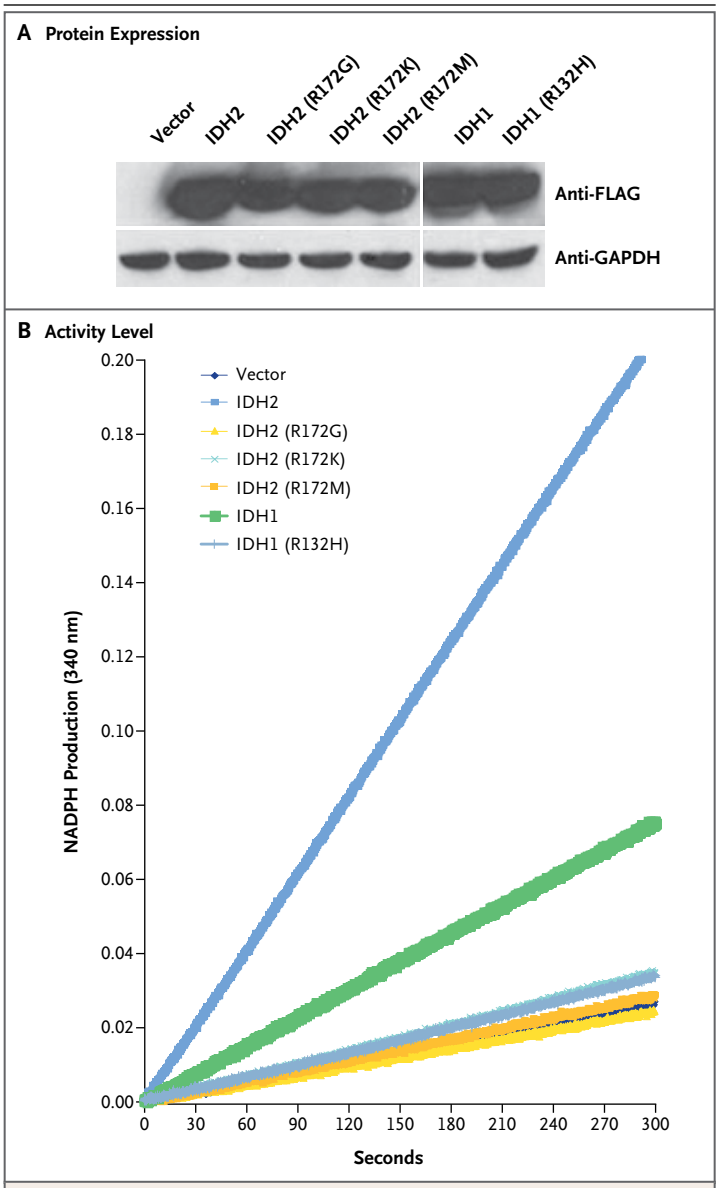

Figure 2. Enzymatic Activity of Wild-Type and Mutant IDH1 and IDH2 Proteins.

Cell lysates were extracted from a human oligodendroglioma cell line without *IDH1* or *IDH2* mutations that had been transfected with vectors encoding the indicated proteins. Panel A shows the expression of proteins encoded by wild-type and mutant *IDH1* and *IDH2*, as determined by Western blotting, with the use of an anti-FLAG antibody. Panel B shows the activity levels of these proteins, as analyzed by monitoring the production of NADPH. GAPDH denotes glyceraldehyde 3-phosphate dehydrogenase.

terations in *PTEN*, *EGFR*, *CDKN2A*, or *CDKN2B* (Table 2). Conversely, anaplastic astrocytomas and glioblastomas with wild-type *IDH1* and *IDH2* genes had few *TP53* mutations (18%) and more frequent alterations of *PTEN*, *EGFR*, *CDKN2A*, or *CDKN2B* (74%) (P<0.001 for both comparisons by Fisher's exact test). Loss of 1p and 19q was observed in 45 of 53 (85%) of the oligodendrocytic tumors with mutated *IDH1* or *IDH2* but in none of the tumors with wild-type *IDH* genes (P<0.001 by Fisher's exact test).

Patients with anaplastic astrocytomas or glioblastomas with *IDH1* or *IDH2* mutations were significantly younger than were patients with tumors carrying wild-type *IDH1* and *IDH2* genes (median age, 34 years vs. 56 years for patients with anaplastic astrocytomas and 32 years vs. 59 years for those with glioblastomas; P<0.001 for both comparisons by Student's t-test). Despite the lower median age of patients with *IDH1* or *IDH2* mutations, no mutations were identified in glioblastomas from the 15 patients who were under the age of 21 (Fig. 3 in the Supplementary Appendix). In patients with oligodendrogliomas or anaplastic oligodendrogliomas, the median age of the patients with *IDH1* or *IDH2* mutation was 39 years; *IDH1* mutations were identified in two teenagers (14 and 16 years) but not in four younger patients.

Our previous observation of improved outcome for patients whose glioblastomas carried the *IDH1* mutation[16] was confirmed in this larger data set and extended to include such patients with mutations in *IDH2*. Patients with a glioblastoma carrying an *IDH1* or *IDH2* mutation had a median overall survival of 31 months, which was significantly longer than the 15-month survival in patients with wild-type *IDH1* (P=0.002 by the log-rank test) (Fig. 3A). Mutations of *IDH* genes were also associated with improved outcome in patients with anaplastic astrocytomas; the median overall survival was 65 months for patients with mutations and 20 months for those without mutations (P<0.001 by the log-rank test) (Fig. 3B). Differential survival analyses could not be performed in patients with diffuse astrocytomas, oligodendrogliomas, or anaplastic oligodendrogliomas because there were too few tumors of these types without *IDH* gene mutations.

DISCUSSION

Our findings implicate mutations in the $NADP^+$-dependent isocitrate dehydrogenase genes, *IDH1* and *IDH2*, in the pathogenesis of malignant gliomas. Gliomas with *IDH* mutations were clinically and genetically distinct from gliomas with wild-type *IDH* genes. Notably, two subtypes of

Table 2. Frequency of Common Genetic Alterations in Gliomas with Mutated or Wild-Type *IDH1* and *IDH2* Genes.*

Tumor Type and *IDH1* or *IDH2* Mutational Status†	Total Patients	Location of Other Alterations‡				
		TP53	*PTEN*	*EGFR*	*CDKN2A* or *CDKN2B*	1p and 19q
	no.	*no./total no. (%)*				
Astrocytic tumors						
Diffuse astrocytoma (grade II)						
Mutant	27	17/20 (85)	0/19	0/23	0/23	1/19 (5)
Wild-type	3	0/3	0/2	0/3	0/3	0/3
Total	30	17/23 (74)	0/21	0/26	0/26	1/22 (5)
Anaplastic astrocytoma (grade III)						
Mutant	38	23/28 (82)	0/22	0/35	0/33	1/22 (5)
Wild-type	14	3/12 (25)	3/11 (27)	1/12 (8)	4/12 (33)	1/6 (17)
Total	52	26/40 (65)	3/33 (9)	1/47 (2)	4/45 (9)	1/28 (4)
Primary adult glioblastoma (grade IV)						
Mutant	6	5/6 (83)	0/5	0/6	0/6	1/2 (50)
Wild-type	117	23/117 (20)	21/88 (24)	35/86 (41)	39/87 (45)	0/24
Total	123	28/123 (23)	21/93 (23)	35/92 (38)	39/93 (42)	1/26 (4)
Secondary adult glioblastoma (grade IV)						
Mutant	11	8/11 (73)	0/6	0/10	1/8 (12)	NA
Wild-type	2	0/2	1/2 (50)	0/2	1/2 (50)	NA
Total	13	8/13 (62)	1/8 (12)	0/12	2/10 (20)	NA
Oligodendroglial tumors						
Oligodendroglioma (grade II)						
Mutant	43	5/24 (21)	0/20	0/43	2/40 (5)	18/23 (78)
Wild-type	8	0/8	0/7	0/8	0/8	0/7
Total	51	5/32 (16)	0/27	0/51	2/48 (4)	18/30 (60)
Anaplastic oligodendroglioma (grade III)						
Mutant	34	3/30 (10)	0/28	0/33	3/33 (9)	27/30 (90)
Wild-type	2	0/2	0/2	0/2	2/2 (100)	0/2
Total	36	3/32 (9)	0/30	0/35	5/35 (14)	27/32 (84)

* All tumors were analyzed for *IDH1* R132 and *IDH2* R172 mutations. In addition, all pilocytic astrocytomas, diffuse astrocytomas, oligodendrogliomas, anaplastic oligodendrogliomas, anaplastic astrocytomas, secondary glioblastomas, and 96 primary glioblastomas were evaluated for mutations in the remaining coding exons of *IDH1* and *IDH2*. NA denotes not analyzed.
† Tumors were graded according to histopathological and clinical criteria established by the World Health Organization.
‡ Alterations included mutations in *TP53* and *PTEN*, loss of heterozygosity in 1p and 19q, amplification in *EGFR*, and deletion in *CDKN2A* or *CDKN2B*.

gliomas of WHO grade II or III (astrocytomas and oligodendrogliomas) often carried *IDH* mutations but not other genetic alterations that are detectable relatively early during the progression of gliomas. This finding suggests that *IDH* mutations occur early in the development of a glioma from a stem cell that can give rise to both astrocytes and oligodendrocytes. The identification of *IDH1* mutations in 10 of 10 oligoastrocytomas and anaplastic oligoastrocytomas, tumors with mor-

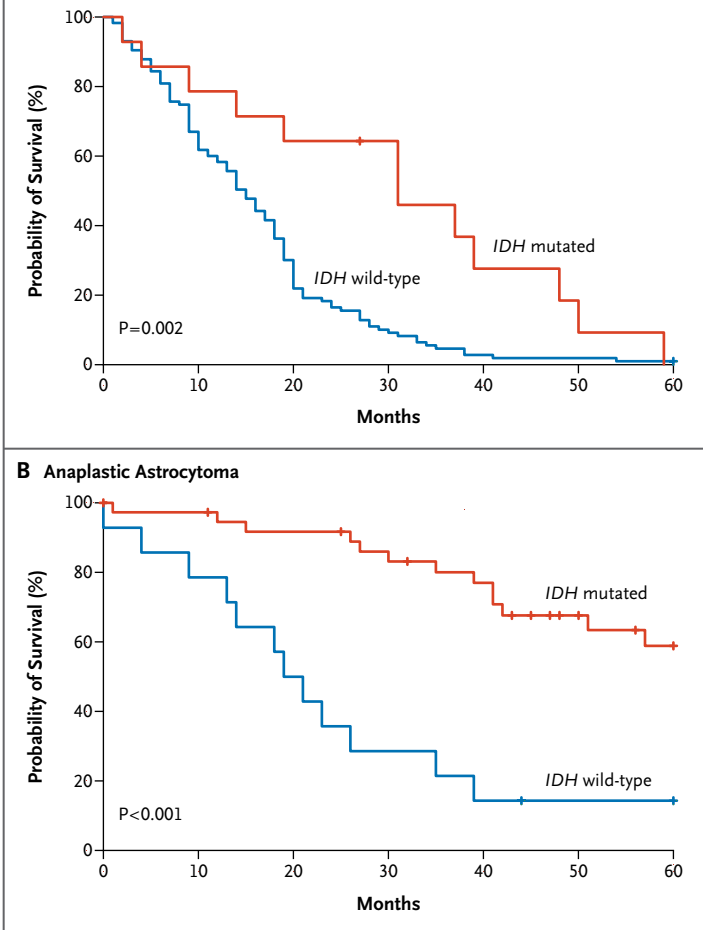

Figure 3. Survival of Adult Patients with Malignant Gliomas with or without *IDH* Gene Mutations.

For patients with glioblastomas, the median survival was 31 months for the 14 patients with mutated *IDH1* or *IDH2,* as compared with 15 months for the 115 patients with wild-type *IDH1* or *IDH2* (Panel A). For patients with anaplastic astrocytomas, the median survival was 65 months for the 38 patients with mutated *IDH1 or IDH2,* as compared with 20 months for the 14 patients with wild-type *IDH1* or *IDH2* (Panel B). Patients with both primary and secondary tumors were included in the analysis. For patients with secondary glioblastomas, survival was calculated from the date of the secondary diagnosis. Survival distributions were compared with the use of the log-rank test.

phologic features of both cell types, supports this conjecture.

Mutations in *IDH1* or *IDH2* were not identified in any pilocytic astrocytomas of WHO grade I, indicating that these tumors arise through a different mechanism. This conclusion is consistent with clinical observations that pilocytic astrocytomas rarely if ever undergo malignant transformation[2] and with recent data indicating that a duplication at 7q34 producing a *BRAF* fusion gene occurs frequently in pilocytic astrocytomas but not higher-grade gliomas.[22]

In each of the tested mutations, the enzymatic activity of the IDH proteins was eliminated. A previous study showed that in vitro substitution of glutamate for arginine at residue 132 of IDH1 (an alteration not observed in patients) resulted in a catalytically inactive enzyme.[23] Although our results demonstrate an effect of the mutations on the function of the IDH1 protein, they do not necessarily mean that the mutations are inactivating. For example, the mutant proteins that preclude the use of isocitrate as substrate could allow other, as-yet-unknown substrates to be used by the enzyme, thereby conferring a gain rather than a loss of activity. If future studies confirm this possibility, mutant IDH could become a target for therapeutic intervention.

Our results have important practical implications. Historically, glioblastomas have been divided into cancers that arise from low-grade gliomas (secondary tumors) and those without such an antecedent (primary tumors).[5,6] Secondary tumors account for only 5% of all glioblastomas. The finding that *IDH1* or *IDH2* is mutated in the vast majority of WHO grade II or III gliomas and in the secondary glioblastomas that develop from these precursors provides a biologic explanation for this clinical categorization: tumors with mutated $NADP^+$-dependent isocitrate dehydrogenases comprise a specific subgroup of glioblastomas.

The localization of *IDH1* and *IDH2* mutations to a single amino acid (R132 and R172, respectively) simplifies the use of this genetic alteration for diagnostic purposes. For example, *IDH* mutation tests could help distinguish pilocytic astrocytomas (WHO grade I) from diffuse astrocytomas (WHO grade II), since these lesions can sometimes be difficult to categorize solely on the basis of histopathological criteria.[2]

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REFERENCES

1. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, eds. WHO classifcation of tumours of the central nervous system. 4th ed. Lyon, France: IARC Press, 2007.
2. Burger PC, Scheithauer BW, Paulus W, et al. Pilocytic astrocytoma. In: Kleihues P, Cavenee WK, eds. Pathology and genetics of tumours of the nervous system. Lyon, France: IARC Press, 2000:45-51.
3. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005;352:987-96.
4. Wen PY, Kesari S. Malignant gliomas in adults. N Engl J Med 2008;359:492-507. [Erratum, N Engl J Med 2008;359:877.]
5. Ohgaki H, Dessen P, Jourde B, et al. Genetic pathways to glioblastoma: a population-based study. Cancer Res 2004;64:6892-9.
6. Ohgaki H, Kleihues P. Genetic pathways to primary and secondary glioblastoma. Am J Pathol 2007;170:1445-53.
7. Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 2008;455:1061-8.
8. Li J, Yen C, Liaw D, et al. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 1997;275:1943-7.
9. Nigro JM, Baker SJ, Preisinger AC, et al. Mutations in the p53 gene occur in diverse human tumour types. Nature 1989;342:705-8.
10. Ueki K, Ono Y, Henson JW, Efird JT, von Deimling A, Louis DN. CDKN2/p16 or RB alterations occur in the majority of glioblastomas and are inversely correlated. Cancer Res 1996;56:150-3.
11. Wong AJ, Bigner SH, Bigner DD, Kinzler KW, Hamilton SR, Vogelstein B. Increased expression of the epidermal growth factor receptor gene in malignant gliomas is invariably associated with gene amplification. Proc Natl Acad Sci U S A 1987;84:6899-903.
12. Wong AJ, Ruppert JM, Bigner SH, et al. Structural alterations of the epidermal growth factor receptor gene in human gliomas. Proc Natl Acad Sci U S A 1992;89:2965-9.
13. Furnari FB, Fenton T, Bachoo RM, et al. Malignant astrocytic glioma: genetics, biology, and paths to treatment. Genes Dev 2007;21:2683-710.
14. Weber RG, Sabel M, Reifenberger J, et al. Characterization of genomic alterations associated with glioma progression by comparative genomic hybridization. Oncogene 1996;13:983-94.
15. Bigner SH, Matthews MR, Rasheed BK, et al. Molecular genetic aspects of oligodendrogliomas including analysis by comparative genomic hybridization. Am J Pathol 1999;155:375-86.
16. Parsons DW, Jones S, Zhang X, et al. An integrated genomic analysis of human glioblastoma multiforme. Science 2008;321:1807-12.
17. Sjöblom T, Jones S, Wood LD, et al. The consensus coding sequences of human breast and colorectal cancers. Science 2006;314:268-74.
18. Wang TL, Diaz LA Jr, Romans K, et al. Digital karyotyping identifies thymidylate synthase amplification as a mechanism of resistance to 5-fluorouracil in metastatic colorectal cancer patients. Proc Natl Acad Sci U S A 2004;101:3089-94.
19. Reifenberger J, Reifenberger G, Liu L, James CD, Wechsler W, Collins VP. Molecular genetic analysis of oligodendroglial tumors shows preferential allelic deletions on 19q and 1p. Am J Pathol 1994;145:1175-90.
20. Batinic-Haberle I, Benov LT. An SOD mimic protects $NADP^+$-dependent isocitrate dehydrogenase against oxidative inactivation. Free Radic Res 2008;42:618-24.
21. Xu X, Zhao J, Xu Z, et al. Structures of human cytosolic NADP-dependent isocitrate dehydrogenase reveal a novel self-regulatory mechanism of activity. J Biol Chem 2004;279:33946-57.
22. Jones DT, Kocialkowski S, Liu L, et al. Tandem duplication producing a novel oncogenic BRAF fusion gene defines the majority of pilocytic astrocytomas. Cancer Res 2008;68:8673-7.
23. Jennings GT, Minard KI, McAlister-Henn L. Expression and mutagenesis of mammalian cytosolic $NADP^+$-specific isocitrate dehydrogenase. Biochemistry 1997;36:13743-7.