IDH1 Mutations as Molecular Signature and Predictive Factor of Secondary Glioblastomas

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Abstract

Purpose: To establish the frequency of *IDH1* mutations in glioblastomas at a population level, and to assess whether they allow reliable discrimination between primary (*de novo*) glioblastomas and secondary glioblastomas that progressed from low-grade or anaplastic astrocytoma.

Experimental Design: We screened glioblastomas from a population-based study for *IDH1* mutations and correlated them with clinical data and other genetic alterations.

Results: *IDH1* mutations were detected in 36 of 407 glioblastomas (8.8%). Glioblastoma patients with *IDH1* mutations were younger (mean, 47.9 years) than those with *EGFR* amplification (60.9 years) and were associated with significantly longer survival (mean, 27.1 versus 11.3 months; $P < 0.0001$). *IDH1* mutations were frequent in glioblastomas diagnosed as secondary (22 of 30; 73%), but rare in primary glioblastomas (14 of 377; 3.7%: $P < 0.0001$). *IDH1* mutations as genetic marker of secondary glioblastoma corresponded to the respective clinical diagnosis in 95% of cases. Glioblastomas with *IDH1* mutation diagnosed as primary had clinical and genetic profiles similar to those of secondary glioblastomas, suggesting that they may have rapidly progressed from a less malignant precursor lesion that escaped clinical diagnosis and were thus misclassified as primary. Conversely, glioblastomas without *IDH1* mutations clinically diagnosed as secondary typically developed from anaplastic rather than low-grade gliomas, suggesting that at least some were actually primary glioblastomas, that may have been misclassified, possibly due to histologic sampling error.

Conclusion: *IDH1* mutations are a strong predictor of a more favorable prognosis and a highly selective molecular marker of secondary glioblastomas that complements clinical criteria for distinguishing them from primary glioblastomas. (Clin Cancer Res 2009;15(19):6002–7)

Glioblastomas, most frequent and malignant brain tumors, may develop rapidly after a short clinical history and without evidence of a less malignant precursor lesion (primary or *de novo* glioblastoma), or slowly through progression from low-grade diffuse or anaplastic astrocytoma (secondary glioblastoma; refs. 1–3). These glioblastoma subtypes constitute distinct disease entities that affect patients of different age, and develop through different genetic pathways (2, 3). Because they are usually indistinguishable histologically (2–4), the distinction between primary and secondary glioblastomas is currently based on clinical data. Tumors are considered primary glioblastomas if the glioblastoma diagnosis is made at the first biopsy, without clinical or histologic evidence of a preexisting, less malignant precursor lesion. The diagnosis of secondary glioblastoma requires histologic evidence of a preceding low-grade or anaplastic astrocytoma. At the population level, only 5% of cases were classified as secondary glioblastoma (2). However, the possibility could not be excluded that some secondary glioblastomas rapidly progressed from less malignant precursor lesions, escaped clinical diagnosis, and were thus misclassified as primary glioblastomas.

IDH1 mutations have recently been identified in an analysis of 20,661 protein-coding genes in glioblastomas (5). Interestingly, many of the glioblastomas carrying *IDH1* mutations had features of secondary glioblastoma and contained *TP53* mutations (5). Subsequent studies showed that low-grade astrocytomas, oligoastrocytomas, oligodendrogliomas, and secondary glioblastomas frequently (>70% of cases) carry an *IDH1* mutation (6–8). *IDH1* mutations have been reported rare or absent in primary glioblastomas, other nervous system tumors (6, 7), or a variety of neoplasms at other organ sites (8, 9).

The objective of the present study was to establish the frequency of *IDH1* mutations in glioblastomas at the population

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Translational Relevance

This study established the frequency of *IDH1* mutations in glioblastomas at a population level, and shows that *IDH1* mutations are the best available molecular markers of secondary glioblastomas. In addition, *IDH* mutations are the most significant factor predictive of a more favorable clinical outcome. The specific location of *IDH1* mutations (all in codon 132) makes this molecular marker very useful as a routine diagnostic procedure. Therefore, these observations may have a significant clinical impact.

level, and to assess to which extent *IDH1* mutations allow a discrimination between primary and secondary glioblastomas. We also assessed mutations in the *IDH2* gene, related to *IDH1*, which was recently reported to be mutated in 4% to 8% of low-grade and anaplastic astrocytic and oligodendroglial gliomas, but in none of the 138 primary or 13 secondary glioblastomas analyzed (8).

Materials and Methods

Tumor samples. A total of 407 glioblastomas analyzed in the present study were from a population-based study in the Canton of Zurich, Switzerland, previously reported (2). They corresponded to 81% of all glioblastomas for which histologic sections were available. The mean age of glioblastoma patients was 59.5 ± 13.9 y and the male to female ratio was 1.4:1. Data on *IDH1* mutations in a subset of 82 glioblastomas were evaluated in a previous study (7).

Tumors were considered to be primary glioblastomas (377 cases) when the glioblastoma diagnosis was made at the first biopsy, without clinical or histologic evidence of a preexisting, less malignant precursor lesion (1, 2). A diagnosis of secondary glioblastoma (30 cases) was made only in cases with histologic evidence of preceding low-grade glioma or anaplastic glioma. For survival analysis, data on 203 patients who were similarly treated with surgical resection aimed at maximum removal followed by radiotherapy (typically 2-Gy fractions and a total dose of 60-Gy) were used.

Single-strand conformational polymorphism (SSCP) analysis and direct DNA sequencing for **IDH1** *and* **IDH2** *mutations.* DNA was extracted from paraffin-embedded sections as previously described (2). PCR-single-strand conformational polymorphism analysis was carried out to prescreen for mutations in exon 4 of the *IDH1* gene as previously described (7), and for mutations in exon 4 of the *IDH2* gene. The primer sequences to detect *IDH1* mutations have been previously reported (7). The primers for *IDH2* mutations were 5′-CCA CTA TTA TCT CTG TCC TC-3′ (sense) and 5′-GCT AGG CGA GGA GCT CCA GT-3′ (antisense). Briefly, PCR was done in a total volume of 10 μL, consisting of 1 μL of DNA solution (~100 ng/μL), 0.5 U of PLATINUM Taq DNA polymerase (Invitrogen), 0.1 mCi of [α-^{33}P]dCTP (ICN Biomedicals, Inc.; specific activity, 3,000 Ci/mmol), 2.5 mmol/L MgCl$_2$, 0.1 mmol/L of each dNTP, 0.25 mmol/L of each primer, 10 mmol/L Tris-HCl (pH 8.3), and 50 mmol/L KCl in a thermal cycler (Biometra) with an initial denaturing step at 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 56°C (for *IDH1*) or 62°C (for *IDH2*) for 30 s, extension at 72°C for 40 s, and a final extension at 72°C for 10 min. PCR products (10 μL) were mixed with 20 μL loading buffer (0.02 N NaOH, 95% formamide, 20 mmol/L EDTA, 0.05% xylene cyanol and bromophenol blue), denatured at 95°C for 10 min and quenched on ice, and then 5.5 μL of this mixture were electrophoresed on a 12.5% polyacrylamide nondenaturing gel containing

10% glycerol at 45 W for 2.5 h with cooling by a fan. Gels were dried at 80°C and autoradiographed for 24 to 36 h. Samples with variant bands in SSCP analyses were further analyzed by direct sequencing on ABI 3100 PRISM DNA sequencer (Applied Biosystems) with the Big DyeTM Terminator cycle sequencing kit (ABI PRISM, Applied Biosystems).

Statistical analyses. Student's *t* test was done to compare the mean age of the patients. The χ^2 test or Fisher's exact test were carried out to analyze the significance of the association between *IDH1* mutation and other genetic alterations, and to compare frequencies of *IDH1* mutations between male and female patients. The Kaplan-Meier method and the log-rank test were used for survival analysis. Cox regression models were used to assess the effect of *IDH1* mutations on the survival of patients who were treated in a similar way, i.e., surgical resection aimed at maximum removal followed by radiotherapy. Adjustment was made for age and gender.

Results

IDH1 *mutations in glioblastomas.* Of a total of 407 glioblastomas analyzed, 36 (8.8%) contained an *IDH1* mutation. All mutations were located at amino acid residue 132, and 83% of them were G395A (Arg->His), followed by C394G (Arg->Gly; 11.1%), C394T (Arg->Cys; 2.8%), and C394A (Arg->Ser; 2.8%). All *IDH1* mutations were heterozygous (Fig. 1). None of 367 glioblastomas analyzed contained an *IDH2* mutation.

Patients with glioblastomas carrying *IDH1* mutations were significantly younger (mean, 47.9 years) than those without *IDH1* mutations (60.6 years; *P* < 0.0001; Table 1). The mean time from the first clinical symptom to glioblastoma diagnosis was significantly longer in patients with *IDH1* mutations (mean, 15.2 months) than in those without *IDH1* mutations (3.9 months; *P* = 0.0003; Table 1). The mean survival time of glioblastoma patients with *IDH1* mutations was 27.1 months, significantly longer than that of patients without *IDH1* mutations (11.3 months; *P* < 0.0001, log-rank test; Fig. 2). Multivariate analysis after adjustment for age and gender confirmed that the presence of *IDH1* mutations is significantly associated with longer survival (Table 1).

For 13 cases with *IDH1* mutations (3 primary and 10 secondary glioblastomas), peritumoral normal brain tissue (10 cases) or a peripheral blood sample (3 cases) was obtained; no *IDH1* mutations were found in these tissues, indicating that the *IDH1* mutations were somatic.

IDH1 *mutations and other genetic alterations.* We correlated *IDH1* mutations found in the present study with other genetic alterations (*TP53* mutations, *EGFR* amplification, *p16*^{INK4a} homozygous deletion, *PTEN* mutations, LOH 10q, LOH 1p, and LOH 19q), which have been previously reported (2, 4). Glioblastomas with *IDH1* mutations also had frequent *TP53* mutations and LOH 19q, whereas those without *IDH1* mutations often showed *EGFR* amplification (Table 1). There were significant associations between *IDH1* mutations and *TP53* mutations (*P* < 0.0001) and between *IDH1* mutations and LOH 19q (*P* < 0.0001), whereas an inverse association was observed between *IDH1* mutations and *EGFR* amplification (*P* = 0.005). Glioblastoma patients with *IDH1* mutations were younger (mean, 47.9 years) than those with *EGFR* amplification (60.9 years; Fig. 3).

IDH1 *mutations and histologic features.* We correlated *IDH1* mutations identified in the present study with previously reported histologic features (4). Oligodendroglial components were significantly more frequent in glioblastomas with *IDH1*
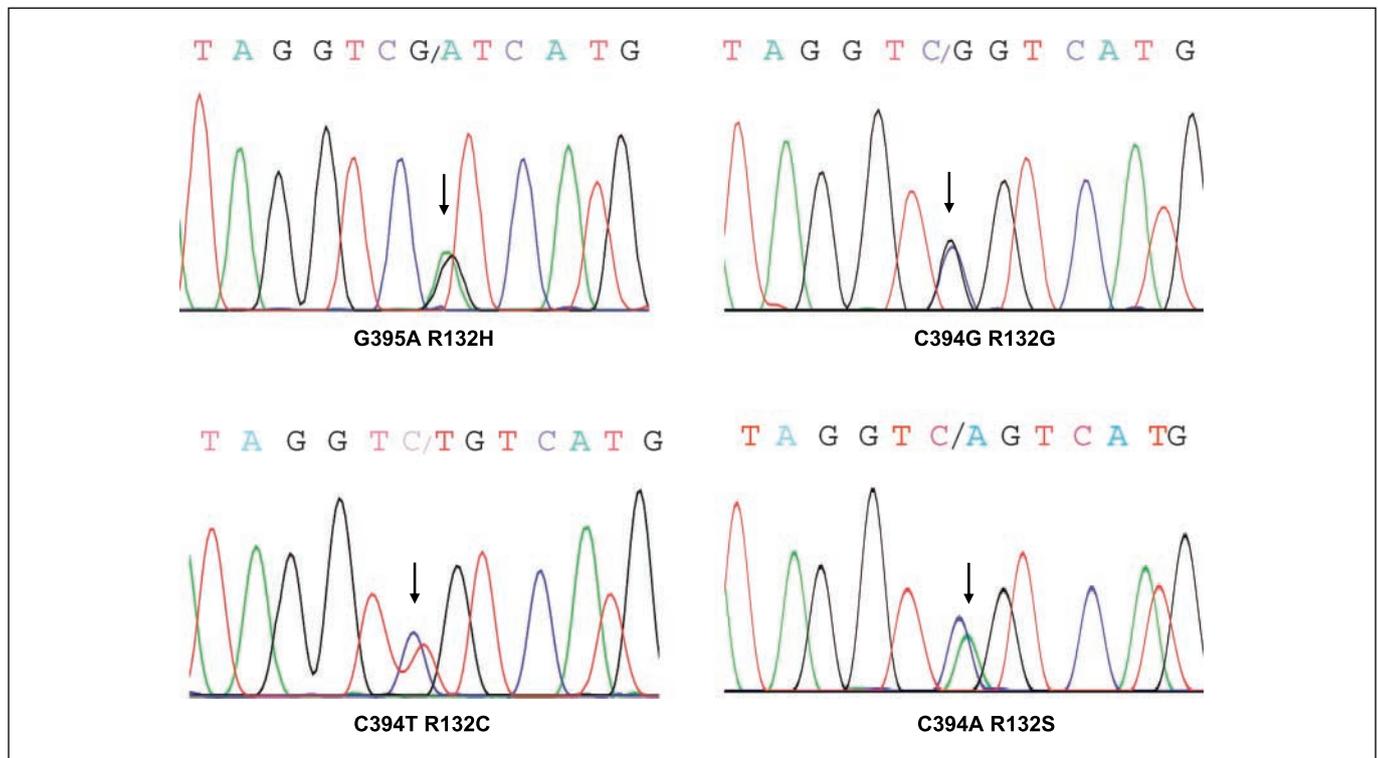
Fig. 1. *IDH1* mutations in glioblastomas. All mutations were heterozygous and located at codon 132.

mutations (54.1% versus 19.5%; *P* < 0.0001), whereas large ischemic and pseudopalisading necroses were histologic hallmarks of tumors without *IDH1* mutations (50.0% versus 90.3%; *P* < 0.0001). There was no significant difference in the frequency of other histologic features, including the presence of glomeruloid vascular proliferation (data not shown).

IDH1 mutations in primary and secondary glioblastomas. *IDH1* mutations were observed in the majority of glioblastomas clinically diagnosed as secondary (22 of 30, 73.3%), but rarely in glioblastomas diagnosed as primary (14 of 377, 3.7%; *P* < 0.0001; Table 2). Taking *IDH1* mutations as a genetic marker of secondary but not primary glioblastomas corresponded to

Table 1. Clinical and genetic features of glioblastomas with and without *IDH1* mutations

	Glioblastomas with *IDH1* mutation	Glioblastomas without *IDH1* mutation	*P*
No. of cases	36 cases (8.8%)	371 cases (91.2%)	
Age at GBM diagnosis	47.9 ± 12.9 y	60.6 ± 13.5 y	<0.0001
Male/female ratio	1.12	1.46	N.s.
Clinical history*	15.2 ± 45.8 mo	3.9 ± 10.8 mo	0.0003
Primary/secondary GBM[†]	14/22 (0.64)	363/8 (45.4)	<0.0001
Median overall survival[‡]	24.0 mo (*n* = 17)	9.9 mo (*n* = 186)	<0.0001
Mean overall survival[‡]	27.1 ± 18.0 mo (*n* = 17)	11.3 ± 7.1 mo (*n* = 186)	<0.0001
Hazard ratio (95% CI)[§]	0.288 (0.163-0.508)	1	<0.0001
Genetic alterations			
TP53 mutation	26/32 (81.3%)	88/331 (26.6%)	<0.0001
EGFR amplification	2/31 (6.5%)	115/329 (35.0%)	0.0005
p16^{INK4a} deletion	5/23 (21.7%)	86/284 (30.3%)	N.s.
PTEN mutation	2/25 (8.0%)	66/274 (24.1%)	N.s.
LOH 1p	5/21 (23.8%)	34/227 (15.0%)	N.s.
LOH 10q	16/22 (72.7%)	145/220 (66.6%)	N.s.
LOH 19q	6/19 (31.6%)	9/230 (3.9%)	<0.0001

Abbreviations: GBM, glioblastoma; N.s., not significant; CI, confidence interval; y, years; mo, months.
*Time from the first clinical symptom to glioblastoma diagnosis.
[†]Tumors were considered primary when a glioblastoma diagnosis was made at the first biopsy, without clinical or histologic evidence of a pre-existing, less malignant precursor lesion. A diagnosis of secondary glioblastoma was made only in cases with clinical and histologic evidence of a preceding low-grade or anaplastic glioma.
[‡]Following surgery and radiotherapy.
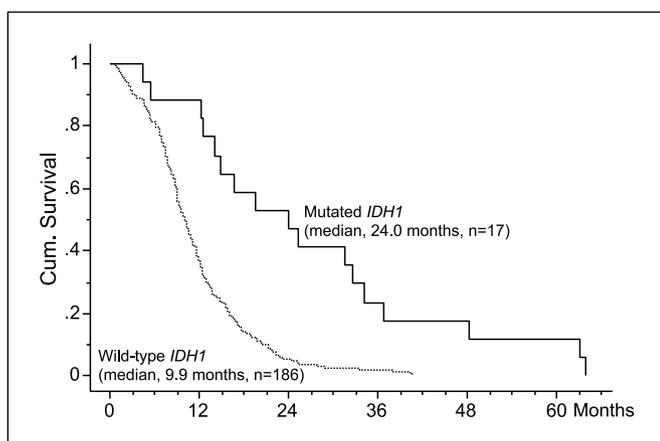[§]Adjusted for age and gender.

Fig. 2. Survival of glioblastoma patients treated with surgery plus radiotherapy. Note that glioblastoma patients carrying an *IDH1* mutation had significantly longer overall survival (log-rank test; Mantel-Cox test; $P < 0.0001$).

the respective clinical diagnosis in 385 of 407 (95%) cases. The sensitivity and specificity for *IDH1* mutations as a molecular marker discriminating secondary glioblastomas from primary glioblastomas were 22 of 30 (73.3%) and 363 of 377 (96.3%), respectively.

Exceptions were 14 glioblastomas with *IDH1* mutations that were clinically diagnosed as primary. These patients were significantly younger than those without *IDH1* mutations (mean, 50.8 versus 60.9 years; $P = 0.0054$), showed a longer clinical history (mean, 28.8 versus 3.8 months: $P < 0.0001$), had frequent *TP53* mutations (75% versus 27%; $P = 0.0009$) and lacked *EGFR* amplification (0% versus 35.4%; $P = 0.0033$; Table 2). Thus, their clinical and genetic profiles were similar to those of secondary glioblastomas.

Conversely, there were eight glioblastomas without *IDH1* mutations that were clinically diagnosed as secondary. These glioblastomas showed less frequent *TP53* mutations (20% versus 85%; $P = 0.0123$) and more frequent $p16^{INK4a}$ deletion (66.7% versus 15.4%; $P = 0.0460$) than those with *IDH1* mutations. Most glioblastomas with *IDH1* mutations that were diagnosed as secondary (16 of 22; 73%) had developed by progression from low-grade glioma WHO grade II (11 low-grade diffuse astrocytomas and 5 oligoastrocytomas), and only 6 cases progressed from anaplastic astrocytomas (WHO grade III), whereas most glioblastomas without *IDH1* mutations that were clinically diagnosed as secondary had developed through progression from WHO grade III anaplastic gliomas (7 of 8 cases; 4 anaplastic astrocytomas, 2 anaplastic oligoastrocytomas, 1 anaplastic oligodendroglioma); only 1 case developed through progression from low-grade astrocytoma WHO grade II.

The mean survival of patients with *IDH1* mutation-positive glioblastoma diagnosed as primary was 30 months, which was significantly longer than for *IDH1* mutation-negative glioblastomas (11.3 months; $P < 0.0001$; Table 2), but was not different from the survival of patients with *IDH1* mutation-positive glioblastoma diagnosed as secondary (23.8 months; $P = 0.4971$).

Discussion

The distinction between primary and secondary glioblastoma was first made by Hans-Joachim Scherer in 1940 (10), but re-

mained conceptual, without use in diagnosis, largely because these subtypes are considered histopathologically indistinguishable. During the past decade, evidence has accumulated that primary and secondary glioblastomas constitute distinct disease entities that affect patients of different age, develop through different genetic pathways (1, 2), show different RNA and protein expression profiles (11–13), and may differ in their response to radiotherapy and chemotherapy.

Both primary and secondary glioblastomas show frequent LOH 10q (63-70%), but differ with respect to other genetic alterations. Changes that are significantly more frequent in primary glioblastomas include LOH 10p (47% versus 8%), *EGFR* amplification (36% versus 8%), and *PTEN* mutations (25% versus 4%; refs. 3, 14). Genetic hallmarks of secondary glioblastomas are frequent *TP53* mutations (28% versus 65%), LOH 19q (6% versus 54%), and LOH 22q (41% versus 82%; refs. 3, 15). However, none of these genetic alterations reliably separate these glioblastoma subtypes. The present study shows that *IDH1* mutations constitute a remarkably reliable molecular signature of secondary glioblastomas. The majority of glioblastomas (95%) were correctly classified as primary or secondary glioblastoma using the presence of an *IDH1* mutation alone as a molecular marker.

Exceptions were some rare glioblastomas with *IDH1* mutations clinically diagnosed as primary (14 cases, 3.7%). They had clinical and genetic profiles similar to those of secondary glioblastomas, i.e., young age, longer survival, frequent *TP53* mutations, and absence of *EGFR* amplification (Table 2), suggesting that at least some of these may actually be secondary glioblastomas that rapidly progressed from less malignant precursor lesions that escaped clinical diagnosis.

Most secondary glioblastomas with *IDH1* mutations (16 of 22; 73%) developed from low-grade astrocytomas or oligoastrocytomas (WHO grade II). In contrast, 7 of the 8 (88%) glioblastomas without *IDH1* mutations clinically diagnosed as secondary progressed from anaplastic astrocytoma, anaplastic oligoastrocytoma or anaplastic oligodendroglioma (WHO grade III). The possibility therefore exists that some of these are actually primary glioblastomas that were misclassified due
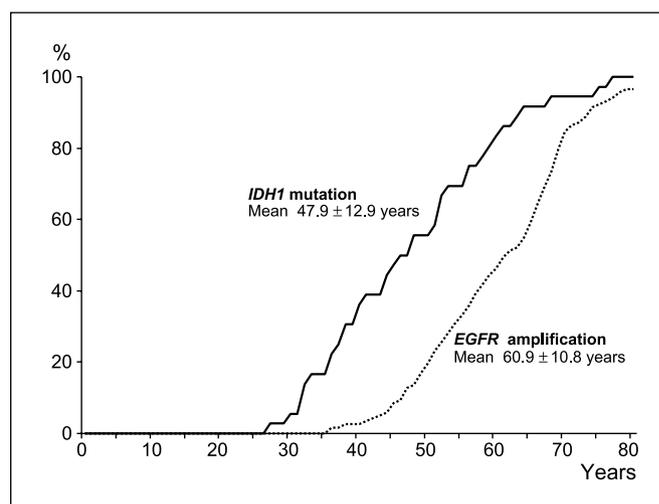

Fig. 3. Cumulative age distribution of glioblastoma patients with *IDH1* mutations and *EGFR* amplification. Glioblastoma patients with *IDH1* mutations were significantly younger than those with *EGFR* amplification.

Table 2. *IDH1* mutations in glioblastomas clinically diagnosed as primary

IDH1 mutation status	IDH1 mutation -	IDH1 mutation +	P
No. of cases	363 (96.3%)	14 (3.7%)	
Age (y)	60.9 ± 13.3	50.8 ± 12.1	0.0054
Male/female ratio	1.49	1.8	N.s.
Clinical history* (mo)	3.8 ± 10.2	28.8 ± 74.1	<0.0001
Median survival (mo)	10.0 ($n = 185$)	31.6 ($n = 9$)	<0.0001
Mean survival (mo)	11.3 ± 7.1 ($n = 185$)	30.0 ± 18.7 ($n = 9$)	<0.0001
Genetic alterations			
TP53 mutations	87/326 (26.7%)	9/12 (75.0%)	0.0009
EGFR amplification	114/322 (35.4%)	0/14 (0%)	0.0033
$p16^{INK4a}$ deletion	82/278 (29.5%)	3/10 (30.0%)	N.s.
PTEN mutations	66/270 (24.4%)	1/10 (10.0%)	N.s.
LOH 1p	34/223 (15.2%)	1/8 (12.5%)	N.s.
LOH 10q	142/216 (65.7%)	8/9 (88.9%)	N.s.
LOH 19q	9/226 (4.0%)	1/7 (14.3%)	N.s.

Abbreviations: N.s., not significant; y, years; mo, months.
*Time from the first clinical symptom to glioblastoma diagnosis.

to a sampling error, e.g., because of the absence of necrosis and/or microvascular proliferation in available histologic sections.

IDH1 mutations are associated with younger age of patients with low-grade diffuse astrocytomas (7), anaplastic astrocytomas (6, 8), anaplastic oligoastrocytomas (6), and glioblastomas (5–8). In line with recent reports (5, 8), we confirmed in a large cohort of patients treated with surgery and radiotherapy, *IDH1* mutations were the most significant predictive factor for longer survival.

However, the molecular mechanisms of these findings remain unclear. The *IDH1* gene encodes isocitrate dehydrogenase (IDH) 1 (16), which catalyzes the oxidative carboxylation of isocitrate to α-ketoglutarate, resulting in the production of NADPH (17) in the citric acid (Krebs) cycle (18). In contrast to other IDHs in the mitochondria, IDH1 is present in the cytosol. It has recently been reported that heterozygous *IDH1* mutations impair the enzyme's affinity for its substrate and dominantly inhibit wild-type IDH1 activity through the formation of catalytically inactive heterodimers (19), and that forced expression of mutant IDH1 in cultured cells reduced formation of α-ketoglutarate, and increased the levels of hypoxia-inducible factor subunit HIF-1α (19), a transcription factor that activates >100 hypoxia-regulated genes, including vascular endothelial growth factor, which induces tumor angiogenesis (20, 21). Glioblastomas with *IDH1* mutations showed less frequent necroses than those without *IDH1* mutations. However, this may reflect the more rapid growth of primary glioblastomas rather than functional neoangiogenesis; the present study also showed that vascular proliferation was similarly frequent in glioblastomas with and without *IDH1* mutations.

In conclusion, the present study strongly suggests that *IDH1* mutations are a significant predictor of a more favorable clinical outcome and constitute a highly selective molecular signature of secondary glioblastomas. The specific location of *IDH1* mutations (all in codon 132) makes this molecular marker very useful as a part of a routine diagnostic procedure. The distinction of primary versus secondary glioblastomas may not influence management decisions in the immediate future. However, it is a crucial factor in clinical trials, because future studies may reveal that because of their different genetic profiles, these glioblastoma subtypes may also significantly differ in response to therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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