Accumulation of 2-hydroxyglutarate is not a biomarker for malignant progression in IDH-mutated low-grade gliomas

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Objectives. To determine whether accumulation of 2-hydroxyglutarate in IDH-mutated low-grade gliomas (LGG; WHO grade II) correlates with their malignant transformation and to evaluate changes in metabolite levels during malignant progression.

Methods. Samples from 54 patients were screened for IDH mutations: 17 patients with LGG without malignant transformation, 18 patients with both LGG and their consecutive secondary glioblastomas (sGBM; n = 36), 2 additional patients with sGBM, 10 patients with primary glioblastomas (pGBM), and 7 patients without gliomas. The cellular tricarboxylic acid cycle metabolites, citrate, isocitrate, 2-hydroxyglutarate, α-ketoglutarate, fumarate, and succinate were profiled by liquid chromatography–tandem mass spectrometry. Ratios of 2-hydroxyglutarate/isocitrate were used to evaluate differences in 2-hydroxyglutarate accumulation in tumors from LGG and sGBM groups, compared with pGBM and nonglioma groups.

Results. IDH1 mutations were detected in 27 (77.1%) of 37 patients with LGG. In addition, in patients with LGG with malignant progression (n = 18), 17 patients were IDH1 mutated with a stable mutation status during their malignant progression. None of the patients with pGBM or nonglioma tumors had an IDH mutation. Increased 2-hydroxyglutarate/isocitrate ratios were seen in patients with IDH1-mutated LGG and sGBM, in comparison with those with IDH1-nonmutated LGG, pGBM, and nonglioma groups. However, no differences in intratumoral 2-hydroxyglutarate/isocitrate ratios were found between patients with LGG with and without malignant transformation. Furthermore, in patients with paired samples of LGG and their consecutive sGBM, the 2-hydroxyglutarate/isocitrate ratios did not differ between both tumor stages.

Conclusion. Although intratumoral 2-hydroxyglutarate accumulation provides a marker for the presence of IDH mutations, the metabolite is not a useful biomarker for identifying malignant transformation or evaluating malignant progression.

Keywords: α-ketoglutarate, IDH1 mutations, liquid chromatography–tandem mass spectrometry, low-grade gliomas, secondary glioblastomas, 2-hydroxyglutarate.
gliomas lacking cytosolic IDH1 mutations were later observed to have mutations in IDH2, the mitochondrial homolog of IDH1.12 IDH mutations are the most commonly mutated genes in many types of gliomas, with incidences of up to 75% in grade II and grade III gliomas.13,14 Further frequent mutations in patients with LGG were recently identified, including inactivating alterations in alpha thalassemia/mental retardation syndrome X-linked (ATRX), inactivating mutations in 2 suppressor genes, homolog of Drosophila capicua (CIC) and far-upstream binding protein 1 (FUBP1), in about 70% of grade II gliomas and 57% of sGBM.15–17 The association between ATRX mutations with IDH mutations and the association between IDH1 mutations are found to be consistently elevated by FUBP1 mutations with LGG standard enzymatic activity of gliomas.13,14 Further frequent mutations in patients with LGG were recently identified, including inactivating mutations of patients, patients with LGG with and without malignant transformation, with use of liquid chromatography–tandem mass spectrometry (LC-MS/MS). Furthermore, we compared the concentrations of the IDH1 mutations in gliomas lacking cytosolic IDH1 mutations and 1p/19q loss are especially common among the grade II-III gliomas and remarkably homogeneous in terms of genetic alterations and clinical characteristics.16 It was thought that IDH mutations might be a prognostic factor in LGG, predicting a prolonged survival from the beginning of the disease.18–23 However, this assumption, as shown in our and other earlier studies, had to be corrected because survival among patients who have LGG with IDH mutations is only improved after transformation to secondary high-grade gliomas.18,19,24 Furthermore, it had already been demonstrated that an IDH mutation is not a biomarker for further malignant transformation in LGG.18 IDH1 and IDH2 catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG) and reduce NADP to NADPH.25 The mutations inactivate the standard enzymatic activity of IDH12 and confer novel activity on IDH1 for conversion of α-KG and NADPH to 2-hydroxyglutarate (2HG) and NADP+, supporting the evidence that IDH1 and 2 are proto-oncogenes. This gain of function causes an accumulation of 2HG in glial and acute myeloid leukemia samples.26,27 The 2HG levels in cancers with IDH mutations are found to be consistently elevated by 10–100-fold, compared with levels in samples lacking mutations of IDH1 or IDH2.26,28 Nevertheless, how exactly the production or accumulation of 2HG by mutant IDH might drive cancer development is not well understood.

In the present study, we postulate that intratumoral 2HG could be a useful biomarker that predicts the malignant transformation of WHO grade II LGG. We therefore screened for IDH mutations in patients with LGG and measured the accumulation of 2HG in 2 populations of patients, patients with LGG with and without malignant transformation, with use of liquid chromatography–tandem mass spectrometry (LC-MS/MS). Furthermore, we compared the concentrations of 2HG in LGG and their consecutive secondary glioblastomas (sGBM) to evaluate changes in metabolite levels during the malignant progression.

### Methods and Materials

#### Patient Population

The tumor database of the Department of Neurosurgery at the University Hospital (Dresden, Germany) was used to identify adult patients who underwent surgery from 1995 through 2005 for WHO grade II LGG and those who underwent surgery from 1995 through 2010 for WHO grade IV sGBM. Furthermore, for a negative control, we identified patients with primary GBM (pGBM) and nonglial brain tumors in the latter period. Consecutive sGBM were categorized on the basis of histological criteria, provided that a WHO grade II tumor had been diagnosed at least 2 years before surgery in the same patient. Histopathology was performed in the Department of Neuropathology at the Dresden University Hospital. In addition, a reference pathological review was performed in all glioma cases. All patients were at least 18 years of age at diagnosis and gave informed written consent for molecular analyses of tumor tissue. The study was approved by the local ethics committee.

According to aforementioned criteria, a total of 72 tumor samples from 54 patients were analyzed (Table 1). The samples were from 17 patients with LGG without malignant transformation, 18 patients with both LGG and consecutive sGBM (n = 36), 2 additional patients with sGBM, 10 patients with pGBM, and 7 patients with nonglioma tumors. The nonglioma

### Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>LGG1</th>
<th>LGG2</th>
<th>sGBM</th>
<th>pGBM</th>
<th>Nonglioma</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>17</td>
<td>18</td>
<td>20</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Ages&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.4 (19–67)</td>
<td>37.1 (22–67)</td>
<td>42.3 (25–70)</td>
<td>55.2 (47–71)</td>
<td>44.7 (31–66)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>12</td>
<td>7</td>
<td>8</td>
<td>2</td>
<td>3</td>
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<tr>
<td>Men</td>
<td>5</td>
<td>11</td>
<td>12</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>IDH1-Mutation</td>
<td>10 (59%)</td>
<td>17 (95%)</td>
<td>18 (90%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IDH2-Mutation</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Oligodendroglial components</td>
<td>1</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviations: LGG, low-grade gliomas; sGBM, secondary glioblastomas; pGBM, primary glioblastomas.
<sup>a</sup>Eighteen Paired IDH- mutated samples from the same patients before (LGG2) and after malignant transformation (sGBM).
<sup>b</sup>Ages presented as medians in years, ranges in parentheses.
samples comprised 3 meningiomas, 2 metastases of breast cancers, 1 cavernoma, and 1 reactive gliosis from a patient with epilepsy.

**DNA Isolation and IDH Mutation Detection**

Tumor tissue samples were taken intraoperatively and were snap frozen at −80°C. To ensure a tumor cell content of at least 80% for nucleic acid extraction, control slides stained with hematoxylin and eosin were examined by the local neuropathologist. *IDH1* and *IDH2* mutations were assessed using direct DNA sequencing, as reported previously.18

**Progression and Survival**

Progression-free survival (PFS) was defined as the time from first diagnosis of an LGG to tumor progression or end of follow-up. Time to malignant transformation was defined as the time from the day of first surgery for an LGG to the day of surgery for malignant progression to a secondary high-grade glioma. Overall survival (OS) was the time from the day of first surgery to death or end of follow-up. All patient data were updated on June 15, 2012.

**LC-MS/MS Analysis of Tricarboxylic Acid Cycle (TCA) Metabolites**

Instrumentation included an AB Sciex QTRAP 5500 triple quadruple mass spectrometer coupled to a high-performance liquid chromatography (HPLC) system from Shimadzu containing a binary pump system, an autosampler, and a column oven. Targeted analyses of citrate, isocitrate, α-ketoglutarate (α-KG), succinate, fumarate (Sigma-Aldrich), and 2-hydroxyglutarate (2HG; SiChem GmbH) were performed in multiple reaction monitoring (MRM) scan mode with use of negative electrospray ionization (-ESI). Expected mass/charge ratios (m/z), assumed as [M-H]^−, were m/z 190.9, m/z 191.0, m/z 145.0, m/z 116.9, m/z 114.8, and m/z 147.0 for citrate, isocitrate, α-KG, succinate, fumarate, and 2HG, respectively. For quantification, ratios of analytes and respective stable isotope-labeled internal standards (IS) (Table 2) were used. For quantification of isocitrate and 2HG, stable isotope-labeled succinate was used as IS because of unavailability of labeled analogs. MRM transitions are summarized in Table 2.

Optimization of compound-dependent source and fragmentation parameters were performed by injection of single standards with use of the instrument-integrated syringe pump (Table 2). Ionization source parameters were further optimized by automatic flow injection analyses provided by the Analyst software package (version 1.51, AB Sciex) with curtain gas (35 psi), ESI voltage (-4500V), temperature (550°C), gas 1 (75 psi), and gas 2 (60 psi). Chromatographical separation of analytes was achieved using an Agilent ZORBAX SB-Aq (150 × 3.0 mm; 3.5 μm) HPLC-column. Mobile phases A and B consisted of 5 mM ammonium formate (pH adjusted with formic acid at pH 2) and acetonitrile, respectively. A 2% proportion of mobile phase B over the first 1.5 min after injection was increased linearly to reach 30% at 6.0 min and then 100% at 6.5 min. Column washing was completed at 7.0 min, at which stage the gradient was reversed to reach the initial 2% proportion of mobile phase B at 7.5 min. This was followed by 2.5 min of column equilibration. Column temperature was maintained at 25°C and autosampler temperature at 5°C.

**Sample Preparation for Targeted LC-MS/MS Analysis of TCA Metabolites**

Aliquots of tumor tissues were weighed and placed in extraction vials. Five hundred microliters of cold methanol and 20 μL of a mixed working solution of IS (2.5 μg/mL each, diluted in methanol) were added to the tissue. Specimen homogenization for cellular metabolite extraction was performed using a SilentCrusher M (Heidolph) with maximum speed under cooled conditions. Further

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### Table 2. MRM transitions and respective fragmentation parameters

<table>
<thead>
<tr>
<th>Compound</th>
<th>Q1</th>
<th>Q3</th>
<th>DP [V]</th>
<th>EP [V]</th>
<th>CE [eV]</th>
<th>CXP [V]</th>
</tr>
</thead>
<tbody>
<tr>
<td>citrate</td>
<td>190.8</td>
<td>111.9+/86.6</td>
<td>−50</td>
<td>−10</td>
<td>−18/−24</td>
<td>−11/−7</td>
</tr>
<tr>
<td>13C2-citrate</td>
<td>192.8</td>
<td>111.9+/86.6</td>
<td>−50</td>
<td>−10</td>
<td>−18/−24</td>
<td>−11/−7</td>
</tr>
<tr>
<td>isocitrate</td>
<td>191</td>
<td>172.7+/155.2</td>
<td>−50</td>
<td>−9</td>
<td>−14/−18</td>
<td>−9/−9</td>
</tr>
<tr>
<td>α-ketoglutarate</td>
<td>145</td>
<td>100.9+/56.8a</td>
<td>−45</td>
<td>−10</td>
<td>−12/−16</td>
<td>−6/−8</td>
</tr>
<tr>
<td>13C2-α-ketoglutarate</td>
<td>149</td>
<td>105</td>
<td>−50</td>
<td>−11</td>
<td>−12</td>
<td>−7</td>
</tr>
<tr>
<td>2-hydroxy-glutarate</td>
<td>147</td>
<td>129+/101</td>
<td>−70</td>
<td>−10</td>
<td>−14/−20</td>
<td>−9/−9</td>
</tr>
<tr>
<td>succinate</td>
<td>116.9</td>
<td>73.1+/98.8</td>
<td>−40</td>
<td>−10</td>
<td>−16/−14</td>
<td>−5/−11</td>
</tr>
<tr>
<td>13C4-succinate</td>
<td>120.9</td>
<td>75.9</td>
<td>−40</td>
<td>−10</td>
<td>−16</td>
<td>−7</td>
</tr>
<tr>
<td>fumarate</td>
<td>114.8</td>
<td>71</td>
<td>−40</td>
<td>−10</td>
<td>−12</td>
<td>−9</td>
</tr>
<tr>
<td>13C2-fumarate</td>
<td>118.8</td>
<td>73.8</td>
<td>−40</td>
<td>−10</td>
<td>−12</td>
<td>−9</td>
</tr>
</tbody>
</table>

**Abbreviations:** CE, collision energy; CXP, cell exit potential; DP, declustering potential; EP, entrance potential; Q1, precursor ion mass; Q3, product ion masses.

*aFragments used as quantifier ions.*
processing included centrifugation of homogenized solutions and vacuum-assisted drying (Savant SC210A Speedvac Concentrator, ThermoFisher Scientific) of supernatants. Residues were reconstituted in 500 μL of initial mobile phase, well mixed, and transferred to analytical vials.

**Statistical Analysis**

Statistical analysis was done using the SPSS statistical software package (SPSS). The Kaplan-Meier technique was used to estimate PFS and OS. The prognostic significance of the IDH1/2 mutations for PFS and OS were first examined using univariate analysis. Logistic regression with stepwise selection was used for multivariate analysis of the relationship between IDH1/2 and other factors (5% significance). Differences in metabolite concentrations and metabolite ratios between IDH1-mutated and nonmutated gliomas and pGBM and nonglioma specimens were determined using Kruskal-Wallis significance tests for independent sample sets. Metabolite concentrations and ratios in LGG and their consecutive sGBM were compared using the Wilcoxon signed-rank test. A Bonferroni-adjusted P value ($P_{\text{adjusted}} = .05/28 = .0018$) was used to determine significance for the 28 pairwise comparisons possible for 8 groups. For all other comparisons, $P < .05$ was considered to be statistically significant.

**Results**

**Patient Characteristics**

According to the malignant progression of the LGG, patients were divided into two groups: group 1 patients with LGG (LGG1) without malignant transformation and group 2 patients with LGG (LGG2) with histologically confirmed malignant progression.

Among 35 patients with LGG who were included in this study, 19 (54%) were women, and 16 (46%) were men. Furthermore, histologically, 6 patients had oligoastrocytomas, and the remaining 29 patients had diffuse astrocytomas.

The median age of all patients with LGG was 37.4 years at the time of the first diagnosis. Patients in LGG2 had a median age of 37.1 years, which did not differ significantly from that of patients in LGG1, who had a median age of 41.4 years.

The median time to malignant transformation among patients in the LGG2 group was 3.35 years (range, 2.5–5.4 years). The median OS among all patients with LGG was 13.1 years (11.4 years in LGG1 and 13.1 years in LGG2; $P = .97$).

**IDH1 Mutation and Outcome**

An IDH1 mutation was detected in 27 of 35 patients with LGG (77.1%), in 10 of 17 patients in LGG1 (59%), and in 17 of 18 patients in LGG2 (95%). In all cases, IDH1 mutations were found on R132. IDH2 mutations were not detected in any of the patients. The IDH1 mutation status was stable during progression from LGG to sGBM in all patients in LGG2. None of the patients with pGBM or nonglioma had an IDH mutation. Patients with LGG with an IDH1 mutation had a median PFS of 3.3 years, which was comparable to that among patients with wild-type LGG (2.8 years; $P > .05$). Furthermore, the OS among patients with LGG with an IDH1 mutation was not statistically different at 13.0 years compared with that among patients with LGG without an IDH1 mutation, who had an OS of 9.3 years ($P = .66$).

**LC-MS/MS Profiling of TCA Metabolites**

TCA metabolites, citrate, isocitrate, α-ketoglutarate, succinate, fumarate, and 2-hydroxyglutarate were measured in glioma samples with and without an IDH1 mutation, in samples identified as primary GBM, and in nonglioma brain tumor specimens (Fig. 1). No differences in citrate, isocitrate, α-KG, succinate, and fumarate concentrations were found when comparing all of the latter groups. Concentrations of 2HG, a side product in IDH1-mutated gliomas, were 20–34-fold higher in IDH1-mutated gliomas (0.64–0.81 μmol/g), compared with non-IDH1-mutated LGG1 ($P \leq .001$). No differences were observed between IDH1-mutated gliomas and IDH1-nonmutated LGG2 and sGBM, caused by strongly elevated 2HG levels in either 1 or 2 samples in these groups, respectively. Furthermore, in IDH1-mutated gliomas, 2HG concentrations were a mean of 20 times higher than in pGBM and nongliomas ($P \leq .001$) (Fig. 1). No differences were observed between the single groups of IDH1-mutated gliomas LGG1, LGG2, and sGBM in relation to 2HG concentration.

To detect possible differences among the IDH1-mutated LGG1, LGG2, and sGBM, the α-KG/isocitrate and 2HG/isocitrate ratios were used in additional tests. Therefore, the direct precursor-product relation would correct for all differences possibly expected during pre-analytical processing. To prove this, analyte ratios of IDH1-mutated and nonmutated gliomas were compared. IDH1-mutated gliomas showed a 2HG/isocitrate ratio that was 13 times higher ($P \leq .001$) (Fig. 2A), which corresponds to a lower accumulation of 2HG in IDH1-nonmutated gliomas. α-KG/isocitrate ratios were determined to be approximately 10-fold higher in IDH1-mutated gliomas than in IDH1-nonmutated gliomas ($P = .005$) (Fig. 2B), which also implies lower accumulation of α-KG in IDH1-nonmutated gliomas.

2HG/isocitrate and α-KG/isocitrate ratios, respectively, were calculated in all 8 specimen groups (Fig. 3). In addition to the differences in 2HG/isocitrate ratios of IDH1-mutated and nonmutated gliomas (Fig. 2A), the ratios in IDH1-mutated gliomas were 4–9 times higher, compared with those in pGBM ($P \leq .001$), and 3–6 times higher, compared with those in non-glioma tumor specimens, which was not
statistically significant (Fig. 3A). In detail, ratios of 2HG and isocitrate were established to be 13, 9.4, and 22 times higher in IDH1-mutated LGG1, LGG2, and their consecutive sGBM, respectively, than in IDH1-nonmutated LGG1 (Fig. 3A). No significant differences were observed between IDH1-mutated gliomas and IDH1-nonmutated LGG2 and sGBM. The comparison of 2HG/isocitrate ratios between IDH1-nonmutated gliomas and IDH1-mutated LGG2 and sGBM showed no statistically significant differences. However, a trend toward higher ratios in IDH1-mutated LGG1 was seen. Furthermore, no differences could be determined by comparing 2HG/isocitrate ratios measured in the groups of IDH1-mutated LGG1 and LGG2. Although 2HG/isocitrate ratios in IDH1-mutated secondary glioblastomas are 1.7 and 2.3 times higher than in the LGG1 and LGG2 groups, respectively, no statistically significant differences were observed.

The absence of a straight trend to higher 2HG/isocitrate ratios during malignant progression is shown by paired analysis of IDH1-mutated LGG2 and their consecutive sGBM (Fig. 3C). Similar findings were observed
using the α-KG/isocitrate ratios. Although significant differences were found, with ratios approximately 10 times higher in IDH1-mutated glioblastomas than in IDH1-nonmutated glioblastomas (Fig. 2B), it was not possible to differentiate among the 3 IDH1-mutated glioblastoma groups LGG1, LGG2, and their consecutive sGBM with use of this analyte ratio (Fig. 3B and D).

Discussion

On the basis of a comprehensive analysis of cellular TCA metabolites from several cohorts of patients with glioma and nonglioma, our study provides evidence that the level of 2HG accumulation is not suitable as an early biomarker for distinguishing patients with LGG in relation to their course of malignancy. To our knowledge, this is the first report of a paired analysis of 2HG levels in LGG and their consecutive sGBM showing stable 2HG accumulation during malignant progression. This fact assumes that malignant transformation of IDH-mutated LGG appears to be independent of their intracellular 2HG accumulation. Considering these results, we could not stratify patients with LGG into subgroups with distinct survival.

To date, little is known about biomarkers that may predict malignant transformation and, consequently, predict survival in patients with LGG. The investigation of biomarkers in this patient group is relevant because treatment interventions can be tailored to prolong survival, minimize treatment-related adverse effects, and, accordingly, maximize quality of life. In many previous studies, IDH mutations, as the most commonly detected mutations in LGG, were observed to be a significant prognostic factor in patients with glioma, often relating to improved survival among patients with LGG.18–23

However, only patients with IDH-mutated LGG with malignant progression have a prolonged OS.18,19,24 In an earlier conducted study, the analysis of 2 groups of patients with LGG with and without malignant transformation failed to provide a significant influence from the IDH mutation, neither on the PFS nor on the OS.18 In agreement with these data, in a recent study, we showed again that PFS and OS among patients with LGG with and without an IDH mutation did not differ significantly, despite the malignant transformation in LGG2. This result is not unexpected because the same patient population was analyzed in both studies.

Accumulation of 2HG in IDH1-mutated gliomas was first described by Dang et al.26 2HG accumulation is an important marker of IDH1/2-mutated gliomas and other neoplasms.26,29,30 Furthermore, it was assumed that 2HG accumulation might be a potential systemic biomarker of gliomas, but it was not detectable in serum samples from patients with glioma.31 Nevertheless, it has become possible in the meantime to detect 2HG production using magnetic resonance spectroscopy in a noninvasive manner to identify patients with IDH1 mutant brain tumors.32

Because 2HG accumulation provides one of the few potential read-outs for mutant IDH enzymatic activity, we suspected that different intratumoral levels of 2HG accumulation in patients with LGG (especially in those with an IDH mutation) may affect their clinical course in relation to malignant transformation. Therefore, intratumoral concentrations of 2HG and other TCA metabolites were quantified by LC-MS/MS, showing concentrations in IDH1-mutated glioma samples that were comparable to the levels described by Dang et al.26 As previously shown,22 no differences in metabolite levels were observed, with the exception of 2HG with increased accumulation in IDH1-mutated gliomas, compared with IDH-nonmutated specimens. However, the fold-difference in 2HG levels between IDH1 mutant and IDH1 wild-type LGG in our study was smaller than in some other studies.26,30 As a reason for this issue, we identified 3 samples of IDH1 wild-type LGG and sGBM with strongly elevated 2HG levels. The fact that some IDH1 wild-type tumors might...
accumulate 2HG was previously described by Wise et al, who reported that the increased IDH-dependent carboxylation of glutamine-derived α-KG in hypoxia is associated with a concomitant increased synthesis of 2HG in cells with wild-type IDH1 and IDH2. Thus, they concluded that, in further support of the increased mitochondrial reductive glutamine metabolism that they observed in hypoxia, the incubation in hypoxia can lead to elevated 2HG levels in cells lacking IDH1/2 mutations.

The ratio of 2HG to isocitrate and the ratio of α-KG to isocitrate were provided. The use of these ratios can function as an internal control because isocitrate is the direct precursor of α-KG and 2HG. However, intracellular accumulation of both metabolites (2HG and of α-KG) did not differ between both IDH-mutated LGG groups with and without malignant progression. In the same way, detection of 2HG concentrations and the 2HG/isocitrate ratios in patients with LGG and their consecutive sGBM were comparable to values during malignant progression. Correspondingly, all other assessed TCA metabolites remained stable during the malignant progression of the LGG to sGBM. Therefore, 2HG accumulation appears, at least for now, merely to represent a highly correlative and stable marker for an emerging class of somatic mutations in the IDH enzymes from the early stage of glioma development and after malignant transformation to high-grade gliomas.

An expected lower level of α-KG in IDH-mutated LGG and sGBM, in comparison with IDH-nonmutated LGG, pGBM, and nonglioma tumors, was not detected in our study. This is in concordance with a similar finding by Dang et al, who showed unaffected α-KG levels in whole-tumor cell lysates. However, the latter finding was in contrast to reported results by Zhao et al, who showed that forced expression of mutant IDH in cultured cells led to a dose-dependent decrease in α-ketoglutarate levels. A possible explanation for this finding is the mono-allelic heterozygous mutations of the IDH1 gene leading to expression of both wild-type and mutant IDH1 in a single cell and, therefore, to production of 2HG and α-KG at the same time in every mutated cell. Another possible reason is the reported...
evidence that the biochemical effects of mutant IDH1 on α-KG-dependent enzymes are not principally attributable to depletion of α-KG but are a competitive antagonism with α-KG. 35–37 Thus, Xu et al postulated that IDH1 mutations alone do not reduce cellular level of α-KG sufficiently to have a significant tumorigenic consequence, but nonetheless these mutations sensitize α-KG–dependent dioxygenases to the inhibitory effect by the large amounts of intratumoral accumulated 2HG. 35

Whether 2HG acts as a mutagen or plays a distinct role in gliomagenesis remains to be determined. Dang et al predicted that patients with LGG may benefit from the therapeutic inhibition of 2HG production, resulting in the slowing or halting of conversion of LGG into a lethal secondary glioblastoma, thus changing the course of the disease. 26 However, our data confirm similar values of 2HG accumulation in the different LGG groups (with and without malignant progression) and present comparable ranges of 2HG in the low-grade and high-grade tumor stages. In addition, our study used the ratio of 2HG to isocitrate, which might provide a more sensitive screening tool for IDH1-mutated LGG than an increase in the absolute concentration of 2HG.

Finally, more work is needed to provide valuable clues about the precise role that 2HG might play in the initiation and progression of LGG. Moreover, the value of 2HG as a useful biomarker for diagnosis or monitoring of the treatment response of LGG has not yet been realized.

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**Conflict of interest statement.** None declared.

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