Diffuse WHO Grade II gliomas are slow-growing tumors that migrate within the brain, with an ineluctable risk of anaplastic transformation. However, spontaneous evolution of these lesions is very heterogeneous, despite an identical initial pathological diagnosis. Some genetic alterations, especially 1p19q codeletion and IDH1/IDH2 mutations, are believed to have an influence on the biological behavior of tumors, and the impact of these alterations on the prognosis of gliomas has extensively been investigated.

Abbreviations used in this paper: FISH = fluorescence in situ hybridization; LOH = loss of heterozygosity; PCR = polymerase chain reaction.

Distinct IDH1/IDH2 mutation profiles in purely insular versus paralimbic WHO Grade II gliomas

Laboratory investigation

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Object. The molecular profile of diffuse WHO Grade II gliomas involving the insular lobe, with a possible impact on outcome, is controversial. The authors undertook this study to investigate a possible difference of molecular patterns between purely insular Grade II gliomas and paralimbic Grade II gliomas that involve both the insular lobe and the frontal and/or temporal structures.

Methods. From a consecutive series of 47 patients who underwent resection of a Grade II glioma invading the insula, 2 subgroups were identified. The first subgroup included 11 patients with a purely insular tumor. The second subgroup included 36 patients with a paralimbic Grade II glioma also involving the frontal and/or temporal lobe. The authors searched systematically for TP53 mutations, 1p19q codeletion, and IDH1/IDH2 mutations.

Results. There was no significant difference between the 2 subgroups with respect to 1p19q codeletion or TP53 mutations rates. Conversely, IDH1/IDH2 mutations were found in all 11 (100%) of the insular Grade II gliomas but only 20 (55%) of 36 paralimbic Grade II gliomas (p = 0.008). Ten (28%) of the 36 patients in the paralimbic tumor group experienced a malignant transformation, and 6 of them died; whereas neither transformation nor death occurred in the insular tumor group (trend toward significance, p = 0.088).

Conclusions. These findings demonstrate for the first time distinct IDH1/IDH2 and consequently distinct “triple-negative” patterns in purely insular versus paralimbic Grade II gliomas. Such findings could explain discrepancies reported in the literature, because insular and paralimbic gliomas have not been separated in previous reports. These results may enable physicians to refine the management of Grade II gliomas involving the insula according to the presence or lack of invasion of the frontal and/or temporal areas.

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KEY WORDS • WHO Grade II glioma • molecular profile • insula • IDH1/IDH2 • surgery • oncology

DIFFUSE WHO Grade II gliomas are slow-growing tumors that migrate within the brain, with an ineluctable risk of anaplastic transformation. However, spontaneous evolution of these lesions is very heterogeneous, despite an identical initial pathological diagnosis. Some genetic alterations, especially 1p19q codeletion and IDH1/IDH2 mutations, are believed to have an influence on the biological behavior of tumors, and the impact of these alterations on the prognosis of gliomas has extensively been investigated.

The 1p19q codeletion has always been associated in WHO Grade II gliomas with favorable outcome whatever way its impact on tumor course has been evaluated: with endpoints like overall survival or progression-free survival5,14,17,18,20,21,25,33,36 or by assessing spontaneous tumor growth velocity12,30.

The prognostic value of IDH1/IDH2 mutations in Grade II gliomas is more controversial. Previous publications have reported an improved overall survival for patients with mutated tumors,6,15,26,31 but more recent reports have concluded that IDH1/IDH2 mutations lack prognostic value.1,20

Interestingly, several studies have shown relationships between cerebral location of Grade II gliomas and their molecular profile.16,22,27,29,40
**IDH1/IDH2 mutations in insular and paralimbic Grade II gliomas**

Because these lesions frequently involve the insular lobe, molecular analyses have specifically been performed in the subset of insular Grade II gliomas compared with other brain locations. Contradictory observations have been reported by different teams. Wu et al. and more recently Ren et al. found no specific molecular profile with respect to 1p19q codeletion for Grade II gliomas involving the insular lobe, while we and others demonstrated a low rate of 1p19q codeletion in insular Grade II glioma. More recently, a new molecular profile called “triple-negative” Grade II glioma—that is, with no 1p19q codeletion, TP53 mutation, or IDH1/IDH2 mutation—was described especially in the insular location.

In the light of these new data, and to try to solve the current controversy, we performed additional molecular analyses, including analyses of 1p19q codeletion, TP53 mutations, and IDH1/IDH2 mutations, in a larger population of Grade II gliomas involving the insular lobe, by differentiating 2 subgroups: 1) lesions that were restricted to the insula, and 2) lesions invading the paralimbic system, that is, the insular lobe plus the frontal and/or temporal structures. We hypothesized that the molecular patterns differ between the “purely insular” and “paralimbic” lesion subtypes.

**Methods**

**Patient Selection**

Patients were selected according to the following criteria: histological diagnosis of WHO Grade II glioma, involvement of the insular lobe only or in association with neighboring frontal or temporal structures, availability of frozen tumor obtained at resection, availability of blood DNA, and availability of written informed consent. Forty-seven patients were eligible for this study.

Using enhanced T1-weighted as well as T2-weighted and FLAIR MRI studies performed before any treatment, 2 subgroups were identified on the basis of tumor location: 1) patients with Grade II gliomas that were restricted to the insular lobe (Fig. 1), and 2) patients with Grade II gliomas that involved frontal and/or temporal structures as well as the insula (Fig. 2). For purposes of this paper, the former group is referred to as the insular tumor group and the latter as the paralimbic tumor group.

Surgery was performed according to a methodology extensively described by our group in previous reports and beyond the scope of this article. There was no mortality or permanent neurological deficit related to the resection.

**Volumetric Analyses**

Volumetric measurement of pre- and postoperative imaging was performed on the basis of FLAIR MR images using a dedicated software (Myrian, Intrasense) to assess tumor volumes (cm³). The extent of resection was calculated as follows: (preoperative tumor volume – postoperative tumor volume) / preoperative tumor volume.

**Patient Outcome Measurements**

Patient follow-up was based on a serial MRI assessment every 6 months. Malignant transformation can be detected by MRI changes, that is, the appearance of new contrast-enhancing nodules, in the absence of any chemotherapy and/or radiation therapy.

**Molecular Biology**

Areas of specimens containing more than 60% tumor cells were selected after examination by a pathologist. Tumor DNA was isolated according to a salting out procedure. Blood DNA was extracted from the patients’ peripheral blood (collected in EDTA-treated tubes) using the MagNA Pure Compact robot (Roche Diagnostics).

Loss of Heterozygosity at 1p19q. Blood and tumor DNA were genotyped for a panel of highly polymorphic microsatellite markers on 1p (D1S2660, D41S450, D1S507, D1S234, D1S2890, D1S230, D1S207, D1S206) and 19q (D19S414, D19S420, D19S903, D19S571) provided by the ABI Prism Linkage Mapping Set version 2.5 (Applied Biosystems).

Screening for IDH1 and IDH2 Mutations. A fragment...
of 254-bp length spanning the catalytic domain of IDH1 including the codon 132 was amplified using the sense primer IDH1f 5'-ACCAATGGCACCATAACA-3' and antisense primer IDH1r 5'-TTCACTTTGCTTTATGGGTGT-3' in PCR conditions previously described.²

For confirmation a 129-bp fragment was amplified by using the sense primer IDH1f 5'-CGGTCTTACGAGAAGGATGGCTA-3' and the antisense primer IDH1r 5'-GGTGCTTACGCATGTTTTGTTT-3' as the same conditions as for the first primer set.

After purification (using multiscreen PCR plates, Millipore), the PCR products were submitted to the sequencing reaction using the Big Dye Terminator version 1.1 sequencing kit. The primers for sequencing were the same as the primers used for PCR amplification of exon 5 through 8 were screened by 5 different PCR reactions. The sequence of the primers used for PCR amplification of exon 5a, exon 5b, exon 6, and exon 8 were as follows: exon 5a: TP53-5AF 5'-GTGGCTTACGAGAAGGATGGCTA-3' and TP53-5AR 5'-TGTGGGAATCAACCCACAGC; exon 5b: TP53-5BF 5'-GTGCAAGTGGTTAGGTATT-3' and TP53-5BR: 5'-GCCCTGTGCTTCTCCAG-3'; exon 6: TP53-6F: 5'-AGCTGGGGCTGGAGAGAC-3' and TP53-6R: 5'-TGGAGGGCCACTGACAAC-3'; exon 7: TP53-7F: 5'-AAAAGGCTCCCTCTGCT-3' and TP53-7R: 5'-TGGAGAGAAATCGGTAAGAGGTTG-3'; exon 8: TP53-8F: 5'-GCCCTTGTGGCTTCTCCTCCTCCT-3' and TP53-8R: 5'-GCCCTTGTGGCTTCTCCTCCT-3'.

The conditions for PCR amplification were as follows: 5 minutes at 94°C for initial denaturation, 30 seconds at 94°C, 1 minute at 59°C, 30 seconds at 72°C for 40 cycles, and 7 minutes at 72°C for final elongation. The PCR products were purified using multiscreen PCR plates (Millipore) and were then submitted to the sequencing reaction using the Big Dye Terminator version 1.1 sequencing kit (Applied Biosystems). Sequencing products were analyzed on a 3130 XL Genetic Analyzer (Applied Biosystems).

Samples that gave negative results for IDH1 mutations were then investigated for mutation in the codon 172 of the exon 4 of the IDH2 gene using the sense primer 5'-CAAGCTGAAGAAGATGTGGAA-3' and the antisense primer 5'-CAAGAGCAAAGAGGATGGCTA-3' as previously described.³⁴

TP53 Mutation Analysis. TP53 mutations in exons 5 through 8 were screened by 5 different PCR reactions. The sequence of the primers used for PCR amplification of exon 5a, exon 5b, exon 6, and exon 8 were as follows: exon 5a: TP53-5AF 5'-GTGGCTTACGAGAAGGATGGCTA-3' and TP53-5AR 5'-TGTGGGAATCAACCCACAGC; exon 5b: TP53-5BF 5'-GTGCAAGTGGTTAGGTATT-3' and TP53-5BR: 5'-GCCCTGTGCTTCTCCAG-3'; exon 6: TP53-6F: 5'-AGCTGGGGCTGGAGAGAC-3' and TP53-6R: 5'-TGGAGGGCCACTGACAAC-3'; exon 7: TP53-7F: 5'-AAAAGGCTCCCTCTGCT-3' and TP53-7R: 5'-TGGAGAGAAATCGGTAAGAGGTTG-3'; exon 8: TP53-8F: 5'-GCCCTTGTGGCTTCTCCTCCTCCT-3' and TP53-8R: 5'-GCCCTTGTGGCTTCTCCTCCT-3'.

The conditions for PCR amplification were as follows: 5 minutes at 94°C for initial denaturation, 30 seconds at 94°C, 1 minute at 59°C, 30 seconds at 72°C for 40 cycles, and 7 minutes at 72°C for final elongation. The PCR products were purified using multiscreen PCR plates (Millipore) and were then submitted to the sequencing reaction using the Big Dye Terminator version 1.1 sequencing kit (Applied Biosystems). Sequencing reactions were run on a 3130 XL Genetic Analyzer (Applied Biosystems).

Statistical Analysis

Because of the small number of patients with insular Grade II glioma, a 2-sided Fisher exact test was used to compare molecular and clinical characteristics with localization of the tumors (purely insular vs paralimbic system), with p ≤ 0.05 considered statistically significant. In other cases, a chi-square test was used. The Bonferroni correction was applied to the comparison between tumor location and IDH1/IDH2 mutation profiles.

### Results

**Patient Population**

All 47 patients included in this study underwent surgery for insular WHO Grade II glioma. The group with purely insular tumors (insular tumor group) comprised 11 patients (8 male and 3 female, male/female ratio 0.726), while the group with paralimbic tumors comprised 36 patients (19 male and 17 female, male/female ratio 0.527). The median age at diagnosis was 38 years (range 23–52 years) in the insular tumor group and 34 years (range 19–58 years) in the paralimbic tumor group. The median preoperative tumor volume evaluated on the MR images obtained immediately before surgery was 63 cm³ in the insular tumor group and was 95 cm³ in the paralimbic tumor group. The median values for residual tumor volume assessed on immediate postoperative MR images were 4 cm³ and 9 cm³ in the insular and paralimbic tumor groups, respectively. The mean extent of resection was 88% in both groups.

The median duration of postoperative follow-up was 36 months (range 19–96 months) in the insular tumor group, with no death and no malignant transformation. In the paralimbic tumor group the median duration of postoperative follow-up was 24 months (range 7–66 months); in this group, 10 patients (28%) had malignant transformation of their tumor, and 6 of them (16%) died.

A summary of patient clinical data is given in Table 1.

**Histological and Molecular Characteristics of the Tumors**

The distribution of histological subtypes was rather

<table>
<thead>
<tr>
<th>Variable</th>
<th>Insular (n = 11)</th>
<th>Paralimbic (n = 36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>age (yrs)</td>
<td>median 38</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>range 23–52</td>
<td>19–58</td>
</tr>
<tr>
<td>sex</td>
<td>male 8</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>female 3</td>
<td>17</td>
</tr>
<tr>
<td>ratio (M/F)</td>
<td>0.726</td>
<td>0.527</td>
</tr>
<tr>
<td>preop tumor vol (cm³)</td>
<td>median 63</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>range 35–190</td>
<td>15–250</td>
</tr>
<tr>
<td>postop tumor vol (cm³)</td>
<td>median 4</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>range 1–75</td>
<td>1–40</td>
</tr>
<tr>
<td>extent of resection (%)</td>
<td>mean 88</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>SEM 11.02</td>
<td>5.3</td>
</tr>
<tr>
<td>follow-up duration (mos)</td>
<td>median 36</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>range 19–96</td>
<td>7–66</td>
</tr>
</tbody>
</table>

* SEM = standard error of the mean.
**IDH1/IDH2 mutations in insular and paralimbic Grade II gliomas**

Homogeneous in the 2 groups: 55% versus 50% of lesions were oligodendrogliomas, 36% versus 28% were oligoastrocytomas, and 9% versus 22% were astrocytomas in the insular and paralimbic tumor groups, respectively.

Only 7 (15%) of the 47 tumors displayed a complete 1p19q codeletion: 1 (10%) of 11 lesions in the insular group and 6 (17%) of 36 in the paralimbic group. TP53 gene mutations in exons 5 through 8 were found in 5 (45%) of 11 cases in the insular group and in 19 (53%) of 36 cases in the paralimbic group and were mutually exclusive with 1p19q codeletion. For both of these genetic markers, the difference between the 2 groups did not reach statistical significance.

Mutations of IDH1 or IDH2 were present in 31 (66%) of the 47 patients. All 11 tumors in the insular group had such a mutation, whereas only 20 (56%) of 36 tumors had this mutation in the paralimbic group (p = 0.008). After application of the Bonferroni correction, owing to the fact that 3 molecular markers were tested against tumor location, the IDH mutation frequency still differed significantly between purely insular and paralimbic Grade II gliomas (p = 0.024). As has been reported many times, the G395A mutation in the IDH1 gene was most common; this mutation was found in 30 of the 31 tumors that had IDH1 mutations. Only 1 paralimbic tumor had an IDH2 mutation (G515A).

As a consequence, no purely insular tumors displayed a triple-negative molecular pattern, defined by the combined absence of 1p19q codeletion, TP53, and IDH1/IDH2 mutations, whereas 7 (19%) of the tumors in the paralimbic group did. Nevertheless, the differences in the rates of triple-negative molecular profile in the 2 groups did not reach statistical significance.

The data are summarized in Table 2.

**Discussion**

To the best of our knowledge, this is the largest series of Grade II gliomas in which an extensive molecular profile (1p19q codeletion, TP53, and IDH1/IDH2 mutations) was compared between 2 close precise tumor localizations: Grade II glioma limited to the insula (the insular tumor group) and insular Grade II glioma with an associated extension to frontal or temporal adjacent structures (the paralimbic tumor group).

Usually when tumors have been studied in relation to their brain location, all tumors invading the paralimbic system, whatever the extent of the tumor was, have been considered as a whole. They have been indiscriminately designated as “insular” or “paralimbic” tumors. Findings of differences in molecular patterns of the tumors invading the insular lobe might explain the controversy in the literature, due to the mix of both entities in previous reports.

**Molecular Heterogeneity of Grade II Gliomas Involving the Insular Lobe**

First of all, despite the absence of significant differences between purely insular and paralimbic tumors in the 1p19q codeletion rate, we confirm a low rate of 1p19q codeletion in these locations (15%) when compared with results reported in large series of gliomas irrespective of location and histological subtype. In a recent study including only Grade II gliomas, Kim et al. reported an average rate of 1p19q codeletion of 42% in a series of 360 tumors. In 2 other large series containing more than 300 tumors each, but mixing low- and high-grade gliomas, global average 1p19q codeletion rates were higher than in our series. In the first series, which included a subset of 45.6% of WHO Grade III and IV gliomas, the 1p19q codeletion frequency was 36.6%. In the second series, which included 62% WHO Grade III or Grade IV gliomas, 25% of tumors displayed a complete 1p19q codeletion. In some molecular studies with a special interest in tumors involving insula, reported results differ from ours with respect to the pattern of 1p19q codeletion.

In a series including only 10 Grade II gliomas and 4 anaplastic (Grade III) gliomas, Wu et al. found an occurrence of 57% of 1p19q codeletion, similar to the frequency reported in other locations. More recently, in Chinese patients, Ren et al. assessed 1p19q codeletion and

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**TABLE 2: Molecular tumor characteristics and brain location**

<table>
<thead>
<tr>
<th>Genetic Alteration</th>
<th>Insular (n = 11)</th>
<th>Paralimbic (n = 36)</th>
<th>p Value</th>
<th>Adjusted p Value†</th>
<th>Total (n = 47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDH1 or IDH2 mutation</td>
<td></td>
<td></td>
<td>0.008</td>
<td>0.024</td>
<td></td>
</tr>
<tr>
<td>present</td>
<td>11</td>
<td>20</td>
<td></td>
<td></td>
<td>31 (66%)</td>
</tr>
<tr>
<td>absent</td>
<td>0</td>
<td>16</td>
<td></td>
<td></td>
<td>16 (34%)</td>
</tr>
<tr>
<td>1p19q deletion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>codeletion</td>
<td>1</td>
<td>6</td>
<td></td>
<td></td>
<td>7 (15%)</td>
</tr>
<tr>
<td>others</td>
<td>10</td>
<td>30</td>
<td></td>
<td></td>
<td>40 (85%)</td>
</tr>
<tr>
<td>TP53 mutation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>present</td>
<td>5</td>
<td>19</td>
<td></td>
<td></td>
<td>24 (51%)</td>
</tr>
<tr>
<td>absent</td>
<td>6</td>
<td>17</td>
<td></td>
<td></td>
<td>23 (49%)</td>
</tr>
<tr>
<td>triple-negative molecular pattern</td>
<td>0</td>
<td>7</td>
<td></td>
<td>0.175</td>
<td>0.525</td>
</tr>
</tbody>
</table>

* Boldface type indicates statistical significance.
† Bonferroni correction.
IDH1/IDH2 mutations in a series of 330 and 280 gliomas, respectively, in relation to tumor location. In a subset of 49 insular gliomas, the authors found no significant difference in incidence of 1p19q codeletion when compared with gliomas in other brain locations studied (frontal, temporal) whatever the histological subtype.

The discrepancies with respect to 1p19q codeletion rates between our previous and present results, on the one hand, and the reports of Wu et al. and Ren et al., on the other, could be at least partially explained by differences in homogeneity of the series and methods for 1p19q codeletion assessment. It is important to note that the studies reported by Ren et al. and Wu et al. involved high-grade gliomas as well as Grade II gliomas, making their series heterogeneous. Moreover, while these authors used FISH to detect 1p19q codeletion, we used LOH with microsatellites markers. The commercial fluorescent probes for FISH, cited in their material and methods sections, interrogate relatively telomeric and limited regions of the 1p (1p36) and 19q (19q13), whereas the microsatellite markers we used spanned the entirety of chromosome arms with nearly centromeric positions for D1S206 (1p21.2) and D19S414 (19q12) on 1p and 19q, respectively. Using FISH tends to overestimate 1p19q codeletion rate because it mistakes partial deletions characterized by loss of only the distal part of the chromosome arm for deletions of the complete chromosome arm.

It is noteworthy that we have been able to replicate the results of our preliminary experience in a larger homogeneous Grade II glioma series using the same LOH method with an extensive interrogation of 1p and 19q chromosome arms.

Interestingly, Wu et al. delineated 4 subgroups in tumors involving insula: they found no difference in 1p19q codeletion rate between tumors restricted to insula (50%) and tumors expanding outside the insula with extension to the frontal or temporal or both (57%). We confirmed this particular point in a larger homogeneous series of Grade II gliomas.

With respect to IDH1 and IDH2, we demonstrated here that mutations were found in all the 11 pure insular tumors (100%) and in only 20 of 36 paralimbic tumors (55%) (p = 0.008 adjusted to p = 0.024 after Bonferroni correction). These results are in accordance with those of Metellus et al., who reported that nonmutated IDH tumors were located mostly in the paralimbic system: 6 of the 7 nonmutated tumors they studied were paralimbic.

Ren et al. highlighted a significantly higher level of IDH1/IDH2 mutations in oligoastrocytic tumors of insular origin than in those of noninsular origin. This difference disappeared when IDH1/IDH2 mutations were considered irrespective of tumor histology. The distinction of insular tumors was not entirely clear in this study, however, because the authors did not provide detailed results in relation to tumor expansion—that is, depending on whether the tumors were restricted to the insular lobe or had invaded the neighboring frontal and temporal structures. As a consequence it is not possible to compare their results with ours, which provide a more accurate molecular analysis.

In addition, determination of TP53 mutations allowed us to count 7 “triple-negative” paralimbic gliomas within our series (7 [15%] of 47), whereas no purely insular glioma was triple negative (0 [0%] of 11). Nevertheless this difference did not reach statistical significance (p = 0.175).

Kim et al. reported, in a large series of 360 Grade II gliomas from different brain localizations, an average rate of triple negatives of 7% (23 of 360 tumors), which is significantly lower than the rate of 15% (7/47) we found in our paralimbic tumor group (p = 0.04). Our higher rate of triple negatives in paralimbic tumors is in accordance with previous findings of Metellus et al. From the 7 triple-negative tumors in a series of 47 Grade II gliomas from various locations studied by Metellus et al., 6 (85.7%) were located in the paralimbic system. Nevertheless, the proportion of triple-negative tumors in the group of Grade II gliomas devoid of IDH1/IDH2 mutations that they reported (7 [100%] of 7) is quite different from that in our series (7 [44%] of 16), even allowing for the fact that our series included only Grade II gliomas in relation with the insula. It is noteworthy that the authors did not perform genotyping for TP53 mutations but rather p53 protein immunostaining.

Clinical and Molecular Impacts of Differences in IDH Mutation Patterns

As expected, paralimbic Grade II gliomas had a larger preoperative volume than Grade II gliomas limited to the insula. The tumors in the paralimbic group showed a trend toward a significantly more rapid malignant transformation (p = 0.088) despite a similar extent of resection in the 2 groups (Table 3). With respect to overall survival, 6 of 36 patients with paralimbic Grade II gliomas died as compared with none in the insular tumor group; the trend is not yet significant despite a shorter duration of follow-up in the former group (24 vs 36 months). At this point, no clear-cut conclusion can be drawn concerning the impact of insular and paralimbic locations on survival; this would require longer follow-up. Similarly, the between-groups difference in IDH1/IDH2 mutation profiles with respect to malignant transformation was not significantly different.

In spite of several studies dedicated to this topic, the prognostic value of IDH1/IDH2 mutations in Grade II glioma is still unclear and remains controversial. The triple-negative profile is also a matter of controversy: Metellus et al. reported a dismal prognosis and distinc-

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**TABLE 3: Effects of IDH1/IDH2 mutations and tumor location on clinical outcome**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Malignant Transformation</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDH1 or IDH2 mutation</td>
<td></td>
<td>0.0684</td>
</tr>
<tr>
<td>present</td>
<td>4</td>
<td>27</td>
</tr>
<tr>
<td>absent</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>tumor location</td>
<td></td>
<td></td>
</tr>
<tr>
<td>insular</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>paralimbic</td>
<td>10</td>
<td>26</td>
</tr>
</tbody>
</table>
tive infiltrative behavior in these tumors whereas Kim et al.\textsuperscript{19} reported no marked difference in survival associated with triple-negative status.

Conversely, recent publications have shown a broad consensus with respect to the role of IDH mutations as decisive genetic events in the first steps of gliomagenesis.\textsuperscript{35,36} IDH1 and IDH2 mutations occur early in Grade II glioma and concern the oligodendrogial as well as the astrocytic lineage. IDH mutations impair the tricarboxylic acid cycle with an accumulation of 2-hydroxyglutarate (2-HG) as a consequence.\textsuperscript{4}

Because of an inhibitory effect of this excessive amount of 2-HG on enzymes involved in epigenetic events (histone demethylases and TET 5-methylcytosine hydroxylases) a link between these metabolic disturbances and the cellular molecular pattern was suggested.\textsuperscript{39} Very recently, Lu et al.\textsuperscript{23} showed an increase in repressive histone methylation marks with a resulting repression of lineage-specific differentiation genes. Simultaneously, Turcan et al.\textsuperscript{35} reported that a single mutation in the IDH1 gene caused a remodeling of the methylene and thus of the transcriptome. The epigenetic alterations resulting from a mutated IDH1 gene regulate key gene expression programs. As a consequence, it makes sense that the presence or absence of IDH1/IDH2 mutations would deeply modify the way the glioma will evolve.

Kim et al.\textsuperscript{19} reported that molecular alterations in the R1 pathway were significantly more frequent in triple-negative Grade II glioma, suggesting that this subset of Grade II gliomas may develop through a distinct genetic pathway. Alterations in the R1 pathway were associated with unfavorable outcome.

In summary, it could be hypothesized that the presence of IDH mutations might act as a switch causing mutated tumors to be predisposed to specific epigenetic deregulations and thus to related genetic defects. In Grade II gliomas, all the molecular events resulting from IDH mutations might not yet be fully acquired, whereas, in contrast, the molecular processes could be achieved in high-grade gliomas. Most of the molecular changes linked to IDH mutational status are not yet identified. It is likely that they could have their own prognostic impact and could interfere with evaluation of the prognostic value of IDH mutations. This might at least partially explain why the prognostic value of IDH mutations is controversial in Grade II glioma and why there is more consensus with respect to its prognostic value in high-grade gliomas. Independent of the individual prognostic value of IDH mutations, which needs to be clarified, the presence or absence of these mutations directs the gliomas in different sequences of molecular events. The expected consequences on the course of Grade II glioma are likely to be quite different in each case.

Conclusions

Relationships between brain tumor localization and molecular patterns seem to be very specific. Separate and accurate molecular analysis of Grade II glioma infiltrating very close brain regions (insulae only or insula plus other surrounding paralimbic structures) demonstrate significantly different rates of IDH1/IDH2 mutations (p = 0.008, p = 0.024 after Bonferroni correction). This study reveals a previously unsuspected molecular heterogeneity in Grade II glioma. The insula is both a surgically challenging region and an important location because of the high frequency of insular Grade II glioma.

If these differences are confirmed, in view of the consequences these mutations imply for the course of disease, it can be argued that these tumors should be more accurately defined and should no longer be considered as a homogeneous group.

Disclosure

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

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