Genetic Signature of Oligoastrocytomas Correlates with Tumor Location and Denotes Distinct Molecular Subsets

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Oligoastrocytomas are heterogeneous tumors that have molecular features that overlap with either oligodendroglialomas or astrocytomas. Differences in the frequency of chromosomal losses of 1p and 19q in oligodendroglialomas are related to tumor location, with a low rate of allelic loss in tumors of the temporal and a high rate in tumors of the frontal, parietal, and occipital lobes. To test the possibility of regional molecular heterogeneity in oligoastrocytoma, we examined a series of 203 gliomas including 68 oligoastrocytomas and two control groups of 73 oligodendroglialomas and 62 astrocytomas for allelic losses of chromosomal arms 1p and 19q8 –12,15 thus genetically resembling oligodendrogliomas, whereas ~30% show mutations in the TP53 gene or LOH 17p10,16 suggesting a relation to astrocytomas. Significantly, LOH 1p and LOH 19q are observed only rarely in gliomas other than oligodendroglioma and oligoastrocytoma.11

Oligoastrocytomas exhibit both astrocytic and oligodendroglial morphologies.14 Because of the rather vague criteria for defining oligoastrocytoma, the incidence of oligoastrocytoma varies considerably between different studies. Based on molecular findings, oligoastrocytomas occupy an intermediate position between oligodendrogliomas and astrocytomas. From 30 to 70% of oligoastrocytomas show LOH 1p and LOH 19q8 –12,15 thus genetically resembling oligodendrogliomas, whereas ~30% show mutations in the TP53 gene or LOH 17p10,16 suggesting a relation to astrocytomas. Significantly, LOH 1p and LOH 19q are inversely associated with TP53 muta-
tions. On microdissection, identical genetic alterations have been identified in astrocytic and oligodendroglial portions, indicating a clonal origin of oligoastrocytomas. Although one might assume that in oligoastrocytomas the astrocytic component implies a less favorable prognosis, most studies could not confirm differences in outcome between oligodendroglioma and oligoastrocytoma.17–19

LOH 1p and LOH 19q have been associated with chemosensitivity and durable responses to chemotherapy in patients with anaplastic oligodendrogliomas. In addition, patients with oligodendrogliomas and anaplastic oligodendrogliomas exhibiting these molecular lesions have longer overall survival from the time of diagnosis. Further, LOH 1p may indicate a better response to chemosensitivity and prolonged survival in a small group of astrocytomas and oligoastrocytomas. In oligoastrocytomas, a subset has been shown to respond favorably to procarbacine lomustine (CCNU)/vincristine-based chemotherapy.23,24

Recently, an association was identified between the incidence of genetic lesions in oligodendroglioma and tumor location.25 Anaplastic oligodendrogliomas located in the frontal, parietal, and occipital lobes were significantly more likely to harbor LOH 1p and LOH 19q than those arising in the temporal lobe, insula, and diencephalon. In addition, LOH 1p and LOH 19q were significantly correlated with a bilateral growth pattern. Because of the strong predictive value of LOH 1p and LOH 19q, these findings may argue for differential therapy approaches in patients with oligodendrogliomas depending on gross tumor localization. The morphological and genetic similarities between oligodendrogliomas and oligoastrocytomas naturally raise the question of whether molecular subsets of oligoastrocytomas correlate with tumor location. The present study was thus conducted to clarify and extend molecular subclassification of oligoastrocytomas. To this end, we analyzed a series of 203 gliomas, including 68 oligoastrocytomas and two control groups of 73 oligodendrogliomas and 62 astrocytomas, for LOH 1p, LOH 19q, and TP53 mutations, with respect to tumor location.

Materials and Methods

Tissue Samples

Two hundred three gliomas, consisting of 37 oligodendrogliomas World Health Organization (WHO) grade II (O II), 36 anaplastic oligodendrogliomas WHO grade III (O III), 38 oligoastrocytomas WHO grade II (OA II), 30 anaplastic oligoastrocytomas WHO grade III (OA III), 28 astrocytomas WHO grade II (A II), and 34 anaplastic astrocytomas WHO grade III (A III), and corresponding blood samples were obtained from patients treated at the Charité Hospital in Berlin, the Helios Klinikum in Buch, the University Hospital in Würzburg, the Neukölln Hospital in Berlin, the University Hospital in Bonn, the University Hospital in Tübingen and the Massachusetts General Hospital in Boston between 1992 and 2001. Because no clearly defined parameters for the diagnosis of oligoastrocytoma have been established, we required for this diagnosis the lesser represented component to amount to at least 20% of the material examined. Among the 203 tumors, 103 were located in the frontal, 53 in the temporal, 17 in the frontotemporal, 11 in the parietal, 6 in the parietotemporal, 7 in the ventricular, 3 in the occipitotemporal, and 1 in the occipital region; 2 were from the spinal cord. All tumors were classified graded by neuropathologists according to the 2000 WHO criteria and all cases were reviewed by one neuropathologist (AvD).30 Thirty-one patients with oligoastrocytomas reported on in an earlier study were included.30 Before extraction of DNA from tumor tissues and leukocytes by standard methods, all tumor samples were examined by frozen sections to exclude contaminating nontumorous portions.11

Microsatellite Analysis for LOH

The microsatellite markers D1S1608 (1p36.31), D1S548 (1p36.23), D1S1597 (1p36.21), D1S1592 (1p36.13), and D1S1161 (1p35.1) were used to identify LOH 1p. For determining LOH 19q, the markers D19S431 (19q12), D19S433 (19q12), D19S559 (D19q13.2), and D19S601 (19q13.33) were used. Amplification conditions and primer sequences are based on corresponding Genome Database entries (www.gdb.org). Polymerase chain reaction products were separated on 8% denaturing acrylamide gels and visualized by silver staining. LOH was scored as previously described.27

Single-Strand Conformation Polymorphism Analysis and Direct Sequencing

For analysis of the TP53 gene, a set of previously published primers for exons 5 to 8 were used. Polymerase chain reaction was performed in a volume of 10 µl containing 10 ng of DNA, 50 mmol/L KCl, 10 mmol/L Tris-HCl, 200 mmol/L of each dNTP, 0.1% gelatin, 20 pmol of each primer, 1.0 to 2.0 mmol/L MgCl₂, and 0.025 U Taq polymerase. Initial denaturation at 94°C for 3 minutes was followed by 30 cycles on an automated thermal cycler (Biometra, Göttingen, Germany). These included denaturation at 94°C for 30 seconds, annealing at 57°C for 40 seconds, and extension at 72°C for 40 seconds. A final extension step at 72°C for 10 minutes was added. Single-strand conformation polymorphism analysis was performed on a sequencing apparatus (BlueSeq 400; Serva, Marburg, Germany) using 8% and 14% acrylamide gels and electrophoresis at 3 to 6 W and variable temperatures for 15 hours. Silver staining of the gels was performed as previously described. Aberrantly migrating single-strand conformation polymorphism bands were excised and the DNA was extracted as described. After reamplification with the same set of primers the polymerase chain reaction products were sequenced on a semiautomated sequencer (model 373A; Applied Biosystems, Foster City, CA) using a Taq cycle sequencing kit (Applied Biosystems). Each amplicon was sequenced bidirectionally.
mutations in exons 5 to 8 of the TP53 tumor suppressor gene. The informative cases had the following alterations: 97 tumors showed LOH 1p (24 O II, 25 O III, 21 OA II, 19 OA III, 2 A II, 6 A III) and 121 cases had LOH 19q (28 O II, 27 O III, 27 OA II, 21 OA III, 4 A II, 14 A III). Combined LOH 1p and LOH 19q was detected in 91 cases (24 O II, 25 O III, 21 OA II, 19 OA III, 2 A II, 6 A III) and 121 cases had LOH 19q (28 O II, 27 O III, 27 OA II, 21 OA III, 4 A II, 14 A III). Combined LOH 1p and LOH 19q was detected in 91 cases (24 O II, 25 O III, 20 OA II, 17 OA III, 2 A II, 3 A III). In 46 tumors a TP53 mutation was detected (1 O II, 3 O III, 10 OA II, 7 OA III, 13 A II, 12 A III). Data are summarized in Table 1.

Oligodendrogliomas from nontemporal sites had significantly more LOH 1p and LOH 19q than those situated in the temporal lobes. Within the respective regions, LOH frequencies in O II did not differ from those in O III. LOH 1p occurred in 36 of 33 frontal, 3 of 4 frontotemporal, 3 of 5 parietal, 1 of 1 parietotemporal, and only 7 of 21 temporal oligoastrocytomas (P < 0.0001, chi-square test). LOH 19q occurred in 27 of 32 frontal, 3 of 4 frontotemporal, 1 of 1 occipitotemporal, 3 of 5 parietal, 1 of 1 parietotemporal, and 13 of 24 temporal oligoastrocytomas. We also divided oligoastrocytomas into the three categories: temporal, nontemporal, and temporal with another lobe. Twenty-nine of 38 nontemporal OA, 4 of 5 OA involving the temporal with another lobe, and 13 of 24 temporal oligoastrocytomas were detected between LOH 1p and TP53 mutation. In 57 oligoastrocytomas with both LOH 1p and TP53 data, a significant inverse association was detected between LOH 1p and TP53 mutation (P < 0.001, Fisher’s exact test). This inverse association was also seen for LOH 19q and TP53 mutation (P = 0.004, Fisher’s exact test). Because 31 cases from an earlier study had been included that showed a similar distribution, the analyses were repeated excluding those cases: the remaining 26 oligoastrocytomas again demonstrated that LOH 1p (P < 0.005, Fisher’s exact test) and LOH 19q (P < 0.005, Fisher’s exact test) tend not to occur with TP53 mutations. Interestingly, patients with tumors of the temporal lobe (mean, 36 years) were younger (P < 0.05, t-test) than patients with nontemporal tumors (mean, 41 years). Within the temporal tumor group, patients with TP53 mutations were rare and not associated with specific brain regions.

### Table 1. Molecular Alterations in Oligodendrogliomas, Oligoastrocytomas, and Astrocytomas

<table>
<thead>
<tr>
<th>Histology</th>
<th>Molecular alteration</th>
<th>Frequency for distinct tumor sites</th>
</tr>
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<tbody>
<tr>
<td>O</td>
<td>LOH 1p</td>
<td>49/70</td>
</tr>
<tr>
<td></td>
<td>LOH 19q</td>
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<td>LOH 1p/19q</td>
<td>49/70</td>
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<td>LOH 19q</td>
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</tr>
<tr>
<td></td>
<td>TP53 mut</td>
<td>17/61</td>
</tr>
<tr>
<td></td>
<td>LOH 1p</td>
<td>8/55</td>
</tr>
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<td>LOH 19q</td>
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O, Oligodendrogliomas; OA, oligoastrocytoma; A, astrocytoma; LOH, loss of heterozygosity; TP53 mut, detection mutation in exons 5 to 8 of TP53; –, no data available. Numbers of cases with alterations are given in respect to informative cases (for LOH data) and in respect to cases examined (for TP53 mutation data).

### Statistical Analysis

For statistical analysis Statview 4.0 (SAS, Cary, NC) was used. The analysis of nominal and independent variables, chi-square and Fisher’s exact tests, were applied. The distribution of age and nominal variables was analyzed by t-test.

### Results

Two hundred three gliomas and corresponding blood samples were analyzed for LOH 1p and LOH 19q and for mutations in exons 5 to 8 of the TP53 tumor suppressor gene. The informative cases had the following alterations: 97 tumors showed LOH 1p (24 O II, 25 O III, 21 OA II, 19 OA III, 2 A II, 6 A III) and 121 cases had LOH 19q (28 O II, 27 O III, 27 OA II, 21 OA III, 4 A II, 14 A III). Combined LOH 1p and LOH 19q was detected in 91 cases (24 O II, 25 O III, 20 OA II, 17 OA III, 2 A II, 3 A III). In 46 tumors a TP53 mutation was detected (1 O II, 3 O III, 10 OA II, 7 OA III, 13 A II, 12 A III). Data are summarized in Table 1.

Oligodendrogliomas from nontemporal sites had significantly more LOH 1p and LOH 19q than those situated in the temporal lobes. Within the respective regions, LOH frequencies in O II did not differ from those in O III. LOH 1p occurred in 36 of 44 frontal, 2 of 2 ventricular, 0 of 1 occipital, 2 of 2 parietal, and only 3 of 13 temporal oligodendrogliomas (P = 0.0001, chi-square test). LOH 19q occurred in 36 of 44 frontal, 2 of 2 ventricular, 1 of 1 occipital, 2 of 2 parietal, and only 4 of 13 temporal oligodendrogliomas (P = 0.0004, chi-square test).

Because those tumors involving both the temporal and another lobe seemed to reflect the alterations seen in nontemporal tumors, we divided the oligodendrogliomas into the three categories: temporal, nontemporal, and temporal with another lobe. Indeed, 40 of 49 nontemporal O, 6 of 8 O involving the temporal and another lobe, and 3 of 13 temporal O exhibited LOH 1p (P = 0.0002, chi-square test). The data for LOH 19q were: 43 of 51 nontemporal O, 8 of 9 O involving the temporal with another lobe, and 0 of 3 temporal O exhibiting LOH 19q (P = 0.0006, chi-square test). TP53 mutations were rare and not associated with specific brain regions.

Oligoastrocytomas of nontemporal origin had significantly more LOH 1p than those situated in the temporal lobes. Within the respective regions, LOH frequencies in OA II did not differ from those in OA III. LOH 1p occurred in 26 of 33 frontal, 3 of 4 frontotemporal, 3 of 5 parietal, 1 of 1 parietotemporal, and only 7 of 21 temporal oligoastrocytomas (P < 0.0001, chi-square test). LOH 19q occurred in 27 of 32 frontal, 3 of 4 frontotemporal, 1 of 1 occipitotemporal, 3 of 5 parietal, 1 of 1 parietotemporal, and 13 of 24 temporal oligoastrocytomas. We also divided oligoastrocytomas into the three categories: temporal, nontemporal, and temporal with another lobe. Twenty-nine of 38 nontemporal OA, 4 of 5 OA involving the temporal with another lobe, and 13 of 24 temporal OA exhibited LOH 1p (P < 0.004, chi-square test). The data for LOH 19q were: 30 of 37 nontemporal OA, 5 of 6 OA involving the temporal with another lobe, and 13 of 24 temporal OA exhibited LOH 19q (P = 0.06, chi-square test). TP53 mutations were seen in 7 of 34 nontemporal OA, in 0 of 5 OA affecting the temporal with another lobe, but in 1 of 22 temporal OA (P < 0.05, chi-square test). Figure 1 depicts a representative case of temporal oligoastrocytoma with TP53 mutation. In 57 oligoastrocytomas with both LOH 1p and TP53 data, a significant inverse association was detected between LOH 1p and TP53 mutation (P < 0.0001, Fisher’s exact test). This inverse association was also seen for LOH 19q and TP53 mutation (P = 0.0004, Fisher’s exact test). Because 31 cases from an earlier study had been included that showed a similar distribution, the analyses were repeated excluding those cases: the remaining 26 oligoastrocytomas again demonstrated that LOH 1p (P < 0.005, Fisher’s exact test) and LOH 19q (P < 0.005, Fisher’s exact test) tend not to occur with TP53 mutations.
Astrocytomas did not exhibit different frequencies of either LOH 1p and LOH 19q or TP53 mutations with respect to tumor localization. LOH 19q occurred more frequently in A III than in A II. Only 4 of 26 A II but 14 of 32 A III exhibited LOH 19q (P < 0.025, Fisher’s exact test). The LOH 1p frequencies did not differ for A II (2 of 25) and A III (6 of 28). TP53 mutations occurred in 13 of 26 A II and 12 of 27 A III.

Discussion

To clarify the nosological position of oligoastrocytoma among the diffuse gliomas, we analyzed a series of 203 gliomas, including 68 oligoastrocytomas, and two control groups of 73 oligodendrogliomas and 62 astrocytomas, for LOH 1p, LOH 19q, and TP53 mutations, with particular emphasis on correlations with tumor location. Both control groups—the pure astrocytomas and oligodendrogliomas—displayed molecular genetic features similar to those reported in the literature. Oligodendrogliomas and oligoastrocytoma had LOH 1p and LOH 19q in the majority of the cases. The overall low frequency of TP53 mutations in oligodendrogliomas also confirmed previous studies. Although LOH 1p was a rare event in astrocytomas, evenly distributed among WHO II and III grades, LOH 19q was significantly associated with higher grade, thereby further supporting the suggestion that a progression-associated tumor suppressor gene resides on this chromosomal arm.

We next correlated these molecular findings with tumor location. Astrocytomas did not exhibit any associations between molecular genetic features and location. Furthermore, in oligodendrogliomas, TP53 mutations did not correlate with tumor site. Nonetheless, as previously reported, temporal lobe oligodendrogliomas have significantly less frequent LOH 1p and LOH 19q than their morphologically indistinguishable nontemporal counterparts. Although such differences can be assessed easily for tumors occupying a single site, such as the temporal lobe, a considerable percentage of lesions involve both the temporal lobe and portions of either the frontal, parietal, or occipital lobes. We therefore placed those oligodendrogliomas involving more than one lobe into a separate category; these tumors had genetic features similar to the group of nontemporal oligodendrogliomas. The findings indicate that those oligodendrogliomas without LOH 1p and LOH 19q predominantly arise in the temporal lobes.

Oligoastrocytomas showed a similar distribution of LOH 1p and LOH 19q with respect to tumor location as...
that noted for oligodendrogliomas. Allelic losses of 1p and 19q were significantly less frequent in temporal oligoastrocytomas, whereas those oligoastrocytomas affecting temporal and additional lobes were similar to non-temporal oligoastrocytomas. However, oligoastrocytomas within the temporal lobe had significantly more frequent TP53 mutations than the oligoastrocytomas affecting other sites. This may reflect the general problem of separating mixed oligoastrocytomas from astrocytomas and may indicate that temporal oligoastrocytomas not only differ with respect to LOH 1p and LOH 19q, but are also enriched by a fraction of tumors possibly resembling astrocytoma. This line of argument is supported by the observation of an inverse association of LOH 1p and TP53 mutations in oligoastrocytomas. Although 32 of 57 oligoastrocytomas had LOH 1p without TP53 mutation and 13 of 57 had TP53 mutations without LOH 1p, only 2 of 57 exhibited both LOH 1p and TP53 mutations (P < 0.0001, Fisher’s exact test). This clearly demonstrates the existence of different pathogenetic pathways in the genesis of oligoastrocytomas and also confirms our previous study.16 Analysis excluding 31 cases already studied in the previous series16 further demonstrated the same two molecular subsets in the remaining 21 samples (P < 0.005, Fisher’s exact test), thereby confirming the initial study16 using an independent series of tumors. In fact, only one of those 10 temporal oligoastrocytomas with TP53 mutation had LOH 1p. Taken together, these data indicate extensive genetic overlap between oligodendroglialomas and oligoastrocytomas in non-temporal sites, raising the question of whether these tumors represent variants of the same entity. In the temporal lobe, approximately half of the oligoastrocytomas share genetic features with astrocytomas, ie, presence of TP53 mutation and absence of LOH 1p and 19q, suggesting that these tumors may indeed be astrocytomas with some histological features resembling oligodendroglioma. It is thus possible that there are three molecular subsets of oligodendroglial tumors, differing not by morphology but on molecular grounds. Such a model would include a set of predominately extratemporal oligodendroglial tumors with LOH 1p and LOH 19q, and a set of predominately extratemporal oligodendroglial tumors without LOH 1p/LOH 19q that may be used for molecular classification. Such an approach results in pooling oligodendroglial tumors based on the presence or absence of LOH 1p and OLH 19q, and, raise the radical possibility of dismissing temporal oligoastrocytomas with TP53 mutations as astrocytomas. Clinical reports are supportive for a molecular classifica-
tion of oligodendroglial tumors. LOH 1p and LOH 19 q have been demonstrated as powerful tools to predict survival. Studies on anaplastic oligodendrogliomas demonstrated these molecular parameters to be the most powerful predictors of response to chemotherapy. The regional heterogeneity of molecular parameters in oligodendroglial tumors also finds clinical support in the observation that patients with frontal oligodendroglial tumors have better outcomes. Taken together, a subclassification on molecular grounds provides a cogent approach to unifying previous findings about prognosis, behavior, response to therapy, genotype, and location in oligodendroglial tumors. The emerging clinicogenetic associations suggest that oligodendroglial tumors will require molecular subdivision in the near future.

Acknowledgments

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