Glioblastomas with an Oligodendroglial Component: A Pathological and Molecular Study

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Abstract. Glioblastoma (GBM) is considered by the WHO classification to represent the most malignant grade of the astrocytic tumors. However, a subset of GBM includes recognizable areas with oligodendroglial features, suggesting that some GBM may also have an oligodendroglial origin. The aim of this study was to analyze the molecular profile of GBM associated with an oligodendroglial component (GBMO). We analyzed a series of 25 GBMO. Loss of heterozygosity (LOH) on 1p and 19q, known as common markers of oligodendroglial tumors, were observed in 40% and 60% of cases, respectively; 72% of the tumors displayed one or both of these markers. All but 4 tumors (84%) showed alterations known to be preferentially involved in the progression of astrocytic tumors to GBM, such as EGFR amplification (44%), P16 deletion (48%), LOH on 10q (64%), PTEN (20%), and TP53 (24%) mutations. Therefore, GBMO displayed all the genetic aberrations found in “standard” GBM with a comparable incidence, but differed from GBM by having a higher rate of LOH on 1p and 19q. These results suggest that GBMO might represent a subgroup of tumors of oligodendroglial origin that is distinct from the “standard” GBM in terms of tumorigenesis pathway.

Key Words: 1p; 10; 19q; EGFR; Glioblastoma; Oligodendroglioma; P16.

INTRODUCTION

Glioblastomas (GBM) are the most frequent glial tumors. The current WHO classification considers GBM to represent the highest grade of malignancy of diffuse astrocytic tumors (astrocytoma grade IV) (1). However, GBM are known to be phenotypically heterogeneous. Several authors have described glial tumors that display both typical GBM and oligodendroglial features (2–5), making difficult the inclusion of these tumors in either the GBM or the oligodendroglia subgroups of the WHO classification, an issue of practical importance since oligodendroglial tumors are chemo-sensitive. The existence of such tumors also raises the possibility that some GBM may represent the ultimate level of malignancy in the oligodendroglial lineage. This hypothesis, still a matter of controversy, is supported by the observation that some oligodendroglias may progress to a highly anaplastic tumor that may be morphologically indistinguishable from GBM (5, 6). In addition, a human GBM-derived cell population (Hu-O-2A/Gb1) expressing many properties otherwise uniquely expressed by the oligodendrocyte progenitor cells has been recently isolated (7).

The aim of our study was to analyze the molecular profile of glioblastomas characterized by the presence of a recognizable oligodendroglial component (GBMO). A selection of 25 tumors that fulfilled the criteria of GBMO was analyzed with specific attention for genetic changes characteristic of oligodendrogiomas (such as chromosome 1p, 19q deletions) and glioblastomas (such as EGFR amplification, P16/CDKN2A homozygous deletion, TP53 mutation, PTEN/MMAC1, and chromosome 10 deletion). To our knowledge, this study represents the first molecular analysis devoted to GBMO, which is not yet recognized as a separate entity by the WHO (1, 8).

MATERIALS AND METHODS

Tumor Selection and Pathology Review

A set of 142 surgically resected gliomas from the Pitié-Salpêtrière’s Hospital (Paris) was selected, based on the availability of paired blood and frozen tissue for molecular analysis. These tumors were reviewed, classified morphologically, and scored according to the WHO guidelines (6) by 3 independent neuropathologists (K.M., M.K., J.P.). Consensus morphology and grade were established for each glioma. The diagnostic criterion of GBMO was based on the association in the same tumor of 2 well distinguishable parts. The first consisted of highly anaplastic oligodendrogial-like cells with networks of branching capillaries and microvascular proliferation (corresponding to grade III oligodendrogioma). The second was represented by poorly differentiated astrocytic tumor cells showing nuclear atypia, mitotic activity, vascular proliferation, and necrosis (corresponding to grade IV astrocytoma). We excluded the GBM with scattered oligodendroglial cells.

Microsatellite Analysis for Loss of Heterozygosity (LOH) on Chromosome 1p, 1q, and 10q

Blood and Tumor DNA, Isolated According to Standard Procedures, Were Screened for LOH on Chromosomes 1p, 1q,
and 10q, Respectively, Using the Following Polymorphic Markers: D1S450, D1S2667 (located on 1p36); D1S234, D1S255 (located on 1p34); D1S2797, D1S2890 (located on 1p32); D1S206 (located on 1p21–13); D1S249 (located on 1q); D1S9425 (located on 19q11); D19S219, D19S888, D19S412, D19S418 (located on 19q13.1–13.4); D10S537, D10S541, D10S597, D10S1693, D10S212 (located on 10q21.2, 10q23.3, 10q23.24, 10q24.25 and 10qter, respectively). One of the primers was labeled with the Hex, Fam, or Ned fluorochromes (Perkin Elmer, Norwalk, CT). The samples were run on an automatic sequencer and analyzed with the Gene Scan program (Abi-prism, Perkin Elmer).

Screening of the TP53 and PTEN/MMAC1 Gene Mutations

TP53 mutations in exons 5 through 8 were screened by 5 different PCR reactions and followed by denaturing gradient gel electrophoresis (DGGE). Oligonucleotide primers and DGGE running conditions were as described (9). PTEN/MMAC1 mutations were screened by the DGGE technique in the entire coding sequence of the 9 exons and their corresponding splice junctions using primers previously described (10). DNA showing altered DGGE profiles in the TP53 and PTEN/MMAC1 genes were sequenced bidirectionally using the Perkin Kit and sequencer. When a DNA variant was found, the corresponding blood DNA was sequenced in order to differentiate somatic events from constitutional variants (polymorphism or germline mutation).

Search for Homozygous Deletion of P16/CDKN2A and EGFR Amplification

Tumor DNA was screened for P16/CDKN2A gene homozygous deletions and for EGFR amplification by multiplex PCR. For the P16/CDKN2A gene analysis, in addition to the P16 exon 2, the microsatellite D9S196 marker was co-amplified as described (11). For the EGFR gene analysis, the cystic fibrosis gene was co-amplified as described (12). The PCR ethidium bromide fluorescence signal response was quantified by scanning.

RESULTS

Twenty-five of the 142 malignant gliomas selected from the bank for pathological reviewing fulfilled the criteria of GBMO (Fig. 1). Twenty tumors were “primary” (de novo) GBMO and 5 were “secondary” GBMO as defined by Kleihues et al (1, 33). These last tumors were a recurrence of previously operated tumors with histologic evidence of progression from low grade or anaplastic gliomas to GBMO. The initial tumor was an oligodendroglioma (grade II) in 3 cases, an anaplastic oligodendroglioma (grade III) in 1 case, and a gemistocytic astrocytoma (grade II) in 1 case. Figure 2 summarizes the main clinical data of the patients and the genetic alteration findings for each tumor. The median age of the patients was 54 yr (range: 30–78 yr), and the median survival from the diagnosis of GBMO was 11.5 months (range: 3 to 28 months).

The incidence of the different genetic alterations are summarized in Table 1. LOH 1p, 19q, and 10q were observed in 40%, 60%, and 64%, respectively, P16 homozygous deletions in 48%, EGFR amplification in 44%. TP53 and PTEN mutations were less frequent and detected in 24% and 20%, respectively. The details of the mutations are shown in Table 2. Two subgroups of tumors could be considered according to the presence or absence of “oligodendroglioma markers” (i.e. LOH 1p and/or LOH 19q). Group A (LOH 1p+ and/or LOH 19q+) included 18 tumors (72%) (tumors T8 to T25), which displayed one or both “oligodendrogliomal markers,” including 7 tumors (28%) with both LOH 1p and 19q, 3 tumors (12%) with LOH 1p without 19q, and 8 tumors (32%) with LOH 19q without LOH 1p. Fifteen of these 18 tumors (83%) presented additional alterations considered to be more specific of GBM, such as EGFR amplification (8/18; 44%), P16 deletions (7/18; 39%), PTEN mutation (2/18; 11%); LOH 10q (11/18; 61%), TP53 mutation (5/18; 28%) (Fig. 3). Group B (LOH 1p- and LOH 19q-) (T1 to T7) included 18 tumors characterized by the absence of both “oligodendroglioma markers.” Nevertheless, these tumors did not differ from those of group A for the other genetic aberrations (Fig. 4). One tumor did not show any genetic alteration, 1 tumor displayed P16 deletion as the only genetic defect, the other 5 tumors demonstrated a more typical molecular profile of “standard” GBM combining multiple aberrations such as EGFR amplification (3 cases), or P16 deletion (4 cases), TP53 mutation (1 case), PTEN mutations (3 cases), and LOH 10q (5 cases). The comparison of the molecular profile of “primary” and “secondary” GBMO showed

Fig. 1. Histological features of GBMO. Oligodendroglial differentiated areas and “GBM like” areas were photographed from the same specimen in 4 tumors. Tumor T22: (A) Densely cellular GBM area in the vicinity of perinecrotic palisading; (B) anaplastic oligodendroglioma area with relatively uniform nuclei and perinuclear halos and endothelial proliferation (hematoxylin-phloxin). Tumor T12: (C) Densely cellular GBM area with nuclear polymorphism and abnormal mitosis; (D) oligodendroglial differentiated area (hematoxylin-phloxin). Tumor T25: (E) Densely cellular GBM area with characteristic perinecrotic palisading; (F) low-grade oligodendroglioma area with microcalcification (hematoxylin-phloxin). Tumor T17: (G) GBM area at high magnification shows tumor cells with an astrocytic differentiation having marked cellular anaplasia and brisk mitotic activity; (H) in another area of the same case the tumor cells have an oligodendroglial differentiation with rounded hyperchromatic nuclei and perinuclear halos (hematoxylin and eosin). Original magnifications: A, B, D, F, ×20; C, ×40; E, ×10; G, H, ×120.
no clear difference between the 2 groups except for *EGFR* amplification (0/5 in secondary GBMO versus 11/20 in primary GBMO) (*p* = 0.02, Chi² test).

**DISCUSSION**

Clinical and neuropathological studies of GBMO are scarce. Little is known about the exact incidence of GBMO. In one study, which used morphonuclear profile criteria to distinguish GBMO from GBM, the incidence of GBMO was estimated to represent about 4% of all the GBM (4). This incidence is probably underestimated. In the present study, GBMO represented 25 out of 142 gliomas (17%). In order to exclude a selection bias, we also reviewed 60 consecutive unselected tumors classified as GBM according to the WHO and found that 9/60 (15%) tumors fulfilled our criteria for the diagnosis of GBMO (unpublished data). The existence of such tumors suggests that some GBM might have an oligodendroglial cell origin and represent, therefore, a specific entity distinct from the “standard” GBM. In order to test this hypothesis, and because to date there is no clear immunohistochemical lineage marker able to distinguish an astrocytic from an oligodendroglial tumor, we analyzed the molecular profile of a series of GBMO.

The genetic alterations that characterize GBM and oligodendrogliomas have been studied extensively over the last few years (13–15). Interestingly, GBM and oligodendrogliomas display a distinct pattern of molecular alterations. Hence, *EGFR* amplification, *P16* homozygous deletions, *TP53* mutations, *PTEN* mutations, and LOH on 10q are all involved in the progression of astrocytic tumors to anaplasia and far more common in GBM than in...
Fig. 3. Representative molecular genetic characteristics of GBMO from the group A (tumor T25) (C: Control). A: LOH of chromosome 1p (D1S450 and D1S2667) and 19q (D19S425 and D19S219) (arrows). B: EGFR amplification as shown by increased signal intensity. CF corresponds to the cystic fibrosis control PCR fragment. C: P16 homozygous deletion as shown by the absence of P16 gene-specific band. The lower band is the control PCR product (D9S196).
Incidence of the Molecular Alterations in GBMO Compared with Anaplastic Oligodendrogliomas (OIII) and GBM in the Literature

<table>
<thead>
<tr>
<th>O III (references)</th>
<th>GBM (references)</th>
<th>GBMO n = 25</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOH 1p</td>
<td>50%–70% (29)</td>
<td>40% (10/25)</td>
</tr>
<tr>
<td>LOH 19q</td>
<td>50%–80% (5, 29, 34)</td>
<td>60% (15/25)</td>
</tr>
<tr>
<td>EGFR amplification</td>
<td>&lt;10% (18)</td>
<td></td>
</tr>
<tr>
<td>P16 deletion</td>
<td>5%–25% (19, 25)</td>
<td></td>
</tr>
<tr>
<td>PTEN mutation</td>
<td>&lt;5% (10, 20)</td>
<td></td>
</tr>
<tr>
<td>LOH 10q</td>
<td>30% (10, 21)</td>
<td></td>
</tr>
<tr>
<td>TP53 mutation</td>
<td>&lt;10% (16)</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 2

PTEN and TP53 mutations in GBMO

<table>
<thead>
<tr>
<th>Patients</th>
<th>Gene</th>
<th>Location</th>
<th>Codon</th>
<th>Nucleotid change</th>
<th>Consequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2</td>
<td>PTEN</td>
<td>IVS4</td>
<td>—</td>
<td>IVS4 → A</td>
<td>Splice mutation</td>
</tr>
<tr>
<td>T3</td>
<td>PTEN</td>
<td>EX 5</td>
<td>139</td>
<td>TTA → TTC</td>
<td>Leu → Phe</td>
</tr>
<tr>
<td>T9</td>
<td>PTEN</td>
<td>EX 7</td>
<td>261</td>
<td>CAG → TAG</td>
<td>Gln → Stop</td>
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<tr>
<td>T19</td>
<td>PTEN</td>
<td>EX 8</td>
<td>317</td>
<td>del TACT</td>
<td>frameshift</td>
</tr>
<tr>
<td>T5</td>
<td>PTEN</td>
<td>EX 9</td>
<td>347</td>
<td>del T</td>
<td>frameshift</td>
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<tr>
<td>T17</td>
<td>TP53</td>
<td>EX 5</td>
<td>161</td>
<td>GCC → ACC</td>
<td>Ala → Thr</td>
</tr>
<tr>
<td>T2</td>
<td>TP53</td>
<td>EX 5</td>
<td>158</td>
<td>CGC → CAC</td>
<td>Arg → His</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EX 7</td>
<td>248</td>
<td>CGG → CTG</td>
<td>Arg → Leu</td>
</tr>
<tr>
<td>T14</td>
<td>TP53</td>
<td>EX 6</td>
<td>203</td>
<td>ins AATTTCGC</td>
<td>frameshift</td>
</tr>
<tr>
<td>T11</td>
<td>TP53</td>
<td>EX 7</td>
<td>248</td>
<td>CGG → CAG</td>
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<tr>
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<tr>
<td>T23</td>
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<td>241</td>
<td>TCC → TTC</td>
<td>Ser → Phe</td>
</tr>
</tbody>
</table>

Abbreviations: EX = exon; IVS = intervening sequence; del = deletion; ins = insertion.

oligodendrogial tumors (14, 16–22). Conversely, LOH 1p and 19q predominate in oligodendrogliomas with an incidence ranging from 50% to 80% (23–26), but are rare in GBM, (27–29) suggesting that 1p and 19q contain yet unidentified tumor suppressor genes whose inactivation is involved in the development of oligodendrogial tumors (30, 31). In our series of 25 GBMO, all the tumors except 4 showed one or several genetic aberrations commonly observed in GBM. EGFR amplification, P16 deletions, LOH 10q, PTEN, and TP53 mutations were detected in 44%, 48%, 64%, 20%, and 24%, respectively. Moreover, the incidence of each of these aberrations in GBMO is comparable with those observed in “standard” GBM (Table 1). Interestingly, GBMO displayed LOH 1p and 19q in 40% and 60% of the cases, respectively. The incidence of these alterations is clearly higher than that reported in “standard” GBM. Kraus et al reported an 8% and 25% incidence of LOH on 1p and 19q, respectively, in a large series of 79 GBM (29). Analysis of the individual profile of our GBMO showed that 72% of the tumors displayed one or both of the “oligodendroglioma markers,” i.e. LOH 1p and 19q (group A) (Table 1). More than half of the cases of GBMO presenting LOH on 1p were associated with P16 deletions. In contrast to what has been reported in anaplastic oligodendrogliomas (25), we did not show that both alterations were mutually exclusive in GBMO. In a recent molecular study of 54 oligodendroglial tumors, Bigner et al described a subgroup of 5 tumors that fulfilled the criteria of GBMO (5). Three of them shared the molecular profile of our group A of GBMO. One tumor displayed LOH on 1p associated with TP53 mutation, LOH on 10q, and LOH on 9p (which contains the P16 gene); 1 tumor showed LOH on 19q associated with TP53 mutation and CDK4 amplification; and 1 tumor demonstrated LOH on 1p and 19q associated with LOH on 9p. The 2 other tumors demonstrated molecular profiles similar to those of our group B of GBMO; 1 tumor

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Fig. 4. Representative molecular genetic characteristics of GBMO from the group B (tumor T5) (C: Control). A: No LOH of chromosome 1p (D1S450 and D1S2667) and 19q (D19S425 and D19S219). B: PTEN mutation in exon 9 detected by denaturing gradient gel electrophoresis (DGGE) showing an altered profile. C: EGFR amplification. CF corresponds to the cystic fibrosis control PCR fragment. D: P16 homozygous deletion as shown by the absence of P16 gene-specific band. The lower band is the control PCR product (D9S196).
GLIOBLASTOMAS WITH AN OLIGODENDROGLIAL COMPONENT

A/

Chromosome 1p

Chromosome 19q

blood

tumor

T5

D1S450
no LOH

D1S2667
no LOH

D19S425
no LOH

D19S219
no LOH

B/

PTEN exon 9

C/

EGFR

CF

D/

Pl6

D9S196
showed LOH on 9p, 10q and EGFR amplification and the other LOH on 10q and CDK4 amplification. Finally, when comparing the genetic alterations between primary and secondary GBMO, we observed an absence of EGFR amplification and PTEN mutation in secondary tumors in contrast to primary tumors as reported in standard GBM (32, 33).

Since LOH on 1p and 19q are considered to be early events in the tumorigenesis of oligodendrogial tumors (5, 23, 28, 29, 34), we can hypothesize that GBMO involves a specific molecular pathway characterized by the occurrence of LOH on 1p and/or 19q as a first step, followed by one or several aberrations, such as EGFR amplification, PI6 deletion, PTEN mutations, LOH10q, and TP53 mutations that allow the oligodendrogial tumor to progress to the GBM phenotype. A comparative molecular analysis of the oligodendrogial and GBM components of the tumors using microdissection techniques would support this hypothesis. However, about 1/3 of the GBMO (group B) do not display any of the oligodendroglioma markers (LOH on 1p, 19q) and show a typical molecular profile of GBM. This suggests the possibility of alternative molecular pathways for these tumors that do not involve LOH on 1p and 19q, probably requiring yet unknown genetic alterations in addition to the common genetic alterations specific to GBM. The existence of such an alternative pathway is supported by a recent comparative genomic hybridization (CGH) study of Jeuken et al (40). These authors have identified a subgroup of high-grade oligodendrogial tumors, which may represent glioblastomas with prominent oligodendrogial differentiation, displaying gain of chromosome 7 (which contains the EGFR gene) and loss of chromosome 10, in the absence of loss of chromosomes 1p and 19q.

For clinicians, GBMO are of particular interest because it has become evident over the last decade that anaplastic oligodendrogial tumors are more chemosensitive than GBM (35). In addition, the presence of chromosome 1p and 19q deletions has recently been shown to be molecular markers predictive of a good chemosensitivity and a better outcome in oligodendrogliomas (25, 36, 41–43). In our study, the age and survival of patients suffering from GBMO were similar to those of GBM and we did not show that the tumors with LOH on 1p had a better outcome than those without LOH on 1p. However, the small size of our series and the heterogeneity of the treatment delivered to our patients did not allow us to make a definitive conclusion concerning the prognostic value of the presence of an oligodendrogial component in GBM.

In summary, we provide evidence for a specific molecular profile associated with GBMO that differs from that of “standard” GBM by frequent LOH 1p and 19q, commonly observed in oligodendrogliomas. This suggests that GBMO represents a subgroup of tumors of oligodendrogial origin that is distinct from the “standard” GBM in terms of tumorigenesis pathway. These results encourage the analysis of larger and preferably prospective series to appreciate whether this subgroup has a different course from “standard” GBM.

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REFERENCES

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