The Definition of Primary and Secondary Glioblastoma

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Abstract
Glioblastoma is the most frequent and malignant brain tumor. The vast majority of glioblastomas (~90%) develop rapidly de novo in elderly patients, without clinical or histologic evidence of a less malignant precursor lesion (primary glioblastomas). Secondary glioblastomas progress from low-grade diffuse astrocytoma or anaplastic astrocytoma. They manifest in younger patients, have a lesser degree of necrosis, are preferentially located in the frontal lobe, and carry a significantly better prognosis. Historically, primary and secondary glioblastomas are largely indistinguishable, but they differ in their genetic and epigenetic profiles. Decisive genetic signposts of secondary glioblastoma are IDH1 mutations, which are absent in primary glioblastomas and which are associated with a hypermethylation phenotype. IDH1 mutations are the earliest detectable genetic alteration in precursor low-grade diffuse astrocytomas and in oligodendrogliomas, indicating that these tumors are derived from neural precursor cells that differ from those of primary glioblastomas. In this review, we summarize epidemiologic, clinical, histopathologic, genetic, and expression features of primary and secondary glioblastomas and the biologic consequences of IDH1 mutations. We conclude that this genetic alteration is a definitive diagnostic molecular marker of secondary glioblastomas and more reliable and objective than clinical criteria. Despite a similar histologic appearance, primary and secondary glioblastomas are distinct tumor entities that originate from different precursor cells and may require different therapeutic approaches.

Introduction

Historical perspective
From a biologic and clinical point of view, the secondary glioblastomas developing in astrocytomas must be distinguished from "primary" glioblastomas. They are probably responsible for most of the glioblastomas of long clinical duration.

H.-J. Scherer (1940; ref. 1).

In a series of publications on gliomas, Hans-Joachim Scherer (1906–1945), a young German neuropathologist working in exile at the Institut Bunge (Antwerp, Belgium) was decades ahead of his contemporaries in his insight into the pathology and biology of brain tumors (2). The distinction between primary and secondary glioblastomas was a remarkable observation at that time. In the first edition of the World Health Organization (WHO) Classification of Tumors of the Nervous System, published 40 years later, glioblastomas were not even recognized as astrocytic neoplasms but listed in a group of “poorly differentiated and embryonal tumors” (3). Only after the introduction of immunohistochemistry was their astrocytic origin unequivocally established (4). Although it was recognized in clinical practice that some glioblastomas developed after resection of low-grade or anaplastic astrocytomas, Scherer’s distinction remained conceptual as histopathologically these subtypes could not reliably be distinguished.

In 1996, we reported evidence that primary and secondary glioblastomas carry distinct genetic alterations (5). TP53 mutations were found to be uncommon in primary glioblastomas but occurred with a high incidence in secondary glioblastomas; EGF receptor (EGFR) overexpression prevailed in primary glioblastomas but was rare in secondary glioblastomas. Only 1 of 49 glioblastomas showed TP53 mutation and EGFR overexpression, indicating that these alterations are mutually exclusive events defining 2 different genetic pathways in the evolution of glioblastoma (5). Subsequently, many studies provided evidence that primary and secondary glioblastomas develop through distinct genetic pathways (6, 7). Typical for primary glioblastomas are EGFR amplification, PTEN mutation, and entire loss of chromosome 10 (6–8). Genetic alterations more common in secondary glioblastomas include TP53 mutations and 19q loss (6, 7, 9). However, until the identification of IDH1 mutation as a molecular marker of secondary glioblastoma (10–13), the patterns of genetic alterations, though different, did not allow an unequivocal separation of the 2 subtypes.
**IDH1 Mutations as Initiator and Lineage Marker in Gliomagenesis**

**IDH1/2 mutations in neural tumors**

*IDH1* mutations were first reported by Parsons and colleagues (14) in 2008, and in this first study the authors already pointed out that "mutations in *IDH1* occurred in a large fraction of young patients and in most patients with secondary glioblastomas and were associated with an increase in overall survival." Subsequent studies showed that these mutations are very frequent in secondary (>80%) but very rare in primary glioblastomas (<5%; refs. 10–13). *IDH1* mutations as a genetic marker of secondary, but not primary, glioblastoma closely correspond to the respective clinical diagnosis in 385 of 407 (95%) of cases (13). It is now agreed that *IDH1* mutation is a definitive diagnostic molecular marker of secondary glioblastomas and more reliable and objective than clinical and/or pathologic criteria.

*IDH1* mutations are frequent (>80%) in diffuse astrocytoma WHO grade II and anaplastic astrocytoma WHO grade III, the precursor lesions of secondary glioblastomas, as well as in oligodendrogial tumors including oligodendroglioma WHO grade II, anaplastic oligodendroglioma WHO grade III, oligoastrocytoma WHO grade II, and anaplastic oligoastrocytoma WHO grade III (10–12, 15). In contrast, *IDH1* mutations are very rare or absent in pilocytic astrocytomas, as well as in most other CNS neoplasms, including ependymomas, medulloblastomas, and meningiomas (10–12, 15). *IDH2* mutations are less frequent and prevail in anaplastic oligodendrogliomas (~5%) and oligoastrocytomas (~6%; ref. 12).

All *IDH1* mutations reported are located at the first or second base of codon 132 (10, 11, 14). Most frequent is R132H (CGT→CAT), observed in 83% to 91% of *IDH1* mutations in astrocytic and oligodendrogial gliomas (10–12). Other mutations are rare, including R132C (CGT→TGT; 3.6%–4.6%; refs. 10–12), R132G (0.6%–3.8%; refs. 10–12), R132S (0.8%–2.5%; refs. 10–12), and R132L (0.5%–4.4%; refs. 10, 12). *IDH2* mutations are located at codon 172 (10, 12), with R172K being most frequent.

**IDH1/2 mutations in non-neural tumors**

*IDH1/2* mutations are absent or very rare in most tumors at other organ sites, including bladder, breast, stomach, colorectum, lung, liver, ovary, and prostate (12, 15). Exceptions are central chondrosarcomas (~55%; ref. 16), intrahepatic cholangiocarcinomas (23%; ref. 17), acute myelogenous leukemia (AML; 15%–20%; refs. 18–23), angioimmunoblastic T-cell lymphoma (AITL; ~20%; ref. 24), malignant melanoma (~10%; ref. 25), and anaplastic thyroid cancer (~10%; ref. 26). This suggests that *IDH1/2* mutations may confer a growth advantage in specific cell lineages at defined stages of development and differentiation.

**Are there primary glioblastomas with *IDH1* mutations?**

In a population-based study, only 14 of 407 glioblastomas clinically diagnosed as primary (3.4%) carried an *IDH1* mutation (13). These patients were 10 years younger and their genetic profiles were similar to those of secondary glioblastomas, including frequent *TP53* mutations and absence of *EGFR* amplification (13). Similarly, several hospital-based studies showed that primary glioblastomas with *IDH1* mutations were 13 to 27 years younger than those without *IDH1* mutations (10, 12, 27, 28). Toedt and colleagues (27) showed that primary glioblastomas with *IDH1* mutations have gene expression profiles similar to those of *IDH1*-mutated secondary glioblastomas. Glioblastomas with *IDH1* mutations clinically diagnosed as primary may have rapidly progressed from precursor lesions that escaped clinical diagnosis and are likely to have been misclassified as primary glioblastoma.

**Are there secondary glioblastomas that do not have *IDH1* mutations?**

Secondary glioblastomas lacking *IDH1* mutations have infrequent *TP53* mutations and patients have a shorter clinical history (13). Furthermore, most secondary glioblastomas lacking *IDH1* mutations (7 of 8) had developed through progression from an anaplastic glioma (WHO grade III), whereas the majority of secondary glioblastomas with *IDH1* mutations had progressed from a WHO grade II glioma (13). The possibility therefore exists that some tumors diagnosed as anaplastic astrocytoma were actually primary glioblastomas that were misdiagnosed because of a sampling error. In the absence of diagnostic hallmarks, that is, necrosis and/or microvascular proliferation, pathologists hesitate to make a diagnosis of glioblastoma even if MRI suggests this.

**Timing of *IDH1/2* mutations in the pathway to secondary glioblastoma**

*IDH1/2* mutations are an early event in gliomagenesis and persist during progression to secondary glioblastoma. In addition to frequent *IDH1/2* mutations, about 65% of diffuse astrocytomas carry a *TP53* mutation, whereas oligodendrogliomas show frequent 1p/19q loss (>75%; refs. 11, 29–33). *IDH1/2* mutations are likely to occur before *TP53* mutation or 1p/19q loss, as low-grade diffuse gliomas carrying only *IDH1/2* mutations are more frequent (17%) than those carrying only a *TP53* mutation (2%) or those showing only 1p/19q loss (3%; ref. 33). Furthermore, the analysis of multiple biopsies from the same patient revealed that there were no cases in which an *IDH1* mutation occurred after the acquisition of a *TP53* mutation or loss of 1p/19q (11, 33).

Acquisition of 1p/19q loss in cells with *IDH1/2* mutations may be the driving force toward oligodendrogial differentiation in low-grade diffuse glioma (11, 31, 33). It has been shown that tumors with the typical histologic signature of oligodendroglioma (e.g., honeycomb appearance of most neoplastic cells) showed loss at 1p/19q in the vast majority of cases (>90%; ref. 31). Furthermore, exomic sequencing recently revealed that mutations in the *CIC* gene (homolog of the *Drosophila* gene capicua) at 19q13.2 and in the *FUBP1* gene at 1p are frequent in oligodendrogliomas but rare or absent in diffuse astrocytomas (34–36).
Astrocytomas typically develop in cells with IDH1/2 mutations that subsequently acquire TP53 mutations. Recent studies have also described mutations in the ATRX (α-thalassemia/mental-retardation-syndrome-X-linked) gene that are often copresent with IDH1/2 mutations and TP53 mutations in diffuse astrocytomas WHO grades II/III and secondary glioblastomas (36, 37). Our current concept of the genetic pathways leading to astrocytic and oligodendrogial gliomas is summarized in Figure 1.

Only 7% of WHO grade II diffuse gliomas had none of these genetic alterations (i.e., IDH1/2 mutations, TP53 mutations, and 1p/19q loss) and were termed "triple negative" (33). These cases are still poorly understood; a minor fraction shows loss of cell-cycle control regulated by the RB1 pathway (38). The possibility cannot be excluded that they are derived from a different precursor cell population.

**IDH1 mutations in gliomas associated with the Li-Fraumeni syndrome**

As indicated earlier, IDH1 mutations precede TP53 mutations in sporadic astrocytic tumors. Patients with Li-Fraumeni syndrome (LFS) carry a germline TP53 mutation that is present in every somatic cell. Thus, by definition TP53 mutations would be the first event in LFS-associated gliomas, which account for 12% to 13% of all tumors occurring in LFS families (39, 40). In patients from 3 families with LFS, we identified IDH1 mutations in 5 astrocytic gliomas that developed in carriers of a TP53 germline mutation. Without exception, all contained the R132C (CGT-->TGT) mutation (41), which in sporadic astrocytic tumors amounts to less than 5% of all IDH1 mutations (10–12). This remarkably selective occurrence suggests a preference for R132C mutations in neural precursor cells that already carry a germline TP53 mutation.

**Biologic Consequences of IDH Mutations**

The mechanisms by which IDH1 mutations contribute to the development and malignant progression of astrocytic and oligodendrogial tumors are still not fully understood. Conditional IDH1 (R132H) knockin mice with expression in all hematopoietic cells or cells of the myeloid lineage caused an increased number of early hematopoietic progenitors with splenomegaly, anemia, and extramedullary hematopoiesis (42), whereas brain-specific IDH1 (R132H)-conditional knockin mice exhibited hemorrhage and perinatal lethality (43). Like EGFR amplification in primary glioblastomas, IDH1 mutations in secondary glioblastomas are typically lost during culture in vitro (44). This is enigmatic, since selective suppression of endogenous mutant IDH1 expression in a fibrosarcoma cell line with a native IDH1R132C heterozygous mutation significantly inhibits cell proliferation (45). Only recently, using a neurosphere culture method, it has been possible to establish a brain tumor stem cell line from an IDH1-mutant anaplastic oligoastrocytoma with an endogenous IDH1 mutation and detectable production of 2-hydroxyglutarate (2HG; ref. 46). This may suggest that IDH1-mutant glioma cells have stem cell–like features that confer a growth advantage under neurosphere culture conditions. Alternatively, there may be intratumoral heterogeneity of IDH1 mutations, and
neoplastic cells lacking IDH1 mutations are positively selected during culture.

**Impaired enzymatic activity and accumulation of 2-hydroxylutarate**

The IDH1 gene encodes isocitrate dehydrogenase 1, an enzyme participating in the citric acid (Krebs) cycle (47, 48), the metabolic pathway used by aerobic organisms to generate usable energy (49). IDH1/2 mutations reduce the wild-type activity of the enzyme, that is, the conversion of isocitrate to α-ketoglutarate (α-KG), and increase levels of hypoxia-inducible factor-1α (HIF-1α), a transcription factor, and its targets (e.g., GLUT1, VEGF, and PGK1; ref. 50). Importantly, IDH1/2 mutations are gain-of-function mutations that also produce the oncometabolite 2HG from α-KG (51, 52). The production of 2HG is a function shared by all the commonly occurring IDH1/2 mutants analyzed (51–53). Malignant IDH1mut gliomas contain an increased (up to 100-fold) concentration of 2HG (51). Cells from brain-specific IDH1 R132H-conditioned knockin mice also show high levels of 2HG that are associated with inhibited prolyl-hydroxylation of HIF-1α and upregulation of its target genes (43). 2HG also blocks prolyl-hydroxylation of collagen, causing a defect in collagen protein maturation, leading to basement-membrane aberrations that may play a role in glioma progression (43).

**Hypermethylation phenotype**

Noushmehr and colleagues (54) first reported that IDH1/2mut glioblastomas displayed concerted CpG island methylation at a large number of loci. A similar hypermethylation phenotype was also observed in IDH1/2mut diffuse astrocytomas (55) and oligodendrogial tumors (55), as well as in IDH1/2mut AML (56). Turcan and colleagues (57) introduced mutant IDH1 into primary human astrocytes, causing alteration of specific histone markers and induction of extensive DNA hypermethylation, suggesting that the presence of an IDH1 mutation is sufficient to establish a hypermethylation phenotype in glioma. This is supported by the observation that the expression of IDH1 R132H in cells of myeloid lineage in knockin mice resulted in hypermethylated histones and changes in DNA methylation similar to those observed in human IDH1/2mut AML (42). Stable transfection of a 2HG-producing IDH1 mutant into immortalized astrocytes resulted in progressive accumulation of histone methylation, which was associated with repression of the inducible expression of lineage-specific differentiation genes and a block to differentiation, suggesting that 2HG-producing IDH1/2 mutants can prevent the histone demethylation that is required for lineage-specific progenitor cells to differentiate into terminally differentiated cells (58). Duncan and colleagues (59) knocked in a single copy of the IDH1 R132H into a human cancer cell line and profiled changes in DNA methylation in more than 27,000 CpG dinucleotides. Heterozygous expression of mutant IDH1 was sufficient to induce widespread alterations in DNA methylation, including hypermethylation of 2,010 and hypomethylation of 842 CpG loci, many of which were consistent with those observed in IDH1-mutant and glioma–CpG island methylator phenotype (G-CIMP)+ primary gliomas (59).

**IDH1 Mutations and the Proneural Signature of Glioblastomas**

Glioblastomas have been classified on the basis of cDNA expression profiles, with distinct proneural, neural, classical, mesenchymal, and proliferative patterns (60, 61). Most IDH1mut glioblastomas (11 of 12, 92%) show a proneural expression signature; conversely, approximately 30% of glioblastomas with a proneural signature are IDH1mut (61). Glioblastomas lacking IDH1 mutations have all been identified as classical, mesenchymal, neural, or proneural (61).

These observations suggest that secondary glioblastomas are a rather homogeneous group of tumors characterized by a proneural expression pattern, whereas primary glioblastomas are heterogeneous, with several distinct expression profiles. Diffuse astrocytomas, oligodendrogliomas, and oligoastrocytomas all show the typical proneural signatures (62), again supporting the view that these neoplasms share common neural progenitor cells.

**Incidence of Secondary Glioblastomas**

Until the discovery of IDH1 mutations as a molecular marker, the distinction between primary and secondary glioblastomas was based on clinical observations. Tumors were considered primary if the diagnosis of glioblastoma was made at the first biopsy, without radiologic or histologic evidence of a preexisting, less malignant precursor lesion. The diagnosis of secondary glioblastoma required neuroimaging and/or histologic evidence of a preceding low-grade or anaplastic astrocytoma (6, 7).

In developed countries, the annual incidence of glioblastoma is usually in the range of 3 to 4 cases per 100,000 persons per year (7, 63–65). In a population-based study in Switzerland, using clinical criteria and histopathologic evidence, only 5% of all glioblastomas diagnosed were secondary (7, 63). Similarly, a study by the University of Alabama (Tuscaloosa, AL) showed that 19 of 392 (5%) cases of glioblastoma had a histologically proven prior low-grade glioma (66). When IDH1 mutations are used as a genetic marker, secondary glioblastomas accounted for 9% of all glioblastomas at the population level (13) and for 6% to 13% in hospital-based studies (10, 12, 67, 68).

The combined incidence rates of low-grade and anaplastic astrocytomas are approximately twice as high as that of clinically diagnosed secondary glioblastoma or IDH1mut glioblastoma (30, 64, 69). This may be explained at least in part by the fact that some patients with low-grade or anaplastic astrocytoma succumb to the disease before progression to glioblastoma occurs. Furthermore, cases with rapid progression from low-grade or anaplastic astrocytoma may be misclassified as primary glioblastoma.

**Age and Sex Distribution**

There is a striking difference in the age distribution of patients with primary and secondary glioblastoma. At a
population level, the mean age of patients with glioblastoma clinically diagnosed as primary was 62 years, whereas secondary glioblastomas developed in younger patients (mean, 45 years; refs. 7, 63). Similarly, the mean ages of patients with or without \( IDH1 \) mutations were 61 and 48 years, respectively (Table 1; ref. 13). Several hospital-based studies showed that patients with \( IDH1^{mut} \) glioblastomas are significantly younger (mean, 32–41 years) than those without \( IDH1 \) mutations (mean age, 56–59 years; refs. 12, 67, 70).

Glioblastomas predominantly affect males, with a population-based M/F ratio ranging from 1.28 (7) to 1.32 (64, 65). In contrast, diffuse astrocytomas (WHO grade II) have a less pronounced male predominance, with M/F ratios of approximately 1.17 (29, 65). Because secondary glioblastomas typically develop from diffuse astrocytomas, one would expect that they have a similar gender ratio. This is indeed the case. In a population-based study, \( IDH1^{mut} \) secondary glioblastomas had an M/F ratio of 1.12, significantly lower than the ratio of 1.46 in patients with primary glioblastoma (7). Several hospital-based studies also showed a tendency toward a lower M/F ratio of patients with secondary glioblastomas (5, 71–74). In a recent multicenter study, 49 of 618 glioblastomas (7.9%) carried an \( IDH1 \) mutation and had an M/F ratio of 0.96, in contrast to a ratio of 1.63 for nonmutated (primary) glioblastomas (68).

### Clinical History

At a population level, the majority of patients with primary glioblastoma (68%) had a clinical history of less than 3 months (6). The mean duration of the clinical history of patients with primary and secondary glioblastoma was 6.3 and 16.8 months, respectively (7, 63). Similarly, patients with glioblastomas lacking \( IDH1 \) mutations had a mean duration of preceding clinical symptoms of 3.9 months, significantly shorter than patients with \( IDH1^{mut} \) glioblastoma (mean, 15.2 months; \( P = 0.0003; \) ref. 13). Glioblastomas clinically diagnosed as primary had a mean clinical history of about 4 (\( IDH1^{wt} \)) and 29 months (\( IDH1^{mut} \)), respectively (13). It remains to be analyzed why the precursor lesions of these \( IDH1^{mut} \) secondary glioblastomas escaped clinical detection despite their long clinical history.

### Localization

Lai and colleagues (68) reported that glioblastomas lacking \( IDH1 \) mutations show widespread anatomic distribution, whereas \( IDH1^{mut} \) glioblastomas have a striking

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**Table 1. Primary and secondary glioblastomas: comparison of clinical versus genetic diagnosis**

<table>
<thead>
<tr>
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<th>Primary glioblastoma</th>
<th>Secondary glioblastoma</th>
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</thead>
<tbody>
<tr>
<td><strong>Clinical criteria</strong></td>
<td>IDH1wt</td>
<td>IDH1mut</td>
</tr>
<tr>
<td>Fraction in a population</td>
<td>94.7%</td>
<td>5.3%</td>
</tr>
<tr>
<td>Mean age, y</td>
<td>59–62</td>
<td>33–45</td>
</tr>
<tr>
<td>Male/female ratio</td>
<td>1.33–1.5</td>
<td>0.65–2.3</td>
</tr>
<tr>
<td>Mean clinical history, mo</td>
<td>6.3</td>
<td>16.8</td>
</tr>
<tr>
<td>Median overall survival, mo</td>
<td>9.9</td>
<td>15.2</td>
</tr>
<tr>
<td>Surgery + radiotherapy</td>
<td>4.7b</td>
<td>7.8b</td>
</tr>
<tr>
<td>Surgery + radio/chemo</td>
<td>15</td>
<td>31</td>
</tr>
<tr>
<td><strong>Histologic features</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oligodendroglial comp.</td>
<td>18%</td>
<td>42%</td>
</tr>
<tr>
<td>Necrosis</td>
<td>89%</td>
<td>63%</td>
</tr>
<tr>
<td><strong>Genetic alterations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDH1 mutations</td>
<td>4–7%</td>
<td>73–88%</td>
</tr>
<tr>
<td>TPS3 mutations</td>
<td>17–35%</td>
<td>60–88%</td>
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<tr>
<td>ATRX mutations</td>
<td>4–7%</td>
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<tr>
<td>EGFR amplification</td>
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<td>CDKN2A deletion</td>
<td>31–52%</td>
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<td>PTEN mutations</td>
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</tr>
<tr>
<td>10q loss</td>
<td>70%</td>
<td>63%</td>
</tr>
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</table>

*References:

1. Lai and colleagues (68) reported that glioblastomas lacking \( IDH1 \) mutations show widespread anatomic distribution, whereas \( IDH1^{mut} \) glioblastomas have a striking

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*Data from population-based study: all the patients who were treated in different ways were included.*
predominance of frontal lobe involvement, in particular in the region surrounding the rostral extension of the lateral ventricles. Stockhammer and colleagues (75) showed that IDH1/2mut WHO grade II astrocytomas tend to develop in a frontal location, and that seizures were the initial symptom in approximately 70% of patients. According to Zlatescu and colleagues (76) oligodendrogliomas with 1p/19q losses occur most frequently in the frontal lobe and have a tendency for widespread growth across the midline. Similarly, Laigle-Donadey and colleagues (77) showed that oligodendrogliomas with 1p/19q loss were located predominantly in the frontal lobe. These observations suggest that oligodendrogliomas, astrocytomas, and secondary glioblastomas derived thereof originate from precursor cells located in or migrating to the frontal lobe.

**Extent of Necrosis**

Already in his 1940 publication, Scherer noted “the absence of extensive necrosis and peritumoral brain swelling in secondary and their almost constant presence in primary glioblastomas may play a certain role in the clinical behavior of the two types” (1). He attributed this to the slower growth rate of secondary glioblastomas. It is now understood that a hypoxia-mediated activation of the coagulation system causes intravascular thrombosis, further increases intravascular hypoxia, and leads to abnormal endothelial cell proliferation and tumor necrosis (78, 79). Microvascular proliferation is induced by VEGF, which shows a markedly higher expression in primary than in secondary glioblastomas (71).

Histopathologically, large areas of ischemic and/or pseudopalisading necrosis are more frequent in primary (89%) than secondary glioblastomas (63%; P = 0.0014; ref. 80) and glioblastomas without IDH1 mutations (90% vs. 50%; P < 0.0001; ref. 13). This was confirmed in clinical MRI studies showing that necrosis was less frequent in IDH1mut glioblastomas, while exhibiting more frequent nonenhancing tumor components, larger size at diagnosis, lesser extent of edema, and increased prevalence of cystic and diffuse components (68).

**Histologic Features**

According to the 2007 WHO classification, histologic criteria for the diagnosis of glioblastoma include nuclear atypia, cellular pleomorphism, mitotic activity, vascular thrombosis, microvascular proliferation, and necrosis (29). Glioblastomas may show significant intertumoral and intratumoral heterogeneity, both histologically and genetically (80–82) and this may also apply to glioma-initiating cells (82, 83). This heterogeneity reflects genomic instability and, occasionally, focal new clones arising as a result of additional genetic alterations can be seen histologically (84, 85). Areas with oligodendroglioma-like components are significantly more frequent in secondary than primary glioblastomas (42% vs. 18%; P = 0.0138; ref. 80) and, accordingly, more frequent in IDH1mut glioblastomas than in IDH1wt glioblastomas (54% vs. 20%; P < 0.0001; ref. 13; Table 1). An increase in the fraction of tumor cells with oligodendrogial morphology in IDH1mut glioblastomas was also reported in a large study of 618 cases (68). This is not surprising as secondary glioblastomas assumedly share IDH1mut precursor cells with oligodendrogliomas (Fig. 1).

**Clinical Outcome**

In a population-based study, the median overall survival of clinically diagnosed secondary glioblastoma was 7.8 months, significantly longer than the survival of patients with primary glioblastoma (4.7 months; P = 0.003; ref. 7). Similarly, the analysis of patients with glioblastoma who were treated with surgery and radiotherapy showed that the mean overall survival time of patients with IDH1mut glioblastoma was 27.1 months, more than twice as long as that of patients with IDH1wt glioblastoma (11.3 months; P < 0.0001; ref. 13). Yan and colleagues (12) reported that IDH1mut glioblastomas treated with radio/chemotherapy had an overall survival time of 31 months, again twice as long as IDH1wt tumors. A different response to therapy is also supported by the observation that dose enhancement did not improve the outcome of glioblastomas with a pronuclear signature (containing IDH1mut cases), in contrast to glioblastomas with neural, classical, or mesenchymal signature, which all profited from more intensive therapy (61). If confirmed in prospective clinical trials, this may eventually allow for dose deescalation in patients with secondary glioblastoma.

**Origin of Primary and Secondary Glioblastomas**

Before the discovery of IDH1 mutations as a lineage marker, it was assumed that primary and secondary glioblastomas developed from the same precursor cell population but showed distinct clinical and biologic behavior due to the acquisition of different genetic alterations (6). There is now increasing evidence that despite their similar histologic features, primary and secondary glioblastomas develop from different cells of origin. The evidence supporting this hypothesis includes the observations that: (i) only secondary glioblastomas but not primary glioblastomas share common IDH1/2 mutations with oligodendrogliomas; (ii) primary and secondary glioblastomas develop in patients of different age groups and have a different sex distribution; (iii) primary and secondary glioblastomas are located in different brain regions; and (iv) primary and secondary glioblastomas have a significantly different clinical outcome. All these data suggest that primary and secondary glioblastomas are in fact different tumor entities that are derived from distinctly different neural precursor cells.

There is also evidence that cancer stem cells in primary and secondary glioblastomas may also be different. In one study, the relative content of CD133+ cells was significantly higher in primary than in secondary glioblastomas, and CD133+ expression was associated with neurosphere formation only in primary but not secondary glioblastomas (86).
Phenotype/Genotype Correlations

Despite differences in clinical history and genetic, epigenetic, and expression profiles, primary and secondary glioblastomas are histologically largely indistinguishable, except that extensive necrosis is more frequent in primary glioblastomas and oligodendroglioma components are more frequent in secondary glioblastomas (80; Table 1). This similarity may be attributable to genetic alterations that are common to both primary and secondary glioblastomas.

The most frequent genetic alteration shared by both primary and secondary glioblastomas is LOH at 10q (~60% of cases; refs. 6–8, 87–89), the most commonly deleted region being 10q25-qter, distal to D10S1683 (8). Because mutations of the PTEN gene located at 10q23.3 prevail in primary glioblastomas (~25%), but are rare in secondary glioblastomas (<5% refs. 6, 7, 90), loss of function of gene(s) other than PTEN is likely to be responsible for the common malignant phenotype. Circumscribed glioblastoma foci in low-grade diffuse astrocytoma or anaplastic astrocytoma show additional deletions at 10q25-qter, distal to D10S597, including the DMBT1 and FGFR2 loci (84). This suggests that the acquisition of a highly malignant glioblastoma phenotype is associated with loss of putative tumor suppressor gene(s) on 10q25-qter.

Pace of Malignant Progression to Secondary Glioblastoma

The ability to predict the pace of progression from low-grade diffuse astrocytoma to secondary glioblastoma would be clinically very important by giving oncologists a rational basis for deciding whether and at which stage to administer adjuvant radiotherapy. Histopathologically, this is not possible, as Scherer noted already in 1940: "Why certain astrocytomas become transformed into glioblastomas while others remain pure, is still obscure. No morphological sign explaining or announcing this tendency could be found" (1).

When commonly deleted genes at 10q25-qter in IDHwt and IDHmut glioblastomas were searched for in The Cancer Genome Atlas (TCGA; ref. 91), 10 genes were identified with log-ratio thresholds of −1.0, and, of these, DMBT1 at 10q26.13 was the only homozygously deleted gene in glioblastomas with or without IDH1 mutations (12.5% vs. 8.0%; ref. 92). DMBT1 homozygous deletion was detected at a similar frequency in an independent set of primary and secondary glioblastomas (20% vs. 21%; ref. 92). A small fraction (11.3%) of diffuse astrocytomas WHO grade II also showed a DMBT1 homozygous deletion, and this was significantly associated with shorter overall patient survival (92).

A similar approach was used to search for commonly amplified genes in IDHwt and IDHmut glioblastomas in the TCGA database. A total of 25 genes were identified, of which 21 were located at 7q31-34 (93). Further analyses revealed gain of the MET gene at 7q31.2 in primary glioblastomas (47%) and secondary glioblastomas (44%). Interestingly, MET gain is also common in diffuse astrocytomas (38%), and was associated with shorter survival (93). These results indicate that several genetic alterations frequent in both primary and secondary glioblastomas may be responsible for the common histologic phenotype and, if already present in diffuse astrocytomas, may predict unfavorable clinical outcome (92, 93). Whole-genome DNA sequencing of diffuse astrocytomas (WHO grade II) and anaplastic astrocytomas (WHO grade III) in patients with favorable or poor outcome is likely to identify further genetic alterations that drive the malignant progression to secondary glioblastoma.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: H. Ohgaki, P. Kleihues

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): H. Ohgaki

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): H. Ohgaki, P. Kleihues

Writing, review, and/or revision of the manuscript: H. Ohgaki, P. Kleihues

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H. Ohgaki, P. Kleihues

Study supervision: H. Ohgaki

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