Review

Clinical Cancer Research

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. Histologically, 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. *Clin Cancer Res; 19(4); 764–72. ©2012 AACR.*

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.

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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 oligodendroglial 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 oligodendroglial 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 (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 oligodendroglial 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 oligodendroglial 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 oligodendroglial 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

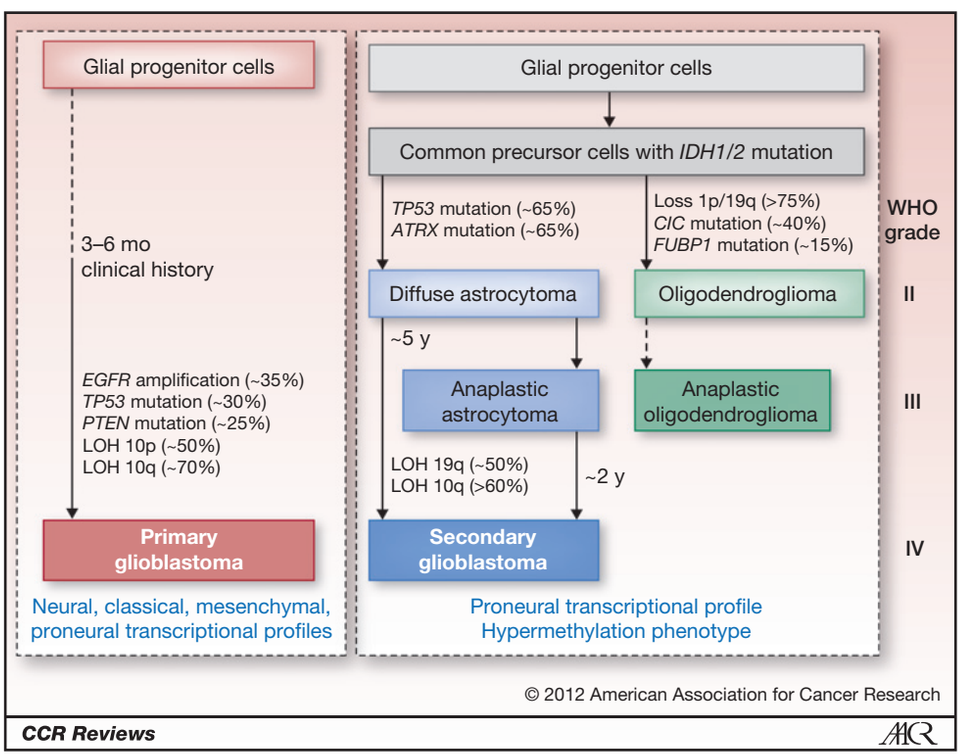

Figure 1. Genetic pathways to primary and secondary glioblastomas. Note that only secondary glioblastomas share common origin of cells with oligodendrogliomas.

neoplastic cells lacking *IDH1* mutations are positively selected during culture.

Impaired enzymatic activity and accumulation of 2-hydroxyglutarate

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 $IDH1^{mut}$ gliomas contain an increased (up to 100-fold) concentration of 2HG (51). Cells from brain-specific *IDH1* (R132H)-conditional 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 $IDH1/2^{mut}$ glioblastomas displayed concerted CpG island methylation at a large number of loci. A similar hypermethylation phenotype was also observed in $IDH1/2^{mut}$ diffuse astrocytomas (55) and oligodendroglial tumors (55), as well as in $IDH1/2^{mut}$ 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 $IDH1/2^{mut}$ AML (42). Stable transfection of a 2HG-producing *IDH* 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 $IDH1^{mut}$ glioblastomas (11 of 12, 92%) show a proneural expression signature; conversely, approximately 30% of glioblastomas with a proneural signature are $IDH1^{mut}$ (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 glioblastomas 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 $IDH1^{mut}$ 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 $IDH1^{mut}$ glioblastoma are significantly younger (mean age, 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 (IDH^{wt}) and 29 months (IDH^{mut}), respectively (13). It remains to be analyzed why the precursor lesions of these IDH^{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

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

	Primary glioblastoma		Secondary glioblastoma		
	Clinical criteria[a]	Genetic criteria ($IDH1^{wt}$)	Clinical criteria[a]	Genetic criteria ($IDH1^{mut}$)	References
Fraction in a population	94.7%	91.2%	5.3%	8.8%	(7, 13)
Mean age, y	59–62	56–61	33–45	32–48	(7, 12, 13, 67, 70)
Male/female ratio	1.33–1.5	1.2–1.46	0.65–2.3	1.0–1.12	(7, 12, 13, 70)
Mean clinical history, mo	6.3	3.9	16.8	15.2	(7, 13)
Median overall survival, mo					(7, 12, 13)
Surgery + radiotherapy	4.7[b]	9.9	7.8[b]	24	(7, 13)
Surgery + radio/chemotherapy		15		31	(12)
Histologic features					
Oligodendroglial comp.	18%	20%	42%	54%	(13, 80)
Necrosis	89%	90%	63%	50%	(13, 80)
Genetic alterations					
IDH1 mutations	4–7%	0%	73–88%	100%	(10, 12, 13)
TP53 mutations	17–35%	19–27%	60–88%	76–81%	(7, 10, 12, 13, 67, 91)
ATRX mutations	4–7%		57–80%		(36, 37)
EGFR amplification	36–45%	35–39%	0–8%	0–6.5%	(7, 10, 12, 13, 91)
CDKN2A deletion	31–52%	30–45%	19–20%	7–22%	(7, 12, 13, 91)
PTEN mutations	23–25%	24–26%	4–12%	0–8%	(7, 12, 13)
19q loss	6%	4%	54%	32%	(9, 13)
1p/19q loss	2–8%		0–13%		(10, 12, 67)
10p loss	47%		8%		(8)
10q loss	70%	67%	63%	73%	(7, 13)

[a]Tumors were considered to be primary if the diagnosis of glioblastoma was made at the first biopsy, without clinical or histological evidence of a preexisting, less malignant precursor lesion, whereas the diagnosis of secondary glioblastoma required histological and/or clinical (neuroimaging) evidence of a preceding low-grade or anaplastic astrocytoma.
[b]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 *IDH1/2*^{mut} 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) oligodendroglial tumors 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 intratumoral 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; Table 1). This was confirmed in clinical MRI studies showing that necrosis was less frequent in *IDH1*^{mut} 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 *IDH1*^{mut} glioblastomas than in *IDH1*^{wt} glioblastomas (54% vs. 20%; $P < 0.0001$; ref. 13; Table 1). An increase in the fraction of tumor cells with oligodendroglial morphology in *IDH1*^{mut} glioblastomas was also reported in a large study of 618 cases (68). This is not surprising as secondary glioblastomas assumedly share *IDH1*^{mut} 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 *IDH1*^{mut} glioblastoma was 27.1 months, more than twice as long as that of patients with *IDH1*^{wt} glioblastoma (11.3 months; $P < 0.0001$; ref. 13). Yan and colleagues (12) reported that *IDH1*^{mut} glioblastomas treated with radio/chemotherapy had an overall survival time of 31 months, again twice as long as *IDH1*^{wt} tumors. A different response to therapy is also supported by the observation that dose enhancement did not improve the outcome of glioblastomas with a proneural signature (containing *IDH1*^{mut} 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 IDH^{wt} and IDH^{mut} 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 IDH^{wt} and IDH^{mut} 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
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