Primary and secondary glioblastomas: From concept to clinical diagnosis[1]

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Glioblastomas may develop de novo (primary glioblastomas) or through progression from low-grade or anaplastic astrocytomas (secondary glioblastomas). These subtypes of glioblastoma constitute distinct disease entities that evolve through different genetic pathways, affect patients at different ages, and are likely to differ in prognosis and response to therapy. Primary glioblastomas develop in older patients and typically show *EGFR* overexpression, *PTEN* (*MMAC1*) mutations, *CDKN2A* (*p16*) deletions, and less frequently, *MDM2* amplification. Secondary glioblastomas develop in younger patients and often contain *TP53* mutations as the earliest detectable alteration. These characteristics are derived largely from patients selected on the basis of clinical history and sequential biopsies. Currently available data are insufficient for a substitution of histologic classification and grading of astrocytic tumors by genetic typing alone. More subtypes of glioblastomas may exist with intermediate clinical and genetic profiles, a factor exemplified by the giant-cell glioblastoma that clinically and genetically occupies a hybrid position between primary (de novo) and secondary glioblastomas. Future research should aim at the identification of criteria for a combined clinical, histologic, and genetic classification of astrocytic tumors.
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Because of their inherent tendency for diffuse infiltration of neighboring brain structures, low-grade astrocytomas invariably recur, often as a higher grade of malignancy and, ultimately, with the histologic and biologic characteristics of glioblastomas. Other glioblastomas develop very rapidly without clinical, radiologic, or morphologic evidence of a less malignant precursor lesion. Although this is commonplace in the clinical setting, the designations "primary" and "secondary" glioblastoma are conceptual rather than diagnostic terms. The main reason for this is that both glioblastoma types share similar morphologic features, and it has remained controversial whether they can be distinguished histologically (e.g., on the basis of a uniform pattern of small and poorly differentiated glioma cells vs. the presence of distinct tumor areas with differentiated neoplastic astrocytes) (Burger and Kleihues, 1989; Russell and Rubinstein, 1989; Scherer 1940a). The lack of histopathologic separation has also made it difficult to estimate the relative frequency at which both subsets of glioblastoma occur. However, it is undisputed that the primary glioblastoma is the prevailing type and probably accounts for more than 80% of glioblastoma cases (Dropcho and Soong, 1996).

Historical Annotation and Nomenclature

The terms *primary* and *secondary* glioblastomas were first used by the German neuropathologist Hans-Joachim Scherer, who, while working in exile at the Institute Bunge in Antwerp (Belgium), published a series of articles on gliomas with an insight into the bio-

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[3]Abbreviations: LOH, loss of heterozygosity.

[4]Ohgaki, H., Watanabe, K., Peraud, A., Biernat, W., von Deimling, A., Yasargil, G., Yonekawa, Y., and Kleihues, P. (1999) A case history of glioma progression. *Acta. Neuropathol.*

[5]Peraud, A., Watanabe, K., Schwechheimer, K., Yonekawa, Y., Kleihues, P., and Ohgaki, H. (1999) Genetic profile of the giant cell glioblastoma. *Lab. Invest.*

logical basis of neoplastic transformation in the nervous system that was far ahead of the knowledge and understanding of his time (Scherer 1938, 1940a, 1940b, 1940c). In 1940, he defined the two major subtypes of glioblastoma as follows (Scherer 1940a):

From a biological 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.

The primary glioblastoma has also been termed de novo. This term refers to the lack of an identifiable precursor lesion rather than to the assumption that this lesion results from a single-step malignant transformation. The *secondary glioblastoma* has been referred to as progressive glioblastoma (Newcomb et al., 1998), but this term is somewhat misleading since the glioblastoma marks the result and end point of malignant progression and is itself no longer progressive. On the other hand, the term s*econdary glioblastoma* is ambiguous for the uninitiated since, in today's oncologic terminology, secondary usually refers to metastatic lesions. However, since glioblastomas very rarely metastasize outside the CNS, the chance of misunderstanding is small. Based on genetic typing, the primary and secondary glioblastomas have also been termed glioblastoma type 2 and 1, respectively (von Deimling et al., 1993, 1995).

Glioma Development as a Multistep Process

Among the most exciting advances in cancer research is the recognition that morphologic changes observed during the process of malignant transformation reflect nothing but the sequential acquisition of genetic alterations (Vogelstein and Kinzler, 1993). Bridging the gaps between phenotype and genotype has led to an increased understanding of features that form the basis of the histologic tumor classification. Among tumors of the CNS, those of astrocytic origin have been studied most extensively. Several of the genes involved have been identified (Ashley and Bigner, 1997; Collins, 1995; Dirks and Rutka, 1997; Kleihues et al., 1997; Louis, 1997; von Deimling et al., 1995), and the correlation among clinical features, histopathology, and genetics has led to the identification of distinct molecular pathways leading to the glioblastoma as the common and most malignant end point (Kleihues and Ohgaki, 1997; Lang et al., 1994; Louis and Gusella, 1995). In addition, the molecular profile of a histologically distinct glioblastoma variant, the giant-cell glioblastoma, has begun to unfold.

Clinical Definition

A short clinical history and the presence of the histologic features of a full-blown glioblastoma at first biopsy are essential criteria for a tumor to be diagnosed as a primary glioblastoma. Conversely, a secondary glioblastoma requires clinical (neuroimaging) or histologic evidence of evolution from a less malignant precursor lesion, i.e., low-grade or anaplastic astrocytoma.

In studies from our laboratory (Biernat et al., 1997a, 1997b; Reyes-Mugica et al., 1997; Tohma et al., 1998a; Watanabe et al., 1996), genetic analyses were carried out on glioblastomas that were selected on the basis of stringent criteria. Primary glioblastomas were included if the clinical history was less than 3 months and histopathological features of glioblastoma were present at the first biopsy. The possibility exists that primary glioblastomas may have a significantly longer preoperative history, but to clearly distinguish between both subsets, the window of eligibility was deliberately kept small. The diagnosis of secondary glioblastoma required at least two biopsies taken at an interval of >6 months to avoid a sampling error and clinical as well as histopathological evidence of progression from low-grade or anaplastic astrocytoma.

Genetic Alterations Operative in the Evolution of Glioblastomas

Although several genetic alterations have been identified in association with the malignant transformation of astrocytes, our understanding of the sequence and types of gene alterations is still far from complete.

TP53

The TP53 protein is a sequence-specific transcription factor that activates genes containing TP53-response elements (Haffner and Oren, 1995). TP53 appears to function as a stress-inducible switch to turn on alternative pathways to G_1 arrest or apoptosis (Hainaut, 1995). *TP53* mutations were among the first genetic alterations identified in astrocytic brain tumors (Nigro et al., 1989). In unselected series of glioblastomas, the reported frequency varies considerably, with a mean of 25–30% (Louis 1994; Ohgaki et al., 1995). A different picture emerges if primary and secondary glioblastomas are analyzed separately. *TP53* mutations are rare in primary glioblastomas (<10%) and have a high incidence (>65%) in secondary glioblastomas, of which approximately 90% are already present in the first biopsy (Watanabe et al., 1996, 1997). The incidence of TP53 protein accumulation is more frequently observed than are *TP53* mutations (Lang et al., 1994; Louis et al., 1993; Newcomb et al., 1993; Watanabe et al., 1997), but is also significantly higher in secondary (>90%) than in primary glioblastomas (<35%) (Watanabe et al., 1996). The percentage of glioma cells with TP53 protein accumulation appears to increase from the first biopsy to recurrent tumors (Reifenberger et al., 1996; Watanabe et al., 1997), and one study suggests that this reflects the clonal expansion of glioma cells carrying a *TP53* mutation (Sidransky et al., 1992).

MDM2

The *MDM2* gene (mouse double minute 2) contains a TP53 DNA-binding site and it has been shown that, in a variety of conditions, the transcription of *MDM2* is induced by wild-type TP53 (Barak et al., 1994; Zauber-

man et al., 1995). The MDM2 protein, in turn, forms a complex with TP53, thereby abolishing its transcriptional activity (Zauberman et al., 1995). Thus, in normal cells, this autoregulatory feedback loop modulates both the activity of the TP53 protein and the expression of the *MDM2* gene (Picksley and Lane, 1993). An increase in TP53 levels would block entry into the cycle at the late G_1 checkpoint; at the same time, TP53 would induce the expression of *MDM2*, resulting in a TP53-MDM2 complex formation that may overcome the G_1 checkpoint and allow entry into the S-phase of the cell cycle (Olson et al., 1993). Therefore, *MDM2* amplification/overexpression constitutes an alternative mechanism to escape from TP53-regulated control of cell growth. Amplification of *MDM2* is present in <10% of glioblastomas (Reifenberger et al., 1993), and these appear to be primary glioblastomas that lack a *TP53* mutation (Biernat et al., 1997a; Reifenberger et al., 1993). Overexpression of *MDM2* was observed immunohistochemically in more than 50% of primary glioblastomas, but the fraction of immunoreactive cells varied considerably (Biernat et al., 1997a; Korkolopoulou et al., 1997; Newcomb et al., 1998). In contrast, less than 10% of secondary glioblastomas showed overexpression of *MDM2*. Thus, overexpression of *MDM2,* with and without gene amplification, is a genetic hallmark of the primary glioblastoma, which typically lacks a *TP53* mutation.

In contrast to low-grade astrocytomas, the majority of glioblastomas (22 of 32; 69%) contain short forms of alternatively spliced *MDM2* transcripts that lack a region containing the TP53 binding domain (Matsumoto et al., 1998). Comparative data on the frequency of these splice transcripts in primary and secondary glioblastomas are not yet available. In human ovarian and bladder tumors, the presence of these *MDM2* transcripts correlates with malignancy (Sigalas et al., 1996), and these variants promote growth when transduced in cultured cells (Sigalas et al., 1996). Thus, splice variants may convey an oncogenic activity of *MDM2*.

MDM2 *and p19^{Arf}*

The *CDKN2A* (*p16* or *INK4a)* locus codes for two gene products (p16 and $p19^{Arf}$) through differences in the first exon and alternative reading frames located in the common second exon. The putative tumor suppressor $p19^{Arf}$ blocks MDM2-induced degradation and transactivational silencing of p53 (Pomerantz et al., 1998; Zhang et al., 1998). Mice lacking functional $p19^{Arf}$ develop tumors, but mutations in human cancers appear to be rare (Kubo et al., 1997; Shiohara et al., 1996).

INK4a, p16, CDK4, and Rb pathways

The *p16* tumor suppressor is also encoded by the *CDKN2A* and exerts growth control by inhibition of the cyclin-dependent kinases CDK4 and CDK6, reducing their capacity to phosphorylate, in conjunction with cyclin D, the Rb protein, and thereby allowing G_1/S-phase transition of the cell cycle. Thus, loss of cell cycle control may result from altered expression of any of these genes: loss of CDKN2A (*p16*) expression, overexpression/amplification of *CDK genes*, or loss of *RB* function. In unselected and selected series of glioblastomas, *CDKN2A* deletion and *RB* alterations were found to be mutually exclusive (Biernat et al., 1997b; Burns et al., 1998; Ueki et al., 1996). Inactivation of genes in this pathway is common in both primary and secondary glioblastomas at an overall frequency of 50 and 39%, respectively (Biernat et al., 1997b). There was no difference in the frequency of loss of RB expression or *CDKN2A* amplification, but *CDKN2A* deletions were significantly more frequent in primary (36%) than in secondary glioblastomas (4%) (Biernat et al., 1997b). Most glioblastomas with *EGFR* (epidermal growth factor receptor) amplification show a *CDKN2A* deletion (Hayashi et al., 1997). This corroborates data from our laboratory showing that homozygous deletion of *CDKN2A* occurs in one-third of primary glioblastomas but is rarely observed in secondary glioblastomas (Biernat et al., 1997b). One-third of glioblastomas with normal CDKN2A (p16) expression showed accumulation of *MDM2* protein ($P<0.05$) (Newcomb et al., 1998).

EGFR

EGFR is involved in the control of cell proliferation and is amplified and overexpressed in more than one-third of glioblastoma cases, sometimes in a truncated and rearranged form (Ekstrand et al., 1992; Libermann et al., 1985; Wong et al., 1992). The most common alteration is a deletion of exons 2–7 from the extracellular domain, resulting in a truncated mutant receptor (Ekstrand et al., 1992; Nishikawa et al., 1994; Sugawa et al., 1990; Wong et al., 1992). This mutant EGFR confers enhanced tumorigenicity on human glioblastoma cells by increasing proliferation and reduced apoptosis (Nagane et al., 1996), apparently through the *Ras-Shc-Grb2* pathway (Prigent et al., 1996). Glioblastomas with *EGFR* amplification typically show a simultaneous loss of chromosome 10 (von Deimling et al., 1992a). In unselected series of glioblastomas, *EGFR* amplification was reported to occur at a frequency of approximately 30–40% (Louis and Gusella, 1995; Ohgaki et al., 1995). In a study from our laboratory, *EGFR* amplification was present in 11 (39%) of 28 of primary glioblastomas, but in 0 of 22 secondary glioblastomas (Tohma et al., 1998a). Immunoreactivity for *EGFR* also prevailed in primary glioblastomas (>60% of cases) versus secondary glioblastomas (<10%). All primary glioblastomas with *EGFR* amplification showed *EGFR* overexpression, and 11 (73%) of 15 showed *EGFR* amplification (Tohma et al., 1998a). Only 1 of 49 glioblastomas showed *EGFR* overexpression *and* a *TP53* mutation (Watanabe et al., 1996). This indicates that overexpression of *EGFR* and mutations of the *TP53* tumor suppressor gene are mutually exclusive events in the evolution of glioblastomas as the common phenotypic end point (Hayashi et al., 1997; Watanabe et al., 1996).

PDGFR

PDGF (platelet-derived growth factor), a major mitogen for connective tissue cells and glia, is a dimer composed of combinations of A and B chains. The ligands are recognized by two types of cell surface receptors, PDGFR-α and PDGFR-β, which belong to the tyrosine kinase

family of receptors (Claesson Welsh, 1994; Heldin and Westermark, 1990). *PDGFR-α* overexpression was found in both low- and high-grade astrocytomas, suggesting that PDGFR-α is involved in tumor cell proliferation in both early and late stages of glioma development. In contrast, amplification of the *PDGFR-α* gene was detected only in a small fraction (16%) of glioblastomas (Hermanson et al., 1996). Comparison of in situ hybridization for *PDGFR-α* with genetic alterations in the same tumor showed a significant correlation between high expression levels of *PDGFR-α* and LOH[3] on 17p (Hermanson et al., 1996). This suggests that amplification and overexpression of *PDGFR-α* is typical in the pathway leading to secondary glioblastomas.

LOH 10 and the PTEN (MMAC1) tumor suppressor gene

LOH on large regions at 10p, 10q23, and 10q25-26 loci or loss of an entire copy of chromosome 10 are the most frequent genetic alterations in glioblastomas (Albarosa et al., 1996; Fults and Pedone 1993; Lang et al., 1994; Louis 1997; Rasheed et al., 1995; Sonoda et al., 1996; Steck et al., 1995; von Deimling et al., 1993).

The tumor suppressor gene *PTEN* (phosphatase and tensin homolog; *MMAC1*) on chromosome 10q23.3 (Li et al., 1997; Steck et al., 1997) is mutated in approximately 30% of unselected glioblastomas (Bostrom et al., 1998; Duerr et al., 1998; Rasheed et al., 1997; Tohma et al., 1998a; Wang et al., 1997). Because mutation is not found in low-grade astrocytomas and is rare in anaplastic astrocytomas, it was initially considered a late event during astrocytoma progression. However, a recent study from our laboratory showed that *PTEN* mutations occur almost exclusively in primary (de novo) glioblastomas (32%) and rarely (4%) in secondary glioblastomas (Tohma et al., 1998a). In support of this observation, *TP53* and *PTEN* mutations in glioblastomas appear to be mutually exclusive (Rasheed et al., 1997; Tohma et al., 1998a). LOH on chromosome 10 in glioblastomas with *TP53* mutations is, therefore, likely to present tumor suppressor loci other than *PTEN*.

LOH 19q

LOH on 19q occurs in 44% of anaplastic astrocytomas and 21–24% of glioblastomas (von Deimling et al., 1992b, 1994). The somewhat lower frequency in unselected glioblastomas may suggest that this LOH is typical for the pathway leading to secondary glioblastomas, which are less frequent. The relative frequency of LOH on 19q in primary and secondary glioblastomas, however, remains to be assessed.

DCC expression

The *DCC* (deleted in colorectal cancer) gene is a candidate tumor suppressor gene located at 18q21. It encodes a 1447-amino acid transmembrane domain protein belonging to a family of neural cell adhesion molecules and is preferentially expressed in the nervous system (Fearon et al., 1990). DCC immunohistochemistry reveals that loss of expression increases during progression from low-grade astrocytoma (7%) to glioblastoma (47%) (Reyes-Mugica et al., 1997). Primary glioblastomas show loss of *DCC* expression somewhat less frequently (23%), suggesting that *DCC* inactivation is preferentially, but not exclusively, lost in the genetic pathway to secondary glioblastoma (Reyes-Mugica et al., 1997).

Necrogenesis and *FAS* Expression

Surgical pathologists judge the presence of tumor necrosis within an astrocytic neoplasm as an essential criterion for the diagnosis of a glioblastoma. Two types of necrosis are typically encountered (Burger and Scheithauer 1994; Lantos et al., 1996). One type consists of multiple small, irregularly shaped, bandlike (Zulch 1986) or serpiginous (Burger and Scheithauer, 1994) necrotic foci surrounded by radially orientated, densely packed, small, fusiform glioma cells in a pseudopalisading pattern. These areas of pseudopalisading necrosis are a histologic hallmark of glioblastomas (Lantos et al., 1996) and are observed at a similar frequency in primary and secondary glioblastomas (Tohma et al., 1998b). The second type consists of large areas of geographic necrosis containing necrotic tumor cells and vessels. In the periphery of such foci, a rim of viable tumor cells is often observed around intact capillaries. This type of necrosis is apparently a result of insufficient blood supply and is, therefore, considered ischemic in nature (Lantos et al., 1996). We observed such large necrosis in all primary glioblastomas, but only one-half of the secondary glioblastoma (Tohma et al., 1998b). This correlates well with reports indicating that the presence and extent of necrosis in glioblastomas correlates with poor clinical outcome (Barker et al., 1996; Burger and Green, 1987; Nelson et al., 1983), and the absence of necrosis is associated with younger patients and a more favorable prognosis (Barker et al., 1996; Burger and Green, 1987). Malignant gliomas with foci of ischemic necrosis easily detectable by neuroimaging are more likely to be primary glioblastomas with rapid growth and poor prognosis. This was already observed by Scherer in 1940 (1940a):

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 different clinical behavior of the two types.

Fas/APO-1 (CD95, APT1) codes for an apoptosis-mediating cell membrane protein and is predominantly expressed in glioma cells surrounding large ischemic necrosis, and thus is significantly more frequent in primary (100%) than in secondary glioblastomas (21%, $P<0.0001$), suggesting that these subtypes of glioblastoma differ both in extent and mechanism of necrogenesis (Tohma et al., 1998b).

Age and Sex Distribution

In unselected series of glioblastomas, lesions with *TP53* mutations occurred relatively more frequently in women and in younger patients (Louis et al., 1993; Rasheed et al., 1994; von Deimling et al., 1993). Primary glioblas-

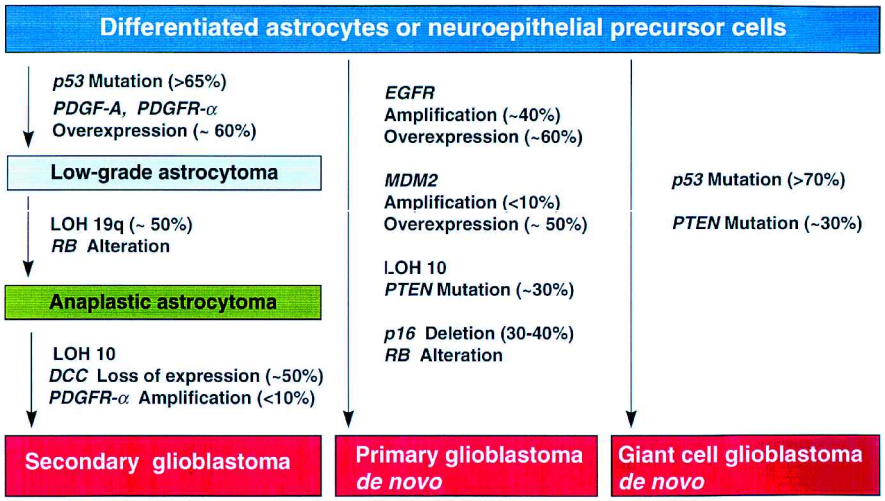

Fig. 1 Genetic pathways operative in the evolution of glioblastomas.

tomas tend to develop in older patients (mean, 50-55 years), while patients with secondary glioblastoma are typically diagnosed at around age 40 (Dropcho and Soong, 1996; Watanabe et al., 1996). We have noticed a trend indicating that the men/women ratio is higher in primary than in secondary glioblastomas (von Deimling et al., 1993; Watanabe et al., 1996).

Dynamics of Glioma Evolution

Primary glioblastoma is characterized by a very rapid development of clinical symptoms. Approximately 50% of glioblastoma patients have a history of less than 3 months, and typically present with large tumors that show central necrosis and extensive perifocal edema on MRI. Sequential neuroimaging that includes early lesions is, therefore, rarely carried out. In such cases, small cortical lesions of ~1 cm in diameter have been documented to develop into large full-blown glioblastomas within 2–3 months (P.C. Burger, personal communication). To define the genetics of primary glioblastomas unambiguously, we analyzed biopsies in our laboratory from patients with a clinical history of less than 3 months (Biernat et al., 1997a, 1997b; Kleihues and Ohgaki, 1997; Tohma et al., 1998a; Watanabe et al., 1996). Thus, the possibility cannot be excluded that glioblastomas with similar histologic and genetic alterations develop more slowly.

Anaplastic astrocytomas are considered to be a lesion of intermediate malignancy in the progression of diffuse low-grade astrocytomas to glioblastomas. This assumption is based on clinical and histologic observations and is fully supported by recent data on the sequential acquisition of genetic alterations (Fig. 1). However, an argument for the existence of an alternative pathway circumventing the anaplastic astrocytoma has also been proposed (van Meyel et al., 1994). Progression of low-grade astrocytoma to anaplastic astrocytoma or

Table 1. Synopsis of clinical and genetic data of primary and secondary glioblastomas

	Primary glioblastoma	Secondary glioblastoma
Clinical onset	de novo	secondary
Preoperative clinical history	1.7 months (Watanabe et al., 1996)	53 months from low-grade astrocytoma (Watanabe et al., 1996)
		25 months from anaplastic astrocytoma (Watanabe et al., 1996)
Sex ratio (M/W)	1.4 (Watanabe et al., 1996)	0.8 (Watanabe et al., 1996)
Age at diagnosis (years)	55 (Watanabe et al., 1996)	39 (Watanabe et al., 1996)
	56[a] (von Deimling et al., 1993)	41[b] (von Deimling et al., 1993)
p53 mutation	2/19 (11%) (Watanabe et al., 1996)	20/30 (67%) (Watanabe et al., 1996)
MDM2 amplification	2/29 (7%) (Biernat et al., 1997a)	0/27 (0%) (Biernat et al., 1997a)
	6/75[c] (8%) (Reifenberger et al., 1993)	
EGFR amplification	11/28 (39%) (Tohma et al., 1998)	0/22 (0%) (Tohma et al., 1998)
p16 deletion	10/28 (36%) (Biernat et al., 1997b)	1/23 (4%) (Biernat et al., 1997b)
PTEN mutation	9/28 (32%) (Tohma et al., 1998)	1/25 (4%) (Tohma et al., 1998)

[a]Tumors with LOH on 17p but without *EGFR* amplification (possibly secondary glioblastomas).

[b]Tumors with *EGFR* amplification but no LOH on 17p (likely to be primary glioblastomas).

[c]None of the tumors with *MDM2* amplification contained a p53 mutation.

glioblastoma occurs at a similar frequency in lesions with (79%) and without (63%) *TP53* mutations, indicating that this genetic alteration is associated with tumor recurrence, but not predictive of progression to a more malignant phenotype (Watanabe et al., 1997).

The course of progression varies considerably, with time intervals ranging from less than 1 year to more than 10 years.[4] In our series of secondary glioblastomas, the mean time for progression from low-grade astrocytoma was 55 months (Watanabe et al., 1997). The time until progression to anaplastic astrocytoma or glioblastoma was somewhat shorter for low-grade astrocytomas carrying a *TP53* mutation (Watanabe et al., 1997).

Data on the survival of patients with primary and secondary glioblastomas are still scarce. For Scherer (1940a) (see quotation above), a more favorable clinical outcome is one of the characteristics of secondary glioblastoma, and this is supported by a study of 188 glioblastomas that showed a significantly longer survival for patients with prior low-grade glioma; the median survival being 40.5 months (Winger et al., 1989). In contrast, a recent clinical epidemiology study showed no significant difference in prognosis (al Sarraj and Bridges, 1995; Dropcho and Soong, 1996). More survival data are needed for a definitive judgment on clinical outcome and response to therapy of both subsets of glioblastomas.

Brain Tumor Classification: Phenotype Versus Genotype

The genetic pathways leading to the evolution of primary and secondary glioblastoma are shown in Fig. 1. Despite some overlapping, the pathogenesis of both subtypes of glioblastomas is quite different, suggesting that they constitute different diseases on the basis of both their clinical and genetic profiles. The most frequent and characteristic genetic alterations in primary glioblastomas are *EGFR* amplification/overexpression, *CDKN2A* deletion, and *PTEN* mutations. The gatekeeper lesions (Kinzler and Vogelstein, 1998) for secondary glioblastomas are *TP53* mutations, since there is increasing evidence that a mutational inactivation of this suppressor gene is the initiating event in the majority of diffuse low-grade astrocytomas.

Note that the data in Table 1 were generated mostly from cohorts of patients carefully selected on the basis of clinical history and, in the case of secondary glioblastoma, multiple biopsies. The genetic data derived from these patients do not by themselves allow the distinction between glioblastoma subtypes. Glioblastoma biopsies from patients with histologically proven progression from low-grade astrocytoma typically contain a *TP53* mutation, but this does not allow the conclusion that *all* glioblastomas with a *TP53* mutation have progressed from a prior low-grade glioma. Similarly, primary glioblastomas with a very short clinical history typically contain an *EGFR* amplification/overexpression, but there are no data showing that *all* glioblastomas with this genetic alteration have developed de novo. More subtypes of glioblastoma may exist with intermediate clinical and genetic profiles. This is exemplified by the giant-cell glioblastoma, a histologically distinct variant that occupies a hybrid position, sharing with primary (de novo) glioblastomas a short clinical history, the absence of a less malignant precursor lesion, and a 30% frequency of *PTEN* mutations. With secondary glioblastomas derived from malignant progression of low-grade astrocytomas, they have in common a younger patient age at manifestation and a high frequency (>70%) of *TP53* mutations (Meyer-Puttlitz et al., 1997; Peraud et al., 1997).[5] Thus, the currently available data are insufficient for a substitution of histological classification and grading by genetic typing (Newcomb et al., 1998). Instead, the aim should be the identification of criteria for a combined clinical, histologic, and genetic profiling of astrocytic tumors.

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