The American Journal of Pathology, Vol. 170, No. 5, May 2007

DOI: 10.2353/ajpath.2007.070011

Review

Genetic Pathways to Primary and Secondary Glioblastoma

Hiroko Ohgaki* and Paul Kleihues†

From the International Agency for Research on Cancer, Lyon, France; and the Department of Pathology,† University of Zurich, Zurich, Switzerland*

Glioblastoma is the most frequent and most malignant human brain tumor. The prognosis remains very poor, with most patients dying within 1 year after diagnosis. Primary and secondary glioblastoma constitute distinct disease subtypes, affecting patients of different age and developing through different genetic pathways. The majority of cases (>90%) are primary glioblastomas that develop rapidly *de novo*, without clinical or histological evidence of a less malignant precursor lesion. They affect mainly the elderly and are genetically characterized by loss of heterozygosity 10q (70% of cases), *EGFR* amplification (36%), *p16*^{INK4a} deletion (31%), and *PTEN* mutations (25%). Secondary glioblastomas develop through progression from low-grade diffuse astrocytoma or anaplastic astrocytoma and manifest in younger patients. In the pathway to secondary glioblastoma, *TP53* mutations are the most frequent and earliest detectable genetic alteration, already present in 60% of precursor low-grade astrocytomas. The mutation pattern is characterized by frequent G:C→A:T mutations at CpG sites. During progression to glioblastoma, additional mutations accumulate, including loss of heterozygosity 10q25-qter (~70%), which is the most frequent genetic alteration in both primary and secondary glioblastomas. Primary and secondary glioblastomas also differ significantly in their pattern of promoter methylation and in expression profiles at RNA and protein levels. This has significant implications, particularly for the development of novel, targeted therapies, as discussed in this review. *(Am J Pathol 2007, 170:1445–1453; DOI: 10.2353/ajpath.2007.070011)*

The distinction of primary and secondary glioblastoma was first made by the German neuropathologist Hans-Joachim Scherer.[1] In 1940, while working as a political refugee at the Institute Bunge in Antwerp (Belgium), he wrote, "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."[2] This was a remarkable observation at that time; as late as 1979, the World Health Organization (WHO) did not consider the glioblastoma as an astrocytic tumor, listing it instead in a group of poorly differentiated and embryonal tumors.[3] With the introduction of immunohistochemistry, the glioblastoma was firmly categorized as astrocytic neoplasm,[4] but the separation of primary and secondary glioblastoma remained conceptual, without being used as diagnostic terms largely because these subtypes are considered histopathologically indistinguishable. During the past decade, evidence accumulated that they constitute distinct disease entities that affect patients of different age, develop through different genetic pathways,[5,6] show different RNA and protein expression profiles,[7–9] and may differ in their response to radio- and chemotherapy.

Epidemiology and Clinical Features

Glioblastoma (WHO grade IV) is the most frequent histological type of brain tumor, accounting for 69% of all incident cases of astrocytic and oligodendroglial tumors, with 3.55 new cases in Switzerland per 100,000 population per year, adjusted to the European Standard Population.[5,6] The incidence rate of glioblastomas in the United States, adjusted to the United States Standard Population, is 2.96 new cases per 100,000 population per year [Central Brain Tumor Registry of the United States: Central Brain Tumor Registry of the United States (CBTRUS); *http://www.cbtrus.org*]. These incident cases do not include secondary glioblastomas that progressed from low-grade or anaplastic gliomas because only the first diagnosis is considered as an incident case.

Primary glioblastomas present at diagnosis as full-blown tumors, without clinical, radiological, or histopatho-

Accepted for publication January 30, 2007.

Address reprint requests to Dr. Hiroko Ohgaki, International Agency for Research on Cancer, 150 cours Albert Thomas, 69372 Lyon Cedex 08, France. E-mail: ohgaki@iarc.fr.

logical evidence of a less-malignant precursor lesion. They are also termed *de novo* glioblastoma, but this does not suggest a single-step transformation; like other human neoplasms, they result from the acquisition of multiple genetic alterations. Secondary glioblastomas develop slowly through progression from low-grade diffuse astrocytoma (WHO grade II) or anaplastic astrocytoma (WHO grade III). The diagnosis of secondary glioblastoma requires clinical (neuroimaging) or histological (bioptic) evidence of an evolution from a less malignant astrocytoma.

At a population level, we found that only 5% of all cases were secondary glioblastomas with histopathological evidence of a precursor low-grade or anaplastic astrocytoma.[5,6] This is consistent with observations at the University of Alabama, in which 19 of 392 (5%) cases of glioblastomas had histologically proven prior low-grade gliomas.[10] The incidence rate of low-grade and anaplastic astrocytomas is approximately two to three times higher than that of secondary glioblastoma[11,12] (CBTRUS; *http://www.cbtrus.org*). This may be explained at least in part by the fact that a significant fraction of patients with low-grade or anaplastic astrocytoma succumb to the disease before progression to glioblastoma occurs. Some cases with very rapid progression from low-grade or anaplastic astrocytoma may have been misclassified as primary glioblastoma. Even when taking into account this possibility, secondary glioblastomas constitute a relatively rare disease when compared with primary glioblastomas.

At the population level, the majority of patients with primary glioblastomas (68%) had a clinical history of less than 3 months. The mean period from first symptoms to histological diagnosis was 6.3 months (Table 1).[5,6] The mean time to progression from anaplastic glioma to glioblastoma was ~2 years, and that from low-grade glioma to glioblastoma was ~5 years (Table 1).[6]

There is a striking difference in the age distribution of patients with primary and secondary glioblastomas (Figure 1). The mean age of primary glioblastoma patients was 62 years, whereas secondary glioblastomas developed in younger patients (45 years).[5,6] At a population level, primary glioblastomas developed more frequently in men (male to female ratio, 1:33), whereas secondary glioblastomas were more frequent in women (male to female ratio, 0:65).[5] A review of several studies also showed a tendency toward a higher male to female ratio in primary than secondary glioblastomas.[7,13–16] This corroborates a previous observation that glioblastomas with *TP53* mutations (a genetic hallmark of secondary glioblastoma) are more common in women.[17] This is surprising because in hospital-based[18,19] and population-based studies[11] (CBTRUS; *http://www.cbtrus.org*), the incidence of precursor low-grade or anaplastic gliomas was reported to be similar to or higher in males than in females. The possibility exists that in female patients gliomas progress more frequently or more rapidly to glioblastoma.

The median survival of secondary glioblastoma patients was 7.8 months, significantly longer than that of primary glioblastoma patients (4.7 months; $P = 0.003$). However, this difference is considered largely due to the younger age of secondary glioblastoma patients, because younger age is a consistent and significant predictive factor of longer survival of glioblastoma patients.[5,6] After age adjustment, multivariate analyses showed no significant difference in survival of patients with primary and secondary glioblastomas.[5,6]

Genetic Pathways to Primary and Secondary Glioblastoma

Genetic pathways to primary and secondary glioblastomas at a population level are summarized in Figure 2. LOH 10q is most frequent in both primary and secondary glioblastomas. Epidermal growth factor receptor (*EGFR*) amplification and *PTEN* mutations are genetic alterations typical of primary glioblastomas, whereas *TP53* mutations are early and frequent genetic alterations in the pathway leading to secondary glioblastomas.

EGFR/PTEN/Akt/mTOR Pathway

The EGFR/PTEN/Akt/mTOR pathway is a key signaling pathway in the development of primary glioblastomas.[20] Amplification of the *EGFR* occurs in ~40% of primary glioblastomas[5,21] but rarely in secondary glioblastomas.[5,15] *EGFR* overexpression is also more common in primary glioblastomas (>60%) than in secondary glioblastomas (<10%).[15] All primary glioblastomas with *EGFR* amplification show *EGFR* overexpression, and 70 to 90% of those with *EGFR* overexpression have *EGFR* amplification.[22,23] The age distribution of cases with *EGFR* amplification closely follows that of primary glioblastoma patients. In a large, population-based study, *EGFR* amplification was not detected in any glioblastoma from patients younger than 35 years of age (Figure 1).[5] This confirms studies on pediatric glioblastomas in which *EGFR* amplification is either absent or very rare.[24] *EGFR* amplicons are often mutated; variant 3 (*EGFRvIII*) with deletion of exons 2 to 7 is the most frequent type. It is associated with constitutive activation of the receptor and failure to attenuate signaling by receptor down-regulation, causes mitogenic effects, and has more powerful transforming activity.[25] The constitutively active *EGFRvIII* can enhance cell proliferation in part by down-regulation of p27 through activation of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway.[26] This variant occurs only in glioblastomas with concurrent wild-type *EGFR* amplification,[21] ie, primary glioblastomas (Table 1).

EGFR becomes activated through the binding of growth factors (epidermal growth factor, transforming growth factor-α) to its extracellular domain, resulting in recruitment of PI3K to the cell membrane (Figure 3). PI3K phosphorylates phosphatidynositol-4,5-bisphosphate to the respective 3-phosphate (PIP3), which activates downstream effector molecules such as AKT (protein kinase B) and mTOR, the mammalian target of rapamy-

Table 1. Incidence, Age, Survival, and Genetic, Epigenetic Changes, and Expression Profiles in Primary and Secondary Glioblastomas

	Primary glioblastoma	Secondary glioblastoma	References
Incidence rate*	3.531	0.199	
Incidence rate†	2.575	0.167	
Mean age	62 years	45 years	6
M/F ratio	1.33	0.65	6
Clinical history	<3 months: 68% 3 to 6 months: 16% >6 months: 16% (mean, 6.3 months)	From grade II: 5.3 years From grade III: 1.4 years	6
Survival	Median, 4.7 months	Median, 7.8 months	5
Genetic alterations			
TP53 mutations	28%	**65%**	5
EGFR amplification	**36%**	8%	5
PTEN mutations	**25%**	4%	5
$p16^{INK4a}$ deletion	31%	19%	5
LOH 1p	12%	15%	67
LOH 10p	**47%**	8%	54
LOH 10q	47%	54%	54
	70%	63%	5
LOH 13q	12%	38%	67
LOH 19q	6%	**54%**	67
LOH 22q	41%	**82%**	66
Promoter methylation			
$p14^{ARF}$	6%	**31%**	38
$p16^{INK4a}$	3%	19%	38
RB1	14%	**43%**	53
MGMT	36%	**75%**	78
TIMP-3	28%	**71%**	66
Expression profiles			
Fas (APO-1/CD95)[a]	**100%**	21%	71
Survivin[a]	**83%**	46%	13
MMP-9[a]	**69%**	14%	14
EGFR[a]	**63%**	10%	15
EGFR[b]	High	Low	8
MDM2[a]	**31%**	0%	37
VEGF[c]	High	Low	16
VEGF fms-related tyrosine kinase 1[d]	High	Low	7
IGFBP2[d]	High	Low	7
Tenascin-X-precursor[b]	High	Low	8
Enolase 1[b]	High	Low	8
Centrosome-associated protein 350[b]	High	Low	8
TP53[a]	37%	**97%**	15
ASCL1[e]	33%	**88%**	85
Loss of TIMP-3[a]	17%	**64%**	66
PDGF-AB[c]	Low	High	16
ERCC6[b]	Low	High	8
DUOX2[b]	Low	High	8
HNRPA3[b]	Low	High	8
WNT-11 protein precursor[b]	Low	High	8
Cadherin-related tumor suppressor homolog precursor[b]	Low	High	8
ADAMTS-19[b]	Low	High	8

M/F, male/female; PDGF-AB, platelet-derived growth factor AB.
Bold indicates significantly more frequent than the other glioblastoma subtype.
*Adjusted to the European Standard Population (per 100,000 persons per year).
†Adjusted to the World Standard Population (per 100,000 persons per year).
[a]Immunohistochemistry, [b]2-DGE, [c]enzyme-linked immunosorbent assay, [d]cDNA array, [e]reverse transcriptase-polymerase chain reaction.

cin; this results in cell proliferation and increased cell survival by blocking apoptosis. PTEN inhibits the PIP3 signal,[27] thereby inhibiting cell proliferation (Figure 3). Response to EGFR kinase inhibitors requires coexpression of EGFRvIII and PTEN.[27]

The *PTEN* (phosphatase and tensin homology) gene, located at 10q23.3,[28,29] encodes a central domain with homology to the catalytic region of protein tyrosine phosphatases, which is important in the function of protein phosphatase[30] and 3′-phosphoinositol phosphatase activities.[31] The amino terminal domain of PTEN, with homology to tensin and auxilin, is important in regulating cell migration and invasion by directly dephosphorylating focal adhesion kinase.[32] The *PTEN*

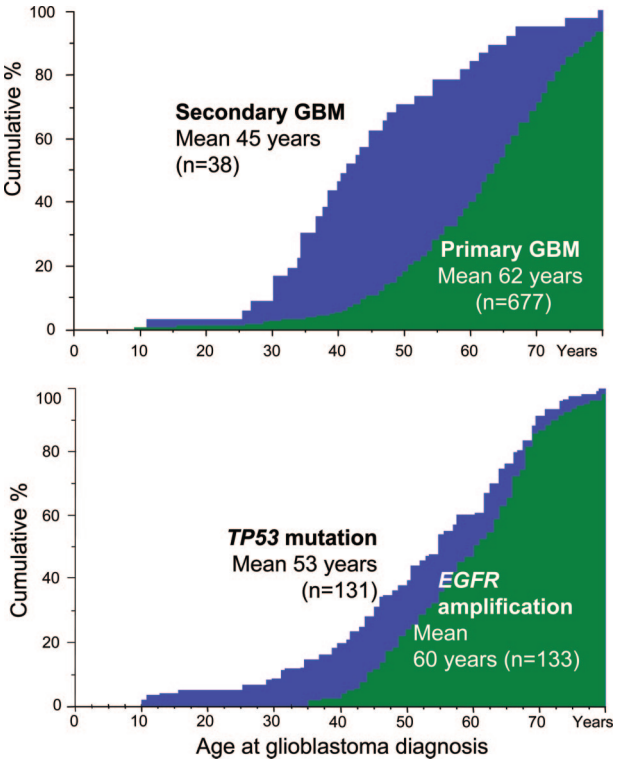

Figure 1. Secondary glioblastomas develop in younger patients than primary glioblastomas. *TP53* mutations occur in patients of any age group, whereas *EGFR* amplification occurs in older patients. Note that there is no single case of glioblastoma with *EGFR* amplification in patients younger than 35 years of age. (Modified from Ohgaki et al[5]).

gene is mutated in 15 to 40% of glioblastomas[33,34] and almost exclusively in primary glioblastomas (Table 1).[5,22]

TP53/MDM2/p14^{ARF} Pathway

The TP53 pathway plays a crucial role in the development of secondary glioblastomas. *TP53* mutations are the first detectable genetic alteration in two-thirds of precursor low-grade diffuse astrocytomas; this frequency is similar to that in anaplastic astrocytomas and secondary glioblastomas derived thereof (Figure 2).[5,15,35] *TP53* mutations also occur in primary glioblastomas, but at a lower frequency (<30% of cases)[5] (Figure 2).

In secondary glioblastomas, 57% of mutations have been reported to be located in the two hotspot codons 248 and 273; however, in primary glioblastomas, mutations were more equally distributed through all exons, with only 17% occurring in codons 248 and 273.[5] Furthermore, G:C→A:T transitions at CpG sites, considered to result from deamination of 5-meC, were significantly more frequent in secondary than in primary glioblastomas.[5] Thus, G:C→A:T mutations at CpG sites, particularly in the hotspot codons 248 and 273, seem to be an early event directly associated with malignant transformation in the pathway to secondary glioblastoma. The less specific pattern of *TP53* mutations in primary glioblastomas may constitute, at least in part, secondary events due to increasing genomic instability during tumor development.

Amplification of *MDM2* is present in <10% of glioblastomas,[36] exclusively in primary glioblastomas that lack a *TP53* mutation.[36,37] Loss of p14^{ARF} expression has frequently been observed in glioblastomas (76%), and this typically correlates with homozygous deletion or promoter methylation of the *p14^{ARF}* gene.[38] Promoter methylation of *p14^{ARF}* was more frequent in secondary than primary glioblastomas, but there was no significant difference in the overall frequency of *p14^{ARF}* alterations (homozygous deletion and promoter methylation) between glioblastoma subtypes.[38] The analysis of multiple biopsies from the same patients revealed *p14^{ARF}* methylation already in one-third of precursor low-grade astrocytomas.[38]

The *TP53* gene at 17p13.1 encodes a 53-kd protein that plays a role in several cellular processes (Figure 3), including the cell cycle, response of cells to DNA damage, cell death, cell differentiation, and neovascularization.[39] After DNA damage, TP53 is activated and induces transcription of genes such as *p21$^{Waf1/Cip1}$*.[40,41] The MDM2 binds to mutant and wild-type TP53 proteins, thereby inhibiting the ability of wild-type TP53 to activate transcription from minimal promoter sequences.[42,43] Conversely, transcription of the *MDM2* gene is induced by wild-type TP53.[44,45] In normal cells, this autoregulatory feedback loop regulates both the activity of the TP53 protein and the expression of MDM2.[46] The *p14^{ARF}* gene product binds to MDM2 and inhibits MDM2-mediated p53 degradation and transactivational silencing.[47–51] Conversely, p14^{ARF} expression is negatively regulated by TP53 and inversely correlates with TP53 function in human tumor cell lines.[41] Thus, loss of normal TP53 function may result from altered expression of any of the *TP53*, *MDM2*, or *p14^{ARF}* genes (Figure 3).

p16^{INK4a}/RB1 Pathway

The p16^{INK4a}/RB1 pathway seems to be important in pathways to both primary and secondary glioblastomas. Homozygous *p16^{INK4a}* deletions were more frequent in primary than in secondary glioblastomas,[38,52] but there was no significant difference in the overall frequency of *p16^{INK4a}* alterations (homozygous deletion and promoter methylation).[38] Promoter methylation of the *RB1* gene was significantly more frequent in secondary (43%) than in primary glioblastomas (14%).[53] There was a significant correlation between loss of RB1 expression and promoter methylation of the *RB1* gene in glioblastomas.[53] *RB1* promoter methylation was not detected in low-grade and anaplastic astrocytoma, indicating that it is a late event during astrocytoma progression.[53]

RB1 protein controls progression through G_1 to S phase of the cell cycle. The CDK4/cyclin D1 complex phosphorylates the RB1 protein, thereby inducing release of the E2F transcription factor that activates genes involved in the G_1→S transition.[40] p16^{INK4a} binds to CDK4, inhibits the CDK4/cyclin D1 complex, and thus inhibits the G_1→S transition.[40] Thus, loss of normal RB1 function may result from altered expression of any of the *RB1*, *p16^{INK4a}*, or *CDK4* genes (Figure 3).

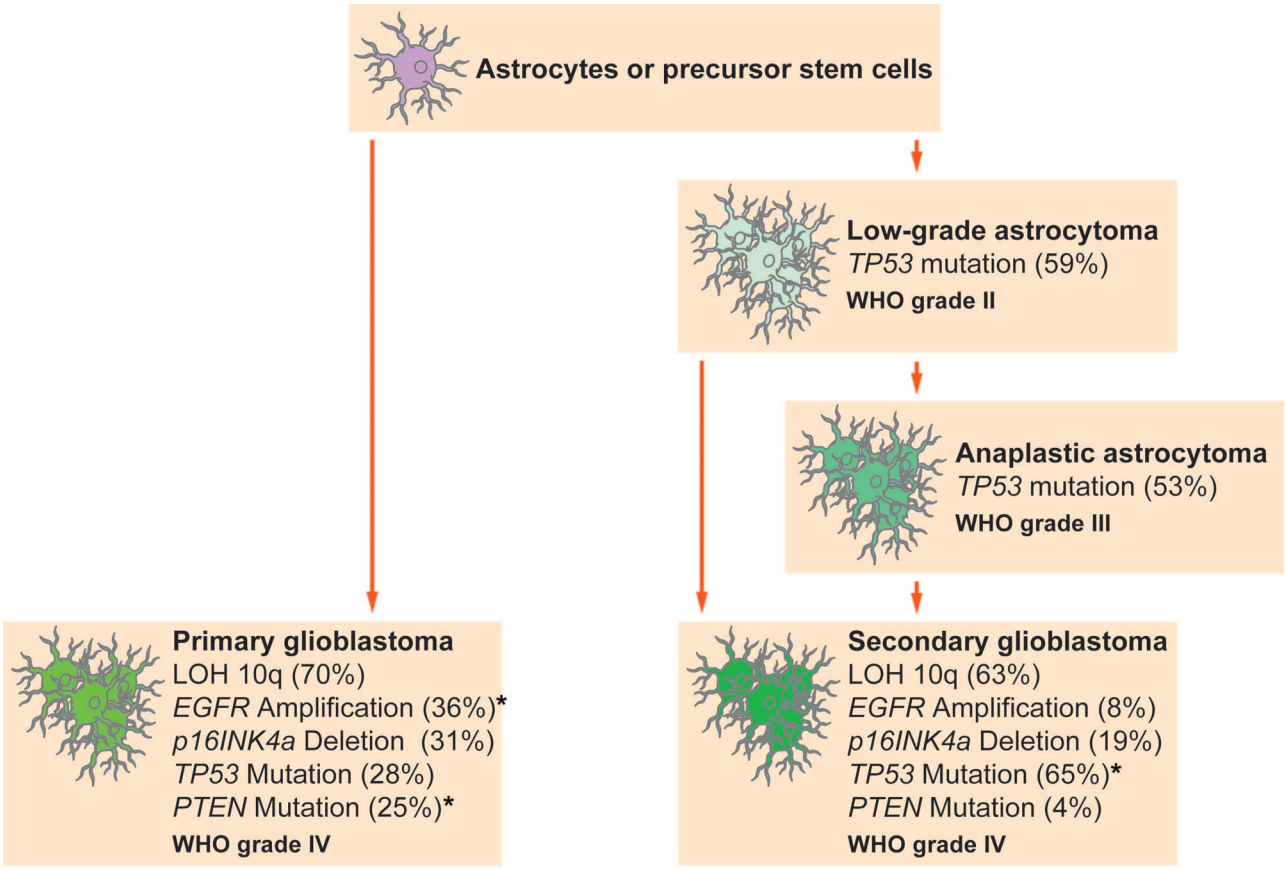

Figure 2. Genetic pathways to primary (*de novo*) and secondary glioblastomas at the population level (Modified from Ohgaki et al[5]). LOH 10q is frequent in both primary and secondary glioblastomas. *TP53* mutations are early and frequent genetic alterations in the pathway leading to secondary glioblastomas. *Genetic alterations that are significantly different in frequency between primary and secondary glioblastomas.

Loss of Heterozygosity (LOH)

LOH 10q is the most frequent genetic alteration occurring in both primary and secondary glioblastomas at similar frequencies (60 to 80%),[5,54–57] with a common deletion at 10q25-qter. In contrast, LOH 10p is largely exclusively present in primary glioblastomas,[54] and complete loss of the entire chromosome 10 is typical for primary glioblastomas. Several LOH studies identified at least three commonly deleted loci, ie, 10p14-p15, 10q23-24 (PTEN), and 10q25-pter, suggesting the presence of several tumor-suppressor genes that may play significant roles in the pathogenesis of glioblastomas.[55–57] Because LOH 10q25-qter is associated with histologically recognized transition from low-grade or anaplastic astrocytoma to glioblastoma phenotypes[58] and is commonly deleted in primary and secondary glioblastomas[5,6] (Figure 2), the tumor suppressor gene(s) at these loci seem to be involved in the pathogenesis of both glioblastoma subtypes. This locus contains several putative tumor suppressor genes, including *LGl1* at 10q24,[59] *BUB3* at 10q24-q26,[60] *MXI1* at 10q25.1,[61] *h-neu* at 10q25.1,[62] *abLIM* or LIMAB1 at 10q25.1,[63,64] and *DMBT1* at 10q26.1[65] genes, but their role remains unclear because mutations of these genes have been rarely detected in glioblastomas.

LOH 22q is significantly more frequent in secondary glioblastomas (82%) than in primary glioblastomas (41%).[66] Characterization of the 22q deletions in primary glioblastomas identified two minimally deleted regions at 22q12.3-13.2 and 22q13.31. The small (957 kb) deletion was also present in 22 of 23 secondary glioblastomas, a region in which the human tissue inhibitor of metalloproteinases-3 (*TIMP-3*) is located. *TIMP-3* promoter methylation was observed with significantly higher frequency in secondary than in primary glioblastomas and correlated with loss of TIMP-3 expression. LOH 19q is more frequent in secondary glioblastomas (54%) than primary glioblastomas (6%),[67] whereas LOH 1p and 13q occurred at a similar frequency in primary and secondary glioblastomas (Table 1).[67]

Co-Presence of Genetic Alterations

LOH 10q typically co-presents with any of the other genetic alterations in glioblastomas.[5] In contrast, *TP53* mutations, *EGFR* amplification, and *PTEN* mutations show inverse associations with each other,[5] whereas there is a positive correlation between $p16^{INK4a}$ deletion and *EGFR* amplification.[5,68,69] These observations suggest that LOH 10q plus at least one or two other genetic alterations

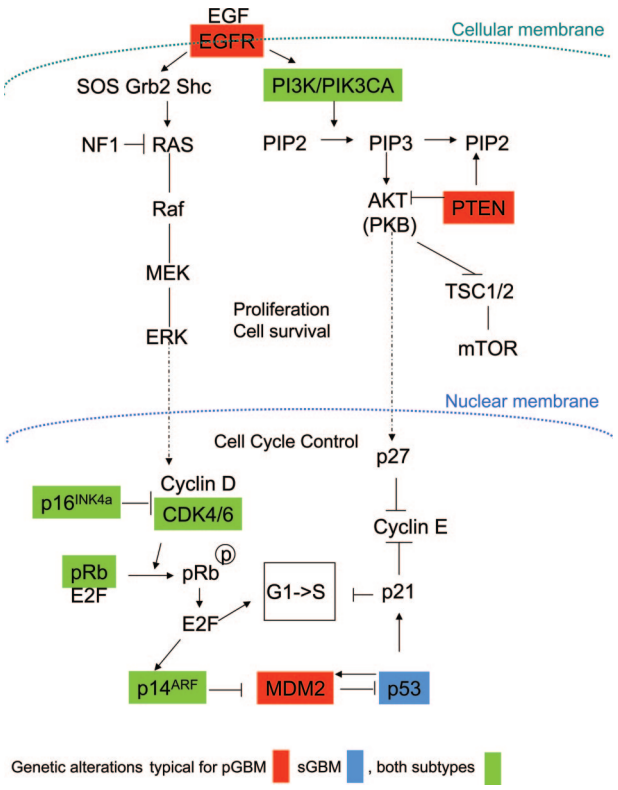

Figure 3. Major signaling pathways involved in the pathogenesis of glioblastomas.

may contribute to the development of both primary and secondary glioblastomas. In the pathway to secondary glioblastoma, *TP53* mutations occur early, and LOH 10q is a late event (Figure 2). However, the sequence of genetic alterations during the development of primary glioblastomas is unknown.

Correlation between Genetic Alterations and Histopathological Features

Glioblastoma is heterogeneous, with remarkable variability of histological features.[18] Some of these tend to be associated with glioblastoma subtypes. The small cell phenotype is often associated with *EGFR* amplification and is thus a typical component of primary glioblastomas.[70] Tumor necrosis, in particular large ischemic necroses, are significantly more frequent in primary glioblastomas,[71,72] whereas secondary glioblastomas more frequently contain an oligodendroglioma component.[72]

Promoter Methylation

Promoter methylation of *p16^{INK4a}*, *p14^{ARF}*, *RB1*, *TIMP-3*, and *MGMT* genes has been comparatively assessed in primary and secondary glioblastomas. Overall, secondary glioblastomas showed a higher frequency of promoter methylation than primary glioblastomas (Table 1). O^6-Methylguanine-DNA methyltransferase (MGMT) is a repair protein that specifically removes promutagenic alkyl groups from the O^6 position of guanine in DNA. MGMT therefore protects cells against carcinogenesis induced by alkylating agents, and an inverse correlation has been reported between MGMT activity and tissue-specific tumorigenesis induced by alkylating agents in rats.[73,74] Repair of O^6-alkylguanine adducts by tumor cells has been implicated in drug resistance because it reduces the cytotoxicity of alkylating chemotherapeutic agents.[75] Loss of *MGMT* expression caused by methylation of promoter CpG islands[76,77] was detected in 75% of secondary glioblastomas, significantly more frequently than in primary glioblastomas (36%).[78] The difference in frequency of *MGMT* methylation between primary and secondary glioblastomas is clinically relevant because patients with glioblastoma containing a methylated *MGMT* promoter were shown to have a substantially greater benefit from adjuvant temozolomide treatment.[79]

A correlation between the presence of *TP53* mutations and *MGMT* promoter methylation has been reported in lung cancer[80] and between the presences of G→A mutations in the K-*ras* gene in colon carcinomas.[81] Similarly, the majority of low-grade astrocytomas with *MGMT* methylation (92%) contained a *TP53* mutation, whereas only 39% of cases without *MGMT* methylation carried a *TP53* mutation.[78] Furthermore, G:C→A:T transition mutations at CpG sites were significantly more frequent in low-grade astrocytomas with *MGMT* methylation (58%) than in those without (11%).[78] These findings suggest the possibility that *TP53* mutations at CpG sites in low-grade gliomas may result, at least in part, from exogenous or endogenous factors that produce DNA adducts at the O^6 position of guanine. Various adducts at this position are substrates for repair by MGMT.[82] Such adducts typically result from exposure to *N*-nitrosamides and related alkylating agents that cause brain tumors in rats,[83] but there is currently no evidence indicating that alkylating carcinogens are involved in the etiology of human brain tumors.

Gene Expression and Proteomic Profiles

Different genetic alterations in primary and secondary glioblastomas reflect different expression patterns at the RNA and protein levels (Table 1). In a cDNA expression array analysis with 1176 cancer-related genes, low-grade astrocytomas showed rather specific and similar expression profiles, whereas primary glioblastomas showed larger variation, and secondary glioblastomas displayed features of both groups.[7] Prominent genes expressed at a significantly higher level in primary than in secondary glioblastomas include vascular endothelial growth factor (VEGF) fms-related tyrosine kinase 1 (involved in angiogenesis) and IGFBP2.[7] Because VEGF is induced by hypoxia-inducible factor (HIF-1), this difference explains the higher frequency and greater extent of necrosis in primary glioblastomas. Using an array containing 14,500 genes, Tso and colleagues[9] showed that secondary glioblastomas primarily include mitotic cell cycle components, suggesting the loss of function in prominent cell cycle regulators, whereas primary glioblastomas preferentially express genes typical of a stromal response,

suggesting the importance of extracellular signaling. Immunohistochemical staining of glioblastoma tissue arrays confirmed expression differences.[9]

EGFR and MDM2 overexpression detected by immunohistochemistry are frequent and typical in primary glioblastomas.[15,37] Immunohistochemistry revealed active matrix metalloproteinase-9 (MMP-9) in 69% of primary glioblastomas but only 14% of secondary glioblastomas.[14] Active MMP-9 expression was strongly correlated with EGFRvIII expression,[14] which is also typical for primary glioblastomas.[21] Survivin (located on 17q25), which was initially identified as a gene with structural homology to a family of genes known as inhibitors of apoptosis,[84] is expressed significantly more frequently in primary (83%) than in secondary glioblastomas (46%).[13] Somasundaram and colleagues[85] reported that ASCL1 is overexpressed in 86% of grade II diffuse astrocytomas and 88% of secondary glioblastomas, whereas the majority (67%) of primary glioblastomas expressed similar to or less than normal brain levels. ASCL1 up-regulation was accompanied by inhibition of Notch signaling as seen by uninduced levels of HES1, a transcriptional target of Notch1, and increased levels of HES6, a dominant-negative inhibitor of HES1-mediated repression of ASCL1.[85] Increased levels of the Notch ligand Delta1 inhibits Notch signaling via formation of intracellular Notch ligand autonomous complexes, suggesting that inhibition of Notch signaling may be an important early event in the pathway to secondary glioblastomas.[85]

A recent proteomics approach identified additional proteins that are differentially expressed between glioblastoma subtypes (Table 1). Using two-dimensional protein gel electrophoresis (2-DGE) and protein sequencing, Furuta and colleagues[8] identified distinct protein patterns in primary and secondary glioblastomas. Proteins unique for primary glioblastomas were tenascin-X precursor (6q21.3), enolase 1 (1pter-p36.13), centrosome-associated protein 350 (1p36.13-q41), and EGFR (7p12.3-p12.1), whereas those unique for secondary glioblastomas were ERCC6 (10q11), DUOX2 (15q15.3), HNRPA3 (10q11.1), WNT-11 protein precursor (11q13.5), cadherin-related tumor suppressor homolog precursor (chromosomal location unknown), and ADAMTS-19 (5q31).[8] Quantification of the cytokines in the supernatant of 30 tissue-correspondent glioma cultures revealed a predominant expression of VEGF-A in primary glioblastomas and a significantly higher expression level of platelet-derived growth factor AB in secondary glioblastomas.[25] This suggests that optimal anti-angiogenic therapy may require targeting of multiple angiogenic pathways that differ significantly between primary and secondary glioblastomas.[16]

Conclusions

Primary and secondary glioblastomas are distinct disease entities that affect different age groups of patients and develop through distinct genetic pathways with different mRNA and protein expression profiles. These differences are important, especially because they may affect sensitivity to radio- and chemotherapy and should thus be considered in the identification of targets for novel therapeutic approaches.

Future Directions

There are several chromosomal regions with LOH that probably contain tumor suppressor genes that need to be identified. The most common and extensive deletions involve chromosome 10q, distal of *PTEN*. It has been suggested that 10q25-qter harbors a putative tumor suppressor that may play a key role in the development of both primary and secondary glioblastomas. Glioblastoma is the first cancer type in The Cancer Genome Atlas project initiated by the United States National Cancer Institute and the National Human Genome Research Institute, which aims at establishing a database of high-resolution expression profiles, LOH, chromosome copy numbers, and sequence alterations in a total of 500 cases. If both primary and secondary glioblastomas were included, this would greatly increase our understanding of their molecular basis and facilitate the development of drugs that specifically target tumor subtypes with divergent genetic profiles.

Acknowledgments

We thank the Foundation for Promotion of Cancer Research, Japan, and the Cancer League of the Canton of Zurich, Switzerland, for their support of population-based studies on glioma patients.

References

1. Peiffer J, Kleihues P: Hans-Joachim Scherer (1906–1945), pioneer in glioma research. Brain Pathol 1999, 9:241–245
2. Scherer HJ: Cerebral astrocytomas and their derivatives. Am J Cancer 1940, 40:159–198
3. Zulch KJ (Ed): Histological typing of tumours of the central nervous system. International Histological Classification of Tumours, vol. 21. Geneva, World Health Organization, 1979
4. Kleihues P, Burger PC, Scheithauer BW (Eds): Histological typing of tumours of the central nervous system. World Health Organization International Histological Classification of Tumours, ed 2. Berlin, Springer-Verlag, 1993
5. Ohgaki H, Dessen P, Jourde B, Horstmann S, Nishikawa T, Di Patre PL, Burkhard C, Schuler D, Probst-Hensch NM, Maiorka PC, Baeza N, Pisani P, Yonekawa Y, Yasargil MG, Lutolf UM, Kleihues P: Genetic pathways to glioblastoma: a population-based study. Cancer Res 2004, 64:6892–6899
6. Ohgaki H, Kleihues P: Population-based studies on incidence, survival rates, and genetic alterations in astrocytic and oligodendroglial gliomas. J Neuropathol Exp Neurol 2005, 64:479–489
7. Godard S, Getz G, Delorenzi M, Farmer P, Kobayashi H, Desbaillets I, Nozaki M, Diserens AC, Hamou MF, Dietrich PY, Regli L, Janzer RC, Bucher P, Stupp R, de Tribolet N, Domany E, Hegi ME: Classification of human astrocytic gliomas on the basis of gene expression: a correlated group of genes with angiogenic activity emerges as a strong predictor of subtypes. Cancer Res 2003, 63:6613–6625
8. Furuta M, Weil RJ, Vortmeyer AO, Huang S, Lei J, Huang TN, Lee YS, Bhowmick DA, Lubensky IA, Oldfield EH, Zhuang Z: Protein patterns and proteins that identify subtypes of glioblastoma multiforme. Oncogene 2004, 23:6806–6814
9. Tso CL, Freije WA, Day A, Chen Z, Merriman B, Perlina A, Lee Y, Dia

EQ, Yoshimoto K, Mischel PS, Liau LM, Cloughesy TF, Nelson SF: Distinct transcription profiles of primary and secondary glioblastoma subgroups. Cancer Res 2006, 66:159–167
10. Dropcho EJ, Soong SJ: The prognostic impact of prior low grade histology in patients with anaplastic gliomas: a case-control study. Neurology 1996, 47:684–690
11. Okamoto Y, Di Patre PL, Burkhard C, Horstmann S, Jourde B, Fahey M, Schuler D, Probst-Hensch NM, Yasargil MG, Yonekawa Y, Lutolf U, Kleihues P, Ohgaki H: Population-based study on incidence, survival rates, and genetic alterations of low-grade astrocytomas and oligodendrogliomas. Acta Neuropathol 2004, 108:49–56
12. Ohgaki H, Kleihues P: Epidemiology and etiology of gliomas. Acta Neuropathol 2005, 109:93–108
13. Xie D, Zeng YX, Wang HJ, Wen JM, Tao Y, Sham JS, Guan XY: Expression of cytoplasmic and nuclear Survivin in primary and secondary human glioblastoma. Br J Cancer 2006, 94:108–114
14. Choe G, Park JK, Jouben-Steele L, Kremen TJ, Liau LM, Vinters HV, Cloughesy TF, Mischel PS: Active matrix metalloproteinase 9 expression is associated with primary glioblastoma subtype. Clin Cancer Res 2002, 8:2894–2901
15. Watanabe K, Tachibana O, Sato K, Yonekawa Y, Kleihues P, Ohgaki H: Overexpression of the EGF receptor and p53 mutations are mutually exclusive in the evolution of primary and secondary glioblastomas. Brain Pathol 1996, 6:217–224
16. Karcher S, Steiner HH, Ahmadi R, Zoubaa S, Vasvari G, Bauer H, Unterberg A, Herold-Mende C: Different angiogenic phenotypes in primary and secondary glioblastomas. Int J Cancer 2006, 118:2182–2189
17. Louis DN, von Deimling A, Chung RY, Rubio MP, Whaley JM, Eibl RH, Ohgaki H, Wiestler OD, Thor AD, Seizinger BR: Comparative study of p53 gene and protein alterations in human astrocytic tumors. J Neuropathol Exp Neurol 1993, 52:31–38
18. Kleihues P, Cavenee WK: Pathology and genetics of tumours of the nervous system. World Health Organization Classification of Tumours. Lyon, IARC Press, 2000
19. Peraud A, Ansari H, Bise K, Reulen HJ: Clinical outcome of supratentorial astrocytoma WHO grade II. Acta Neurochir (Wien) 1998, 140:1213–1222
20. Kita D, Yonekawa Y, Weller M, Ohgaki H: PI3KCA alterations in primary (de novo) and secondary glioblastomas. Acta Neuropathol (Berl) 2007, 113:295–302
21. Ekstrand AJ, Sugawa N, James CD, Collins VP: Amplified and rearranged epidermal growth factor receptor genes in human glioblastomas reveal deletions of sequences encoding portions of the N- and/or C-terminal tails. Proc Natl Acad Sci USA 1992, 89:4309–4313
22. Tohma Y, Gratas C, Biernat W, Peraud A, Fukuda M, Yonekawa Y, Kleihues P, Ohgaki H: PTEN (MMAC1) mutations are frequent in primary glioblastomas (de novo) but not in secondary glioblastomas. J Neuropathol Exp Neurol 1998, 57:684–689
23. Biernat W, Huang H, Yokoo H, Kleihues P, Ohgaki H: Predominant expression of mutant EGFR (EGFRvIII) is rare in primary glioblastomas. Brain Pathol 2004, 14:131–136
24. Kraus JA, Felsberg J, Tonn JC, Reifenberger G, Pietsch T: Molecular genetic analysis of the TP53, PTEN, CDKN2A, EGFR, CDK4 and MDM2 tumour-associated genes in supratentorial primitive neuroectodermal tumours and glioblastomas of childhood. Neuropathol Appl Neurobiol 2002, 28:325–333
25. Huang HS, Nagane M, Klingbeil CK, Lin H, Nishikawa R, Ji XD, Huang CM, Gill GN, Wiley HS, Cavenee WK: The enhanced tumorigenic activity of a mutant epidermal growth factor receptor common in human cancers is mediated by threshold levels of constitutive tyrosine phosphorylation and unattenuated signaling. J Biol Chem 1997, 272:2927–2935
26. Narita Y, Nagane M, Mishima K, Huang HJ, Furnari FB, Cavenee WK: Mutant epidermal growth factor receptor signaling down-regulates p27 through activation of the phosphatidylinositol 3-kinase/Akt pathway in glioblastomas. Cancer Res 2002, 62:6764–6769
27. Mellinghoff IK, Wang MY, Vivanco I, Haas-Kogan DA, Zhu S, Dia EQ, Lu KV, Yoshimoto K, Huang JH, Chute DJ, Riggs BL, Horvath S, Liau LM, Cavenee WK, Rao PN, Beroukhim R, Peck TC, Lee JC, Sellers WR, Stokoe D, Prados M, Cloughesy TF, Sawyers CL, Mischel PS: Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. N Engl J Med 2005, 353:2012–2024
28. Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliaresis C, Rodgers L, McCombie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R: PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 1997, 275:1943–1947
29. Steck PA, Pershouse MA, Jasser SA, Yung WK, Lin H, Ligon AH, Langford LA, Baumgard ML, Hattier T, Davis T, Frye C, Hu R, Swedlund B, Teng DH, Tavtigian SV: Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat Genet 1997, 15:356–362
30. Myers MP, Stolarov JP, Eng C, Li J, Wang SI, Wigler MH, Parsons R, Tonks NK: P-TEN, the tumor suppressor from human chromosome 10q23, is a dual-specificity phosphatase. Proc Natl Acad Sci USA 1997, 94:9052–9057
31. Maehama T, Dixon JE: The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. J Biol Chem 1998, 273:13375–13378
32. Tamura M, Gu J, Matsumoto K, Aota S, Parsons R, Yamada KM: Inhibition of cell migration, spreading, and focal adhesions by tumor suppressor PTEN. Science 1998, 280:1614–1617
33. Dahia PL: PTEN, a unique tumor suppressor gene. Endocr Relat Cancer 2000, 7:115–129
34. Knobbe CB, Merlo A, Reifenberger G: Pten signaling in gliomas. Neuro-Oncology 2002, 4:196–211
35. Watanabe K, Sato K, Biernat W, Tachibana O, von Ammon K, Ogata N, Yonekawa Y, Kleihues P, Ohgaki H: Incidence and timing of p53 mutations during astrocytoma progression in patients with multiple biopsies. Clin Cancer Res 1997, 3:523–530
36. Reifenberger G, Liu L, Ichimura K, Schmidt EE, Collins VP: Amplification and overexpression of the MDM2 gene in a subset of human malignant gliomas without p53 mutations. Cancer Res 1993, 53:2736–2739
37. Biernat W, Kleihues P, Yonekawa Y, Ohgaki H: Amplification and overexpression of MDM2 in primary (de novo) glioblastomas. J Neuropathol Exp Neurol 1997, 56:180–185
38. Nakamura M, Watanabe T, Klangby U, Asker CE, Wiman KG, Yonekawa Y, Kleihues P, Ohgaki H: $P14^{Arf}$ deletion and methylation in genetic pathways to glioblastomas. Brain Pathol 2001, 11:159–168
39. Bögler O, Huang HJ, Kleihues P, Cavenee WK: The p53 gene and its role in human brain tumors. Glia 1995, 15:308–327
40. Sherr CJ, Roberts JM: CDK inhibitors: positive and negative regulators of G_1-phase progression. Genes Dev 1999, 13:1501–1512
41. Stott FJ, Bates S, James MC, McConnell BB, Starborg M, Brookes S, Palmero I, Ryan K, Hara E, Vousden KH, Peters G: The alternative product from the human CDKN2A locus, $p14^{ARF}$, participates in a regulatory feedback loop with p53 and MDM2. EMBO J 1998, 17:5001–5014
42. Momand J, Zambetti GP, Olson DC, George D, Levine AJ: The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. Cell 1992, 69:1237–1245
43. Oliner JD, Kinzler KW, Meltzer PS, George DL, Vogelstein B: Amplification of a gene encoding a p53-associated protein in human sarcomas. Nature 1992, 358:80–83
44. Barak Y, Gottlieb E, Juven Gershon T, Oren M: Regulation of mdm2 expression by p53: alternative promoters produce transcripts with nonidentical translation potential. Genes Dev 1994, 8:1739–1749
45. Zauberman A, Flusberg D, Haupt Y, Barak Y, Oren M: A functional p53-responsive intronic promoter is contained within the human mdm2 gene. Nucleic Acids Res 1995, 23:2584–2592
46. Picksley SM, Lane DP: The p53-mdm2 autoregulatory feedback loop: a paradigm for the regulation of growth control by p53? Bioessays 1993, 15:689–690
47. Kamijo T, Weber JD, Zambetti G, Zindy F, Roussel MF, Sherr CJ: Functional and physical interactions of the ARF tumor suppressor with p53 and Mdm2. Proc Natl Acad Sci USA 1998, 95:8292–8297
48. Pomerantz J, Schreiber-Agus N, Liegeois NJ, Silverman A, Alland L, Chin L, Potes J, Chen K, Orlow I, Lee HW, Cordon-Cardo C, DePinho RA: The Ink4a tumor suppressor gene product, $p19^{Arf}$, interacts with MDM2 and neutralizes MDM2's inhibition of p53. Cell 1998, 92:713–723
49. Fleming TP, Saxena A, Clark WC, Robertson JT, Oldfield EH, Aaronson SA, Ali IU: Amplification and/or overexpression of platelet-derived growth factor receptors and epidermal growth factor receptor in human glial tumors. Cancer Res 1992, 52:4550–4553
50. Nürnberg P, Zischler H, Fuhrmann E, Thiel G, Losanova T, Kinzel D,

Nisch G, Witkowski R, Epplen JT: Coamplification of simple repetitive DNA fingerprint fragments and the EGFR gene in human gliomas. Genes Chromosom Cancer 1991, 3:79–88
51. Mercer WE, Shields MT, Amin M, Sauve GJ, Appella E, Romano JW, Ullrich SJ: Negative growth regulation in a glioblastoma tumor cell line that conditionally expresses human wild-type p53. Proc Natl Acad Sci USA 1990, 87:6166–6170
52. Biernat W, Tohma Y, Yonekawa Y, Kleihues P, Ohgaki H: Alterations of cell cycle regulatory genes in primary (de novo) and secondary glioblastomas. Acta Neuropathol 1997, 94:303–309
53. Nakamura M, Yonekawa Y, Kleihues P, Ohgaki H: Promoter hypermethylation of the RB1 gene in glioblastomas. Lab Invest 2001, 81:77–82
54. Fujisawa H, Reis RM, Nakamura M, Colella S, Yonekawa Y, Kleihues P, Ohgaki H: Loss of heterozygosity on chromosome 10 is more extensive in primary (de novo) than in secondary glioblastomas. Lab Invest 2000, 80:65–72
55. Rasheed BK, McLendon RE, Friedman HS, Friedman AH, Fuchs HE, Bigner DD, Bigner SH: Chromosome 10 deletion mapping in human gliomas: a common deletion region in 10q25. Oncogene 1995, 10:2243–2246
56. Ichimura K, Schmidt EE, Miyakawa A, Goike HM, Collins VP: Distinct patterns of deletion on 10p and 10q suggest involvement of multiple tumor suppressor genes in the development of astrocytic gliomas of different malignancy grades. Genes Chromosom Cancer 1998, 22:9–15
57. Fults D, Pedone CA, Thompson GE, Uchiyama CM, Gumpper KL, Iliev D, Vinson VL, Tavtigian SV, Perry III WL: Microsatellite deletion mapping on chromosome 10q and mutation analysis of MMAC1, FAS, and MXI1 in human glioblastoma multiforme. Int J Oncol 1998, 12:905–910
58. Fujisawa H, Kurrer M, Reis RM, Yonekawa Y, Kleihues P, Ohgaki H: Acquisition of the glioblastoma phenotype during astrocytoma progression is associated with loss of heterozygosity on 10q25-qter. Am J Pathol 1999, 155:387–394
59. Chernova OB, Somerville RP, Cowell JK: A novel gene, LGI1, from 10q24 is rearranged and downregulated in malignant brain tumors Oncogene 1998, 17:2873–2881
60. Cahill DP, da Costa LT, Carson-Walter EB, Kinzler KW, Vogelstein B, Lengauer C: Characterization of MAD2B and other mitotic spindle checkpoint genes. Genomics 1999, 58:181–187
61. Eagle LR, Yin X, Brothman AR, Williams BJ, Atkin NB, Prochownik EV: Mutation of the MXI1 gene in prostate cancer. Nat Genet 1995, 9:249–255
62. Nakamura H, Yoshida M, Tsuiki H, Ito K, Ueno M, Nakao M, Oka K, Tada M, Kochi M, Kuratsu J, Ushio Y, Saya H: Identification of a human homolog of the Drosophila neuralized gene within the 10q25.1 malignant astrocytoma deletion region. Oncogene 1998, 16:1009–1019
63. Roof DJ, Hayes A, Adamian M, Chishti AH, Li T: Molecular characterization of abLIM, a novel actin-binding and double zinc finger protein. J Cell Biol 1997, 138:575–588
64. Kim AC, Peters LL, Knoll JHM, van Huffel C, Ciciotte SL, Kleyn PW, Chishti AH: Limatin (LIMAB1), an actin-binding LIM protein, maps to mouse chromosome 19 and human chromosome 10q25, a region frequently deleted in human cancers. Genomics 1997, 46:291–293
65. Mollenhauer J, Wiemann S, Scheurlen W, Korn B, Hayashi Y, Wilgenbus KK, von Deimling A, Poustka A: DMBT1, a new member of the SRCR superfamily, on chromosome 10q25.3-26.1 is deleted in malignant brain tumours. Nat Genet 1997, 17:32–39
66. Nakamura M, Ishida E, Shimada K, Kishi M, Nakase H, Sakaki T, Konishi N: Frequent LOH on 22q12.3 and TIMP-3 inactivation occur in the progression to secondary glioblastomas. Lab Invest 2005, 85:165–175
67. Nakamura M, Yang F, Fujisawa H, Yonekawa Y, Kleihues P, Ohgaki H: Loss of heterozygosity on chromosome 19 in secondary glioblastomas. J Neuropathol Exp Neurol 2000, 59:539–543
68. Hegi ME, zur Hausen A, Ruedi D, Malin G, Kleihues P: Hemizygous or homozygous deletion of the chromosomal region containing the $p16^{INK4a}$ gene is associated with amplification of the EGF receptor gene in glioblastomas. Int J Cancer 1997, 73:57–63
69. Hayashi Y, Ueki K, Waha A, Wiestler OD, Louis DN, von Deimling A: Association of EGFR gene amplification and CDKN2 (p16/MTS1) gene deletion in glioblastoma multiforme. Brain Pathol 1997, 7:871–875
70. Burger PC, Pearl DK, Aldape K, Yates AJ, Scheithauer BW, Passe SM, Jenkins RB, James CD: Small cell architecture—a histological equivalent of EGFR amplification in glioblastoma multiforme? J Neuropathol Exp Neurol 2001, 60:1099–1104
71. Tohma Y, Gratas C, Van Meir EG, Desbaillets I, Tenan M, Tachibana O, Kleihues P, Ohgaki H: Necrogenesis and Fas/APO-1(CD95) expression in primary (de novo) and secondary glioblastomas. J Neuropathol Exp Neurol 1998, 57:239–245
72. Homma T, Fukushima T, Vaccarella S, Yonekawa Y, Di Patre PL, Franceschi S, Ohgaki H: Correlation among pathology, genotype, and patient outcomes in glioblastoma. J Neuropathol Exp Neurol 2006, 65:846–854
73. Margison GP, Kleihues P: Chemical carcinogenesis in the nervous system. Preferential accumulation of O6-methylguanine in rat brain deoxyribonucleic acid during repetitive administration of N-methyl-N-nitrosourea. Biochem J 1975, 148:521–525
74. Goth R, Rajewsky MF: Persistence of O6-ethylguanine in rat-brain DNA: correlation with nervous system-specific carcinogenesis by ethylnitrosourea. Proc Natl Acad Sci USA 1974, 71:639–643
75. Belanich M, Pastor M, Randall T, Guerra D, Kibitel J, Alas L, Li B, Citron M, Wasserman P, White A, Eyre H, Jaeckle K, Schulman S, Rector D, Prados M, Coons S, Shapiro W, Yarosh D: Retrospective study of the correlation between the DNA repair protein alkyltransferase and survival of brain tumor patients treated with carmustine. Cancer Res 1996, 56:783–788
76. Watts GS, Pieper RO, Costello JF, Peng YM, Dalton WS, Futscher BW: Methylation of discrete regions of the O^6-methylguanine DNA methyltransferase (MGMT) CpG island is associated with heterochromatinization of the MGMT transcription start site and silencing of the gene. Mol Cell Biol 1997, 17:5612–5619
77. Qian XC, Brent TP: Methylation hot spots in the 5′ flanking region denote silencing of the O^6-methylguanine-DNA methyltransferase gene. Cancer Res 1997, 57:3672–3677
78. Nakamura M, Watanabe T, Yonekawa Y, Kleihues P, Ohgaki H: Promoter hypermethylation of the DNA repair gene MGMT in astrocytomas is frequently associated with G:C → A:T mutations of the TP53 tumor suppressor gene. Carcinogenesis 2001, 22:1715–1719
79. Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, Kros JM, Hainfellner JA, Mason W, Mariani L, Bromberg JE, Hau P, Mirimanoff RO, Cairncross JG, Janzer RC, Stupp R: MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 2005, 352:997–1003
80. Wolf P, Hu YC, Doffek K, Sidransky D, Ahrendt SA: O^6-Methylguanine-DNA methyltransferase promoter hypermethylation shifts the p53 mutational spectrum in non-small cell lung cancer. Cancer Res 2001, 61:8113–8117
81. Esteller M, Toyota M, Sanchez-Cespedes M, Capella G, Peinado MA, Watkins DN, Issa JP, Sidransky D, Baylin SB, Herman JG: Inactivation of the DNA repair gene O^6-methylguanine-DNA methyltransferase by promoter hypermethylation is associated with G to A mutations in K-ras in colorectal tumorigenesis. Cancer Res 2000, 60:2368–2371
82. Pegg AE: Repair of O^6-alkylguanine by alkyltransferases. Mutat Res 2000, 462:83–100
83. Kleihues P, Rajewsky MF: Chemical neuro-oncogenesis: role of structural DNA modifications, DNA repair and neural target cell population. Prog Exp Tumor Res 1984, 27:1–16
84. Ambrosini G, Adida C, Altieri DC: A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat Med 1997, 3:917–921
85. Somasundaram K, Reddy SP, Vinnakota K, Britto R, Subbarayan M, Nambiar S, Hebbar A, Samuel C, Shetty M, Sreepathi HK, Santosh V, Hegde AS, Hegde S, Kondaiah P, Rao MR: Upregulation of ASCL1 and inhibition of Notch signaling pathway characterize progressive astrocytoma. Oncogene 2005, 24:7073–7083