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PTEN (MMAC1) Mutations Are Frequent in Primary Glioblastomas (de novo) but not in Secondary Glioblastomas

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Abstract. Loss of heterozygosity (LOH) on chromosome 10 is the most frequent genetic alteration associated with the evolution of malignant astrocytic tumors and it may involve several loci. The tumor suppressor gene *PTEN* (*MMAC1*) on chromosome 10q23 is mutated in approximately 30% of glioblastomas (WHO Grade IV). In this study, we assessed the frequency of *PTEN* mutations in primary glioblastomas, which developed clinically de novo, and in secondary glioblastomas, which evolved from low-grade (WHO Grade II) or anaplastic astrocytomas (WHO Grade III). Nine of 28 (32%) primary glioblastomas contained a *PTEN* mutation and an additional case showed a homozygous *PTEN* deletion. This indicates that after overexpression/amplification of the *EGF* receptor, loss of *PTEN* function is the most common alteration in primary glioblastomas. In this series, 5 of 28 (18%) primary glioblastomas showed both a *PTEN* mutation and *EGFR* amplification. In contrast, only 1 of 25 (4%) secondary glioblastomas contained a *PTEN* mutation, and none of them showed a homozygous *PTEN* deletion. The secondary glioblastoma with a *PTEN* mutation developed from an anaplastic astrocytoma that already carried the mutation. The observation that secondary glioblastomas have a *p53* mutation as a genetic hallmark but rarely contain a *PTEN* mutation supports the concept that primary and secondary glioblastomas develop differently on a genetic level.

Key Words: *MMAC1*; Mutation; Primary glioblastoma; *PTEN*; Secondary glioblastoma.

INTRODUCTION

Loss of heterozygosity (LOH) in large regions, particularly at 10p, 10q23, or 10q25–26 loci, or loss of an entire copy of chromosome 10 are the most frequent genetic alterations in glioblastomas (1–8). The *PTEN* (*MMAC1*) tumor suppressor gene was identified on chromosome 10q23 and was often found to be mutated in a variety of human neoplasms with LOH on chromosome 10 (9, 10). *PTEN* mutations were detected in glioblastomas (WHO Grade IV) and, less frequently, in anaplastic astrocytomas (WHO Grade III), but not in low-grade astrocytomas (WHO Grade II) (9–11, 11–15). Wang et al (12) reported that 15 of 34 (44%) glioblastomas without LOH and 15 of 25 (60%) with LOH on chromosome 10q contained a *PTEN* mutation. Rasheed et al (13) found *PTEN* mutations in 13 of 42 (31%) adult glioblastomas and 3 of 13 (23%) adult anaplastic astrocytomas, but found no *PTEN* mutations in 21 low-grade adult gliomas and none in 22 childhood gliomas of all grades. Bostrom et al (11) reported a *PTEN* mutation in 9 of 36 (25%) glioblastomas, but reported none in 34 meningiomas. Duerr et al (14) found a *PTEN* mutation in 20 of 142 (14%) glioblastomas, but found none in 52 astrocytomas or in 37 oligoastrocytomas. We recently found a *PTEN* mutation in 2 of 11 (18%) anaplastic gemistocytic astrocytomas (WHO Grade III), but found none in 15 gemistocytic astrocytomas (WHO Grade II) (16).

Glioblastomas (WHO Grade IV) may arise rapidly de novo, i.e. without clinical or histologic evidence of a less malignant precursor lesion. These have been designated primary (de novo) glioblastomas (17–21). Less frequently, glioblastomas develop from low-grade or anaplastic astrocytomas; these have been termed secondary glioblastomas (17-21). Several recent studies have shown that the development of these 2 subtypes is different genetically. Primary glioblastomas typically show overexpression of *EGFR* and *MDM2* and p16 deletion, but rarely *p53* mutations; secondary glioblastomas frequently contain *p53* mutations, but rarely a p16 deletion or overexpression of *EGFR* or *MDM2* genes (22–24). The objective of this study is to clarify whether mutational loss of *PTEN* function is involved in the evolution of both primary and secondary glioblastomas, and whether other genetic alterations such as *p53* mutations and *EGFR* overexpression/amplification are associated with *PTEN* mutations.

MATERIALS AND METHODS

Tumor Samples

The surgical specimens were obtained from patients treated in the Department of Neurosurgery, University Hospital, Zürich, Switzerland, between 1976 and 1994. Twenty-eight patients with primary glioblastomas had a clinical history of less than 3 months (mean: 1.7 months), without prior biopsy or excision for lower-grade astrocytomas. Twenty-five patients with secondary glioblastomas had undergone previous operations for low-grade astrocytoma or anaplastic astrocytoma. Eighteen low-grade astrocytomas from 16 patients and 10 anaplastic

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astrocytomas from 9 patients were also screened for *PTEN* mutations. The sex and age of each patient are shown in Table 1.

SSCP and DNA Sequencing Analyses for *PTEN* Mutations

DNA was extracted from formalin-fixed, paraffin sections as described previously (22). Prescreening for mutations in exons 1–9 of the *PTEN* gene was carried out by PCR-SSCP. PCR was carried out with 1 μl DNA solution, 1.5–2.5 pmol of each primer, 2 mM of dNTPs, 0.5 μCi of [α-^{33}P]-dCTP (ICN Biomedicals N.V./S.A, Belgium; specific activity, 3000 Ci/mmol), 10 mM Tris (pH 8.8), 50 mM KCl, $MgCl_2$ (1.5–2.5 mM), and 0.75–1.0 U of Taq polymerase (Life Technologies, Cergy Pontoise, France) in a final volume of 10 μl. PCR was carried out in a robocycler 96 gradient temperature cycler (Stratagene GmbH, Heidelberg, Germany) with an initial denaturation step for 2 minutes (min) at 95°C followed by 35 to 40 cycles of denaturation at 95°C for 60 seconds, annealing at 52–58°C for 50 seconds, and extension at 72°C for 50 seconds. Primer sequences for PCR amplification were reported previously (16), except for exon 5, for which the following additional primers were used: 5′-TTTTACCACAGTTGCACAATA (sense) and 5′-GAAGAGGAAAGGAAAAACATC (antisense) to yield 265 bp product, 5′-TTTTACCACAGTTGCACAATA (sense) and 5′-TATCATTACACCAGTTCGTC (antisense) to yield 163 bp product, and 5′- TAAAGCTGGAAAGGGACGAA (sense) and 5′-5′-GAAGAGGAAAGGAAAAACATC (antisense) to yield 157 bp product. After PCR, 2 μl 0.2 M NaOH and 9 μl sequencing stop solution (USB, Cleveland, Ohio) were added to the 1.5 μl PCR reaction mixture. Samples were heated at 95°C for 10 min and immediately loaded onto a 6% polyacrylamide nondenaturing gel containing 6% glycerol. Gels were run at 40 W for 3–5 hours (h) with cooling by fan at room temperature, dried at 80°C, and autoradiographed for 12–48 hours.

Samples that showed mobility shifts in the SSCP analysis were further analyzed by direct DNA sequencing. After PCR amplification as described above, 10 μl of PCR products were digested with 2 U of shrimp alkaline phosphatase and 10 U of exonuclease I at 37°C for 20 min. After inactivation of these enzymes at 80°C for 15 min, 10 pmol of sequencing primer was added. Template-primer mixture was heated at 100°C for 3 min and then placed in ice-cold water. Two μl of 5 × sequenase buffer (200 mM Tris-HCl, pH 7.5, 100 mM $MgCl_2$, 250 mM NaCl), 0.1 M dithiothreitol, 3.2 U Sequenase version 2.0 (USB, Cleveland, Ohio), and 5 μCi [α-^{33}P]-dCTP were added to samples, which were then divided into 4 wells containing each termination mixture. Samples were incubated at 45–48°C for 10–13 min and mixed with 5 μl stop solution (USB, Cleveland, Ohio). After being heated at 80°C for 5 min, samples were loaded onto a 6% polyacrylamide/7 M urea gel. Gels were dried at 80°C and autoradiographed for 12–48 h. Primers for DNA sequencing are as follows: 5′- TTT TAC CAC AGT TGC ACA ATA (sense), 5′- TAA AGC TGG AAA GGG ACG AA (sense), 5′- GAA GAG GAA AGG AAA AAC ATC (antisense), 5′- TAT CAT TAC ACC AGT TCG TC (antisense) for exon 5 and intron 4, 5′-GGGAGT AAC TAT TCC CAG T (sense) and 5′- CCA ATA CAT GGA AGG ATG AG (antisense) for exon 6 and intron 6, and 5′-TGA GTC ATA TTT GTG GGT TTT (sense) and 5′-CAT GGT GTT TTA TCC CTC TT (antisense) for exon 9.

Differential PCR for *PTEN* Deletion

All glioblastomas were screened for homozygous deletion of the *PTEN* gene by differential PCR using primers for *PTEN* exon 5 (5′-AATTTTTAAAGGCACAAGAGG-3′ and 5′-TCCAGGAAGAGGAAAGGA-3′) together with primers for the *GAPDH* gene (5′-AACGTGTCAGTGGTGGACCTG-3′ and 5′-AGTGGGTGTCGCTGTTGAAGT-3′) (11). Differential PCR was performed with 1.5 μl of DNA solution in 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.1μM of each *GAPDH* primer, 0.3 μM of each *PTEN* primer, and 0.1 U of Taq DNA polymerase in a final volume of 15 μl. Three min initial denaturation was followed by 27 cycles of 1 min at 94°C, 1 min at 54°C, 1 min at 72°C, and a final extension at 72°C for 5 min. After PCR, the entire reaction product was electrophoresed on an 8% polyacrylamide gel and stained by ethidium bromide. Gels were photographed using Polaroid film and the intensities of the *PTEN* fragment (115 bp) and *GAPDH* reference sequence (160 bp) were measured by means of a densitometer GS-670 (Bio-Rad). *PTEN/GAPDH* ratios of less than 0.3 relative to the constitutional control were regarded as evidence for homozygous deletion (11).

Differential PCR for *EGFR* Amplification

For detecting *EGFR* amplification in glioblastomas, differential PCR was carried out as described previously, with some modifications (25). Briefly, 5 μl of template were amplified in 25 μl of a reaction mixture containing 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM dNTPs, 0.18 μM primers for *EGFR* and 0.18 μM primers for cystic fibrosis (CF) reference gene, and 0.625 U Taq DNA polymerase (Boeringher Mannheim, Germany). After denaturing DNA at 95°C for 3 min, 25 cycles of PCR (95°C for 1 min, 55°C for 1 min, and 72°C for 1 min) were carried out with a final extension at 72°C for 5 min. The primer sequences for differential PCR were as follows: 5′-AGCCATGCCCGCATTAGCTC (sense) and 5′-AAAGGAATGCAACTTCCCAA (antisense) for *EGFR*, and primers 5′- GGCACCATTAAAGAAAATATCATCTT (sense) and 5′-GTTGGCATGCTTTGATGACGCTTC (antisense) for CF reference gene (25). After PCR, the entire reaction product was electrophoresed on an 8% polyacrylamide gel and stained by ethidium bromide. Gels were photographed using Polaroid film and the intensities of the *EGFR* fragment (110 bp) and the CF reference sequence (79 bp) were measured by means of a densitometer GS-670 (Bio-Rad). The mean *EGFR/CF* ratios using the DNA from the peripheral blood of healthy adults was 1.31, with a standard variation of 0.20. The threshold value, 3.22, was regarded as evidence of *EGFR* amplification as per Rollbrocker et al (26).

Statistical Analyses

The Fisher's exact test and χ^2 test were carried out to analyze the contingency table for incidence of PTEN mutations between primary and secondary glioblastomas, and to analyze the correlation between PTEN mutation, *p53* mutation, and EGFR amplification.

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TABLE 1
PTEN Mutations in Primary (*de novo*) Glioblastomas

Case No.	Age/Sex	Location	Exon/ Intron	Codon	Predicted effect	*PTEN/ GADPH* ratio	*EGFR* IHC	*EGFR/CF* ratio	*p53* mutation
DN1	65/M	T	Exon 9	404	TGA→TGC, Stop→Cys stop in intron 9	0.91	+	**12.56**	–
DN2	50/M	T	–			1.07	–	1.38	–
DN5	58/M	O	Exon 5	116	GAC→GA, 1 bp deletion, frameshift, stop at codon 133	0.85	–	1.46	–
DN6	61/F	TO	Exon 6	183–184	TTA AAG→TT, 4 bp deletion, frameshift, stop at codon 199	1.07	+	**9.50**	–
DN7	35/F	FT	–			0.75	+	1.74	–
DN8	66/F	F	–			0.83	+	**4.93**	–
DN9	34/M	T	–			0.55	–	1.99	codon 239
DN10	63/M	PT	–			0.79	+	**4.75**	–
DN11	46/F	TO	–			0.46	–	2.45	–
DN12	54/M	TPO	–			0.42	–	1.88	–
DN13	48/M	P	Exon 6	169	CCC→CAC, Pro→His	0.67	+	**5.19**	–
DN14	39/F	T	–			0.65	+	**22.02**	–
DN15	70/F	FT	–			0.64	+	1.51	–
DN16	73/M	FT	–			0.69	–	0.90	codon 248
DN17	69/F	TO	–			0.43	+	**13.16**	–
DN18	51/M	FrT	Exon 6	165	GGA→AGA, Gly→Arg	0.63	+	**10.46**	–
DN20	58/M	T	–			0.80	–	1.17	–
DN21	78/F	P	–			0.82	–	1.05	–
DN24	58/M	FP	–			0.69	–	0.90	codon 169
DN25	55/F	T	–			0.77	–	0.79	–
DN27	58/F	F	–			**0.20**	–	1.24	–
DN28	63/M	T	Exon 6	173	CGC→CAC, Arg→His	0.95	–	0.93	–
DN29	63/M	F	–			1.01	+	**5.45**	codon 273
DN30	69/M	FP	Intron 6	–	TGC Agtaa → TGC Agaaa, 1bp insertion, splicing variant	1.51	–	1.87	–
DN31	63/F	T	–			1.06	+	**7.70**	–
DN34	73/M	PO	Intron 4	–	cag TT GCA→caag TT GCA, 1 bp insertion, splicing variant	0.84	–	1.76	–
DN37	47/M	P	–			1.20	–	1.22	–
DN38	59/F	TPO	Exon 6	199	1 bp deletion, frameshift ATG→AT, stop at codon 220	0.71	+	**6.37**	–

F, frontal; P, parietal; O, occipital; T, temporal; *PTEN/GAPDH* ratios of less than 0.3 were regarded as evidence of homozygous deletion (11). IHC, Immunohistochemistry; *EGFR/CF* ratio of more than 3.22 regarded as evidence of amplification (26). The results of *p53* mutations and *EGFR* IHC were previously reported (22, 23).

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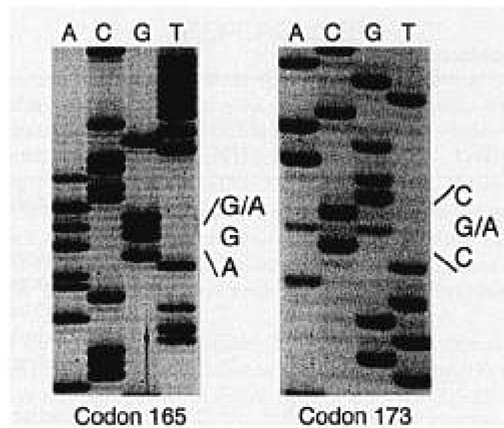

Fig. 1. DNA sequencing autoradiographs of *PTEN* mutations in primary (de novo) glioblastomas. Left: GGA≥AGA mutation with amino acid substitution of Gly≥Arg at codon 165 (case DN18). Right: CGC≥CAC with amino acid substitution of Arg≥His at codon 173 (case DN28).

RESULTS

PTEN Mutation and Homozygous Deletion

PCR-SSCP followed by DNA sequencing revealed that 9 of 28 (32%) primary glioblastomas contained a *PTEN* mutation (Table 1, Fig. 1). In contrast, only 1 *PTEN* mutation was found in a secondary glioblastoma (case 111) that had developed from an anaplastic astrocytoma that already contained the same *PTEN* mutation (1/25, 4%, $P < 0.01$, Table 2). None of the 18 secondary glioblastomas from 16 patients that progressed from low-grade astrocytomas carried a *PTEN* mutation (Table 2), and none of the 18 low-grade astrocytomas from 16 patients contained a *PTEN* mutation.

PTEN mutations were located primarily in exons 6 (5 cases) and 5 (2 cases); others were identified in exon 9, intron 4, and intron 6. Five were point mutations, 3 were deletions, and 2 were insertions (Tables 1, 2).

Differential PCR revealed homozygous *PTEN* deletion in 1 primary glioblastoma (case DN27), but in none of the secondary glioblastomas (Tables 1, 2).

EGFR Amplification

EGFR amplification was detected by differential PCR in 11 of 28 (39%) primary glioblastomas, but in none of 22 secondary glioblastomas ($P = 0.0046$, Tables 1, 2). *EGFR* amplification detected by differential PCR correlated well with the *EGFR* overexpression detected by immunohistochemistry that was reported previously (22): all glioblastomas with *EGFR* amplification showed *EGFR* overexpression, and 11 of 15 (73%) glioblastomas with *EGFR* overexpression showed *EGFR* amplification.

Correlation between *PTEN* Mutation, *EGFR* Amplification, and *p53* Mutation

We correlated *PTEN* mutations with the *p53* mutations previously reported for this case series (22, 23) or with *EGFR* amplification assessed by differential PCR in this study. There was a significant reciprocal correlation between *PTEN* and *p53* mutations ($P = 0.035$). No glioblastoma contained both *PTEN* and *p53* mutations (Tables 1, 2). There was no significant correlation between *PTEN* mutations and *EGFR* amplification. Five of 9 primary glioblastomas with a *PTEN* mutation also showed *EGFR* amplification, while 6 of 19 primary glioblastomas without a *PTEN* mutation showed *EGFR* amplification ($P = 0.4$).

DISCUSSION

In this study, we present evidence that *PTEN* mutations typically occur in primary (de novo) glioblastomas (9/28, 32%), but rarely occur in secondary glioblastomas (1/25, 4%, $P < 0.01$). Rasheed et al (13) reported a high incidence of *PTEN* mutations in glioblastomas from older patients, which is consistent with our finding that more primary glioblastomas occur in older patients (mean age, 55 years) than secondary glioblastomas (mean age, 40 years) (22). The present result is also consistent with the report by Duerr et al (14) in which 6 glioblastomas classified as secondary lacked *PTEN* mutations. The only secondary glioblastoma with a *PTEN* mutation in this study (case 111) developed from an anaplastic astrocytoma that was partially resected 2 years earlier. Histopathology showed atypia and mitotic activity, but no necrosis or microvascular proliferation. CT scans showed some contrast enhancement, but the extent and intensity were considered insufficient for the diagnosis of primary glioblastoma. Thus, this case was classified as a secondary glioblastoma.

Sequence analysis of the open reading frame revealed that *PTEN* encodes a protein with homology to the catalytic domain of tyrosine phosphatase and to the cytoskeletal proteins tensin and auxilin (9, 10). Myers et al (27) demonstrated that recombinant *PTEN* dephosphorylates protein and peptide substrates at serine, threonine, and tyrosine residues, indicating that *PTEN* is a dual-specificity phosphatase. Mutations in the *PTEN* gene identified in primary tumors, cell lines, and a patient with Bananayan-Zonana syndrome resulted in the ablation of phosphatase activity, demonstrating that the enzymatic activity of *PTEN* is necessary in order for it to function as a tumor suppressor (27). The homology of *PTEN* to tensin is also of interest, as tensin appears to bind actin filaments at focal adhesion complexes that contain integrins, focal adhesion kinase, src, and growth factor receptors (28–30).

Loss of heterozygosity on chromosome 10 has been found in 57–67% of glioblastomas with *EGFR* amplification, more frequently than in those without *EGFR* amplification (37–39%) (2, 4). In one study, all glioblastomas with *EGFR* amplification showed LOH on chromosome 10 for all informative loci on both the short

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TABLE 2
PTEN Mutations in Secondary Glioblastomas

Case No.	Age/Sex	Location	1st op	2nd op	3rd op	Exon/ Intron	Codon	Predicted effect	*PTEN/ GADPH* ratio	EGFR IHC	*EGFR/ CF* ratio	*p53* mutation
48	35/M	FT	II	II	IV	–			1.05	–	1.36	codon 175
57	25/F	T	II	II	IV	–			0.69	–	1.20	–
60	29/F	F	II	IV		–			0.74	–	1.08	codon 163
62	51/M	T	II	IV		–			1.22	–	1.17	codon 273
65	25/M	F	II	IV		–			2.20	–	1.11	codon 175
68	29/F	F	II	IV		–			0.91	–	2.43	codon 273
71	32/F	BG	II	IV		–			1.04	–	1.96	codon 163
73	24/M	F	II	IV		–			0.82	–	1.60	–
75	39/F	T	II	IV		–			0.89	–	1.62	codon 275
77	40/M	F	II	IV		–			0.78	+	1.07	–
79	40/F	T	II	IV		–			1.51	–	1.89	codon 256
81	49/F	F	II	IV		–			0.91	+	1.11	codon 278
85	52/M	BG	II	IV		–			1.40	–	1.22	–
87	39/M	F	II	IV		–			0.91	–	1.56	codon 275
89	30/M	F	II	IV		–			1.01	–	1.93	codon 300
91	23/F	F	II	IV		–			1.27	–	2.02	codon 301
104	44/M	TP	III	III	IV	–			1.43	–	1.15	codon 280
111*	45/F	T	III	IV		Exon 5	130	CGA→TGA, Arg→Stop	0.60	–	1.17	–
113	30/F	FP	III	IV		–			1.40	–	1.08	codon 273
115	32/M	FP	III	IV		–			1.19		1.71	–
117	25/F	TP	III	IV		–			1.12	–	0.83	–
119	12/F	BG	III	IV		–			0.82	–	2.89	codon 280
121	74/M	T	III	IV		–			1.62	–	2.07	–
123	45/F	F	III	IV		–			1.98	–	1.12	–
125	23/F	T	III	IV		–			1.25	–	0.81	–

II, low-grade astrocytoma; III, anaplastic astrocytoma; IV, glioblastoma; F, frontal; P, parietal; O, occipital; T, temporal; BG, basal ganglia; *PTEN/GAPDH* ratios of less than 0.3 were regarded as evidence of homozygous deletion (11). *EGFR/CF* ratio of more than 3.22 regarded as evidence of amplification (26). The results of *p53* mutations and *EGFR* IHC were previously reported in (22, 23). * Both anaplastic astrocytoma and glioblastoma from this patient contained the same *PTEN* mutation; op, operation; IHC, immunohistochemistry.

and long arms, indicating the absence of one entire chromosome 10 (31). This suggests a strong association between *EGFR* amplification and LOH on chromosome 10. In contrast, this study and reports by others (13, 15) show that there is no consistent correlation between *EGFR* amplification and *PTEN* mutations in glioblastomas. Since LOH on chromosome 10 is significantly more frequent than *PTEN* mutations, it is likely that other tumor suppressor genes on this chromosome are more frequently associated with *EGFR* amplification in glioblastomas. A candidate gene to be considered is DMBT1, which was recently identified on chromosome 10q25–26 (32).

It has been reported that 50–57% of glioblastomas with *p53* mutations (2, 33) and 44– 63% of those with LOH on chromosome 17p (2, 4, 33) show LOH on chromosome 10. In contrast, this study shows significant reciprocal correlation between *p53* mutations and *PTEN* mutations in glioblastomas. No glioblastoma contained both a *p53* mutation and a *PTEN* mutation. Similarly, Rasheed et al (13) also observed that the 2 mutations never occurred together, except in one instance in which an anaplastic astrocytoma contained both mutations. It appears that glioblastomas with *p53* mutations are typically associated with the loss of tumor suppressor loci on chromosome 10 other than *PTEN*.

In conclusion, this study shows that loss of *PTEN* function is the second most common alteration in primary glioblastomas, second only to *EGFR* amplification. Our observation that secondary glioblastomas have a *p53* mutation as genetic hallmark but rarely contain a *PTEN* mutation supports the concept that primary and secondary glioblastomas develop differently on a genetic level. Since we have used rather stringent criteria for the selection of primary and secondary glioblastomas, it is possible that a population of glioblastoma patients exists that does not meet our definition of either a primary or secondary glioblastoma. It would be of interest to determine whether such tumors have genetic alterations typical for primary or secondary glioblastomas or whether their genetic alterations are unique.

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REFERENCES

1. Albarosa R, Colombo BM, Roz L, et al. Deletion mapping of gliomas suggest the presence of two small regions for candidate tumor-suppressor genes in a 17-cM interval on chromosome 10q. Am J Hum Genet 1996;58:1260–67
2. Lang FF, Miller DC, Koslow M, Newcomb EW. Pathways leading to glioblastoma multiforme: A molecular analysis of genetic alterations in 65 astrocytic tumors. J Neurosurg 1994;81:427–36
3. Fults D, Pedone C. Deletion mapping of the long arm of chromosome 10 in glioblastoma multiforme. Genes Chromosom Cancer 1993;7:173–77
4. von Deimling A, von Ammon K, Schoenfeld D, Wiestler OD, Seizinger BR, Louis DN. Subsets of glioblastoma multiforme defined by molecular genetic analysis. Brain Pathol 1993;3:19–26
5. Rasheed BK, McLendon RE, Friedman HS, et al. Chromosome 10 deletion mapping in human gliomas: A common deletion region in 10q25. Oncogene 1995;10:2243–46
6. Louis DN. A molecular genetic model of astrocytoma histopathology. Brain Pathol 1997;7:755– 64
7. Sonoda Y, Murakami Y, Tominaga T, Kayama T, Yoshimoto T, Sekiya T. Deletion mapping of chromosome 10 in human glioma. Jpn J Cancer Res 1996;87:363–67
8. Steck PA, Ligon AH, Cheong P, Yung WK, Pershouse MA. Two tumor suppressive loci on chromosome 10 involved in human glioblastomas. Genes Chromosomes Cancer 1995;12:255–61
9. Li J, Yen C, Liaw D, et al. *PTEN*, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 1997;275:1943–47
10. Steck PA, Pershouse MA, Jasser SA, et al. Identification of a candidate tumour suppressor gene, *MMAC1*, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nature Genet 1997; 15:356–62
11. Bostrom J, Cobbers JMJL, Wolter M, et al. Mutation of the *PTEN* (*MMAC1*) tumor suppressor gene in a subset of glioblastomas but not in meningiomas with loss of chromosome arm 10q[1]. Cancer Res 1998;58:29–33
12. Wang SI, Puc J, Li J, et al. Somatic mutations of *PTEN* in glioblastoma multiforme. Cancer Res 1997;57:4183–86
13. Rasheed BKA, Stenzel TT, McLendon RE, et al. *PTEN* gene mutations are seen in high-grade but not in low-grade gliomas. Cancer Res 1997;57:4187–90
14. Duerr E-M, Rollbrocker B, Hayashi Y, et al. *PTEN* mutations in gliomas and glioneuronal tumors. Oncogene 1998. Forthcoming.
15. Liu W, James CD, Frederick L, Alderete BE, Jenkins RB. *PTEN/MMAC1* mutations and *EGFR* amplification in glioblastomas. Cancer Res 1997;57:5254–57
16. Watanabe K, Peraud A, Gratas C, Wakai S, Kleihues P, Ohgaki H. *p53* and *PTEN* gene mutations in gemistocytic astrocytomas. Acta Neuropath 1998. Forthcoming.
17. Scherer HJ. Cerebral astrocytomas and their derivatives. Am J Cancer 1940;40:159–98
18. Russell DS, Rubinstein LJ. Pathology of tumours of the nervous system. 5th ed. London: Edward Arnold; 1989
19. Burger PC, Scheithauer BW. Tumors of the central nervous system. Washington: Armed Forces Institute of Pathology, 1994
20. Kleihues P, Burger PC, Plate KH, Ohgaki H, Cavenee WK. Glioblastoma. In: Kleihues P, Cavenee WK, eds. Pathology and genetics. Tumours of the nervous system. Lyon: International Agency for Research on Cancer, 1997;1:16–24
21. Lantos PL, VandenBerg SR, Kleihues P. Tumours of the nervous system. In: Graham DI, Lantos PL, eds. Greenfield's neuropathology. 6th ed. London: Edward Arnold; 1996;9:583–79
22. 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;6:217–24
23. Biernat W, Kleihues P, Yonekawa Y, Ohgaki H. Amplification and overexpression of *MDM2* in primary (de novo) glioblastomas. J Neuropathol Exp Neurol 1996;56:180–85
24. 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;94:303–9
25. Hunter SB, Abbott K, Varma VA, Olson JJ, Barnett DW, James CD. Reliability of differential PCR for the detection of *EGFR* and *MDM2* gene amplification in DNA extracted from FFPE glioma tissue. J Neuropathol Exp Neurol 1995;54:57–64
26. Rollbrocker B, Waha A, Louis DN, Wiestler OD, von Deimling A. Amplification of the cyclin-dependent kinase 4 (CDK4) gene is associated with high cdk4 protein levels in glioblastoma multiforme. Acta Neuropathol 1996;92:70–74
27. Myers MP, Stolarov JP, Eng C, et al. P-TEN, the tumor suppressor from human chromosome 10q23, is a dual-specificity phosphatase. Proc Natl Acad Sci USA 1997;94:9052–57
28. Chuang JZ, Lin DC, Lin S. Molecular cloning, expression, and mapping of the high affinity actin-capping domain of chicken cardiac tensin. J Cell Biol 1995;128:1095–1109
29. Miyamoto S, Teramoto H, Coso OA, et al. Integrin function: Molecular hierarchies of cytoskeletal and signaling molecules. J Cell Biol 1995;131:791–805
30. Miyamoto S, Akiyama SK, Yamada KM. Synergistic roles for receptor occupancy and aggregation in integrin transmembrane function. Science 1995;267:883–85
31. von Deimling A, Louis DN, von Ammon K, et al. Association of epidermal growth factor receptor gene amplification with loss of chromosome 10 in human glioblastoma multiforme. J Neurosurg 1992;77:295–301
32. Mollenhauer J, Wiemann S, Scheurlen W, et al. 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
33. Fults D, Brockmeyer D, Tullous MW, Pedone CA, Cawthon RM. *p53* mutation and loss of heterozygosity on chromosomes 17 and 10 during human astrocytoma progression. Cancer Res 1992;52: 674–79

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