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Advances in Brief

PTEN Gene Mutations Are Seen in High-Grade but not in Low-Grade Gliomas[1]

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Abstract

The *PTEN* gene, located on 10q23, has recently been implicated as a candidate tumor suppressor gene in brain, breast and prostate tumors. In the present study, 123 brain tumors, including various grades and histological types of gliomas occurring in children and adults, were analyzed for *PTEN* mutations by SSCP assay and sequencing. Mutations in the *PTEN* gene were found in 13 of 42 adult glioblastomas and 3 of 13 adult anaplastic astrocytomas, whereas none of the 21 low-grade adult gliomas or the 22 childhood gliomas of all grades showed mutations. The single medulloblastoma with a mutation was a recurrent tumor that also possessed a *p53* mutation. High-grade adult gliomas with *PTEN* mutations included cases that also contained gene amplification or *p53* gene mutations, as well as cases that did not contain either of these abnormalities. There was no obvious relationship between presence of *PTEN* mutation and survival; however, there was a tendency for *PTEN* mutations to occur in older age group patients. This analysis suggest that *PTEN* gene mutations are restricted to high-grade adult gliomas and that this abnormality is independent of the presence or absence of gene amplification or *p53* gene mutation in these tumors.

Introduction

Cytogenetic and LOH[3] studies have demonstrated that the most frequent genetic abnormality in malignant gliomas, particularly glioblastomas, is loss of all or part of chromosome 10 (1, 2). Two recent reports have indicated that a tumor suppressor gene located on 10q23 may account for the high incidence of chromosome 10 loss seen among glioblastoma tumors (3, 4). The protein encoded by this gene has domains homologous to protein phosphatases and the cytoskeletal proteins tensin and auxillin. The incidence of inactivating mutations in primary glioblastoma tumor samples was about 20% (3 of 18 and 6 of 26), whereas glioma cell lines exhibited a combined homozygous deletion and mutation rate approaching 60% (5 of 8 and 10 of 17).

Analysis of other genetic markers in astrocytic neoplasms has shown that glioblastomas can be divided into two varieties. One type, designated "*de novo* tumors," frequently has gene amplification, particularly involving the epidermal growth factor receptor gene and lack mutations of the *p53* gene. The other type shows the reverse pattern with no gene amplification but possessing mutations of the *p53* gene and tends to occur in younger patients. Because these features are shared by lower-grade astrocytomas, this group of glioblastomas is hypothesized to originate from lower-grade lesions and its members are thus termed "progressive tumors" (5, 6).

The present study is designed to evaluate the incidence of *PTEN* gene mutations among glioblastomas, as well as other, lower-grade astrocytomas, other types of gliomas, and medulloblastomas. Because data concerning gene amplification and *p53* gene mutations are available for many of these cases, we also sought to determine the relationship between mutations of the *PTEN* gene and other molecular abnormalities seen in these tumors.

Materials and Methods

Tumor Samples. One hundred twenty-three brain tumor samples were obtained from patients during surgery or biopsy. Tumors were classified according to Burger *et al.* (Ref. 7; Table 1). Some of these tumors were the subject of previous studies evaluating chromosomes 10 and 17 for LOH, presence of TP53 mutation, and gene amplification (8–12). Tissue samples were collected fresh at the time of surgery, snap frozen in liquid nitrogen, and kept frozen at −135°C. Cryostat sections from each tumor specimen were examined histologically, and only those blocks of tumor tissue composed of more than 70% neoplastic cells were selected for subsequent DNA isolation. DNA from tumor tissue and corresponding leukocyte DNA were obtained following standard procedures.

SSCP Analysis and Sequencing. Individual exons of the *PTEN* gene were amplified using previously described primers (4, 13). Each PCR mix contained 20 ng of genomic DNA, 1× PCR buffer (Perkin-Elmer Corp.), 0.4 μM of each primer, 0.5 μCi of [^{33}P]dCTP, and 0.5 unit of Taq polymerase in a total volume of 5 μl. Thirty cycles were used for amplification. Each cycle consisted of 1 min at 94°C, 1 min at annealing temperature (52–55°C), and 1 min of extension at 72°C. After completion of PCR except for exon 8, an equal volume of sequencing stop solution containing 20 mM NaOH was added, heated at 95°C, and rapidly cooled on ice, and 1–1.5 μl were loaded on a 6% acrylamide-0.5× TBE (45 mM Tris-borate, pH 8.3, 2 mM EDTA) gel containing 5% glycerol (14). Electrophoresis was carried out at room temperature (30 W for 3–5 h) with a cooling fan. Gels were dried and autoradiographed for 1–3 days. The amplification product of exon 8 was digested with Taq I at 65°C for 1 h by adding 1 unit of enzyme directly to the PCR reaction before SSCP analysis. Tumor samples exhibiting SSCP shift were subjected to another round of PCR/SSCP along with the corresponding DNA from normal tissue. The individual exon with SSCP shift was PCR amplified, gel purified, and sequenced using a ThermoSequenase cycle sequencing kit (Amersham Corp.).

Results

Fifty GBMs (42 adult and 8 childhood), 15 AAs (13 adult and 2 childhood), 22 medulloblastomas, 7 astrocytomas, 14 oligodendrogliomas, 12 pilocytic astrocytomas, 1 supratentorial primitive neuroectodermal tumor, 1 ependymoblastoma, and 1 ganglioglioma were included in the study. All nine exons of the *PTEN* gene were amplified and subjected to SSCP analysis (Fig. 1). Many tumors showed SSCP shift in exon 8 due to polymorphisms. This was confirmed by comparing the SSCP pattern of tumor DNA with the patient blood DNA. We also sequenced exon 8 because of higher incidence of polymorphisms and exons 5 and 6 because of higher frequency of mutations in all GBM and AA tumors with LOH. In other cases, individual exons showing SSCP shift were amplified from the tumor DNA and sequenced.

High-Grade Astrocytomas (Glioblastomas and AAs). SSCP alterations were detected in 10 of 33 GBM tumors with 10q loss, and *PTEN* mutations in these tumors were confirmed by sequencing the

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[3] The abbreviations used are: LOH, loss of heterozygosity; SSCP, single-strand conformational polymorphism; GBM, glioblastoma multiforme; AA, anaplastic astrocytoma.

Table 1 *PTEN mutations in high-grade gliomas*

Tumor	Sex/age	Type	LOH[a]	Exon	Mutation	TP53[b]	Oncogene	Survival[c]
315	M/69	GBM	+			+		7
318	M/58	GBM	−					1.5
332	F/68	GBM	+	6	173 CGC→TGC		EGFR	4.5
340	M/56	GBM	+					15
342	M/62	GBM	+					1.5
365	F/67	GBM	+	9	377 4-bp deletion			27
395	F/60	GBM	+					22
397	M/53	GBM	+				EGFR	3
398	M/75	GBM	+	4	80 AAA→AAT			9
399	M/62	GBM	−					1
405	M/54	GBM	+					6
409	M/57	GBM	+				EGFR	3
423	M/51	GBM	+	6	173 CGC→TGC		GLI	7+
450	M/64	GBM	+				EGFR	16
452	M/75	GBM	−				EGFR	4.5
457	M/39	GBM	+				EGFR	7.7
471	F/81	GBM	+	6	171 CAG→TAG[d]			0
475	F/59	GBM	+	7	248 CCT→ACCT			6
493	F/60	GBM	+				EGFR	8
519	M/62	GBM	+				EGFR	11
522	F/29	GBM	+			+	CDK4	33+
527	M/61	GBM	+	7	214 CAG→TAG			2
529	M/44	GBM	+			+		5
534	M/63	GBM	+	1	15 2-bp insertion[e]		MDM2 GLI	6.5
561	M/63	GBM	+	9	377 4-bp deletion		EGFR	31.8
566	M/69	GBM	+	8	335 CGA→TGA[d]		CMYC	6
599	M/66	GBM	+				EGFR	19
600	M/59	GBM	+			+	EGFR CDK4	13
601	M/64	GBM	+				PDGFRA	1
628	M/54	GBM	+	6	193 3-bp deletion			8
640	F/32	GBM	−					69+
641	M/67	GBM	+				EGFR	2
651	M/35	GBM	−					46
658	M/66	GBM	+					6.8
675	M/62	GBM	−			ND		2.3
743	M/48	GBM	+	5	96 CCA→CTA[d]	ND		13.3
745	M/39	GBM	ND			ND		14
793	M/20	GBM[f]	+			ND		12
860	M/42	GBM	+			ND		20.4+
892	F/63	GBM	+			ND		13.9
930	M/45	GBM[f]	ND	5	132 GGT→AGT	ND		9.2+
502	M/77	GBM	−			+		1
376	F/43	AA	+				EGFR	23
467	M/38	AA	−			+		39
509	M/63	AA	+	5	126 GCT→CCT[d]			18
532	F/74	AA	+	5	130 CGA→TGA			1.5
535	F/27	AA	+			+	CDK4	65
537	M/25	AA	+			+		79.2+
550	M/33	AA	+					22
579	M/42	AA[f]	+			+		9
636	M/46	AA	−					61.6+
652	M/67	AA	+	5	130 CGA→CAA	+		23.7
806	M/61	AA	−			ND		29.2+
808	M/41	AA[f]	ND			ND		36.3
828	F/35	AA	−			ND		30.9+

[a] +, LOH for 10q alleles; −, no LOH; ND, not done.
[b] +, tumor with TP53 mutation.
[c] Survival in months; +, alive.
[d] There was no SSCP shift. Mutation detected by direct sequencing.
[e] Previously published (3).
[f] Recurrent tumor.

altered exon (Fig. 2). Direct sequencing of exons 5, 6, and 8 detected three additional cases involving exons 5, 6, and 8. Altogether, there were four cases of mutations in exon 6; two each in exons 5, 7, and 9; and one each in exons 1, 4, and 8. There were two SSCP alterations among the 13 adult AA tumors analyzed. Both of these were in exon 5. Direct sequencing revealed an additional case with exon 5 mutation. Altogether, there were three cases of *PTEN* mutations among 8 AA tumors with 10q loss (Table 1).

Other Tumors. One of the 22 medulloblastomas analyzed showed SSCP shift, and sequencing confirmed a mutation in exon 5 (tumor 690, codon 130). None of the astrocytomas (7 cases) or oligodendrogliomas (14 cases) had *PTEN* mutations (Table 2). Among pediatric tumors analyzed, no mutations were detected in GBM (8 cases), AA (2 cases), or pilocytic astrocytomas (12 cases), although SSCP alteration was detected in a childhood GBM (exon 3) and a pilocytic astrocytoma (exon 5). Sequencing showed that the SSCP shift in these two tumors was due to polymorphism in the intron (a 4-bp deletion between exons 2 and 3 and a 1-bp insertion between exons 4 and 5).

Discussion

LOH for chromosome 10 alleles is seen frequently in GBM tumors; the reported incidence ranges from 60 to 90% in reports from different laboratories (15–18). Recent studies have suggested that *PTEN*, located on 10q23, may be the target gene on chromosome 10 in glioma and prostate tumors. In the initial studies, the reported incidence of *PTEN* mutation in primary tumors is approximately 20% (3, 4). If *PTEN* is indeed the target suppressor gene on chromosome 10, a high

incidence of *PTEN* mutation is expected in tumors with LOH for chromosome 10 alleles. SSCP analysis is widely used to detect gene mutations mainly due to its simplicity and ability to screen a large number of samples quickly. The present study is designed to evaluate the incidence of *PTEN* mutations among various grades of gliomas.

Most of the tumors in the present series were the subject of previous studies involving LOH analysis, gene amplification, and TP53 mutations. We have reported LOH for 10q loci in 33 of 40 (82%) GBMs and 8 of 12 (75%) AAs. The overall incidence of *PTEN* mutations was 31% (13 of 42) in adult GBM tumors and 23% (3 of 13) in AA tumors. Among LOH cases, the incidence was 13 of 33 (39%) in GBM tumors and 3 of 8 (38%) in AA. Thus, our data suggest that mutations of the *PTEN* gene are restricted to high-grade adult gliomas (glioblastomas and AAs); no mutations were seen in the 21 low-grade adult gliomas (astrocytomas and oligodendrogliomas) or the 22 childhood gliomas. The lone medulloblastoma with a *PTEN* mutation was a recurrent tumor that had been subjected to radiation and chemotherapy and also possessed a mutation of the *p53* gene, therefore representing an unusual case.

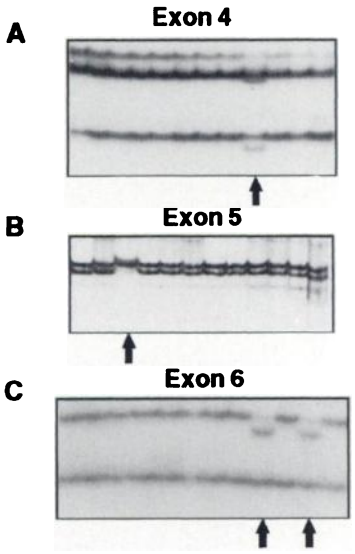

Fig. 1. Examples of SSCP analysis. Portions from autoradiographs of SSCP gels (*A*, exon 4; *B*, exon 5; *C*, exon 6) are shown. *Arrows*, abnormal SSCP band in tumors 398 (exon 4), 690 (exon 5), and 332 and 423 (exon 6).

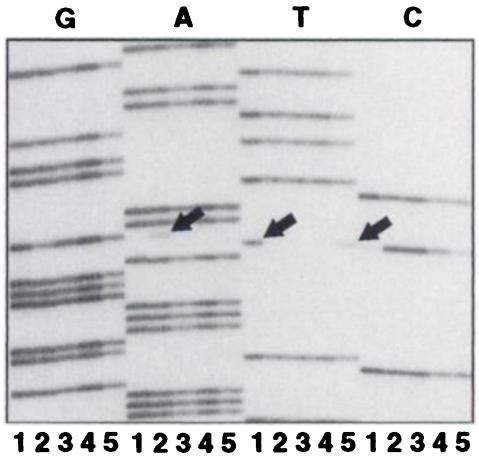

Fig. 2. Examples of sequence analysis. Sequence of nucleotides 373–403 (*bottom* to *top*) in tumors 690, 652, 641, 537, and 532 (*Lanes 1–5*, respectively) is shown. *Arrows*, mutant nucleotide. Exon 5 codon 130 CGA (nucleotides 388–390) is mutated to TGA in tumor 690, CAA in tumor 652, and TGA in tumor 532. There was no change in tumors 537 and 641.

Table 2 *Frequency of PTEN mutations by tumor type*

Tumor type	Mutation frequency
Adult GBM	13/42
Adult AA	3/13
Adult astrocytomas	0/7
Childhood GBM	0/8
Childhood AA	0/2
Medulloblastoma	1/22[a]
Oligodendroglioma	0/9
Anaplastic oligodendroglioma	0/5
Pilocytic astrocytoma	0/12
PNET	0/1
Ependymoblastoma	0/1
Ganglioglioma	0/1

[a] The medulloblastoma tumor (tumor 690) contained exon 5 codon 130 CGA→TGA mutation.

Large homozygous deletions covering the entire *PTEN* gene have been reported in gliomas (3). We have not investigated in detail homozygous deletions of the *PTEN* gene in this series of tumors, and thus the observed frequency of alteration of the *PTEN* gene may represent underestimates of actual incidence. Mutations in the regulatory regions of the gene, as well as methylation of the gene, may also represent additional mechanisms of inactivation of this gene in gliomas. These or other mechanisms may be responsible for inactivation of the *PTEN* gene in the gliomas in this series with 10q LOH in which no *PTEN* mutation was demonstrated. Alternatively, the possibility of another chromosome 10 suppressor gene cannot entirely be excluded.

The 42 adult glioblastomas included 17 cases with gene amplification, 5 cases with *p53* gene mutations, 2 cases with both of these features, 15 cases with neither gene amplification nor *p53* gene mutations, and 7 cases not analyzed. Five of the 13 GBM tumors with *PTEN* mutations also contained gene amplification; another 5 GBMs with *PTEN* mutations did not show either gene amplification or *p53* gene mutation; and in 2 of the cases, the p53 status is not known. None of the 5 GBMs with *p53* mutations had *PTEN* mutations. Among the 13 adult AA tumors, there were 2 cases with gene amplification, 5 cases with *p53* gene mutations, 1 case with both features, 4 cases without either abnormality, and 3 undetermined cases. None of the 3 *PTEN* mutants in the AA group carried gene amplification, although 1 of them had a *p53* mutation.

Multivariate analysis for survival in the present series of tumors was not possible due to the small numbers of patients in each histological and age group and the variable therapy which they received. Although no obvious relationship between survival and presence of *PTEN* mutation was observed, there was a tendency for *PTEN* mutation to occur in older age group patients. In this series, the age of the patient and histological grade of the tumor appeared to be the best predictors of survival.

In the present series, 6 of the 17 mutations detected were in exon 5, and 4 of 17 were in exon 6. Codons 126–132 were mutated in 5 cases, of which codon 130 accounted for 3 cases. Previous studies have shown codon 129 mutation in a GBM (3) and in 2 Cowden disease patients (13) and a codon 130 mutation in a glioma (4). These codons are in the tyrosine phosphatase homologous region of the *PTEN* gene and may represent a hot spot for mutations.

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