MALIGNANT GLIOMA: THE INVOLVEMENT OF LOSS OF ALLELIC HETEROZYGOSITY AND *PTEN* MUTATIONS IN A GROUP OF MALAY PATIENTS

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Abstract. Frequent loss of heterozygosity (LOH) and mutations of the tumor suppressor gene *PTEN* (phosphatase and tensin homologue deleted from chromosome 10) have been found in sporadic gliomas. The most documented regions of allelic losses include 9p21, 10q23-25 and 17p13 whereas *PTEN* aberrations are preferentially found in glioblastoma multiformes. This research aimed to detect the incidence of allelic losses on chromosomes 10q, 9p, 17p and 13q and mutations on exons 5, 6 and 8 of *PTEN* in malignant gliomas. Malignant glioma specimens obtained were classified histopathologically according to the WHO criteria. Each tumor was then subjected to polymerase chain reaction (PCR)-LOH analysis using microsatellite markers and single-stranded conformational polymorphism (SSCP) analysis. Twelve of 23 (52%) malignant glioma cases showed allelic losses whereas 7 of 23 (30%) samples showed aberrant band patterns and mutations of *PTEN*. Four of these cases showed LOH in 10q23 and mutations of *PTEN*. The data on LOH indicated the involvement of different genes in the genesis of glioma whereas mutations of *PTEN* indicated the role of *PTEN* tumor suppressor gene in the progression of glioma in Malay population.

INTRODUCTION

Increasing numbers of genetic alterations are involved in tumor progression. One of the alterations that occur at high frequency in a variety of human tumors is loss of heterozygosity (LOH) at chromosome 10q23. This change appears to occur late in tumor development. Although rarely seen in low-grade glial tumors, LOH at 10q23 occurs in approximately 70% of glioblastomas (Rasheed *et al,* 1997; Ali *et al,*1999). The pattern of LOH and the finding that wild-type chromosome 10 suppresses the tumorigenicity of glioblastoma cells in mice suggest that 10q23 encodes a tumor suppressor gene involved in gliomagenesis (Li *et al,* 1997).

Loss of heterozygosity at non-random frequency of different loci has been consistently reported in sporadic gliomas. The most frequently documented regions of allelic losses include 9p21, 10q23-25 and 17p13 (Watling *et al,*1995). Although not strongly associated with LOH in brain tumors, allelic losses on chromosome 13q in malignant astrocytomas have been documented previously (Lee *et al,*1995). Frequent LOH at 10q23 and mutations of the tumor suppressor gene located on 10q23.3, the *PTEN* (phosphatase and tensin homologue deleted from chromosome 10) gene, have been found in various types of cancer, including gliomas (Li *et al,*1997; Schmidt *et al,*1999). *PTEN* mutations are found distributed along the entire gene (Schmidt *et al,*1999).

In malignant astrocytomas, consistent and frequent allelic losses were detected on chromosomes 10, 13q, 17p and 22q, suggesting the presence of tumor suppressor genes on these chromosomes (Lee *et al,*1995). In glioblastomas with partial loss of chromosome 10, at least three common deletions have been identified, 10p14-pter, 10q23-24 and 10q25-qter, suggesting the presence of multiple tumor suppressor genes (Fujisawa *et al,* 2000). In 40-60% of glioblastomas, deletion was found involving chromosome

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9p21 (Cheng *et al,* 1999). LOH on chromosome 17p was observed in anaplastic oligodendroglioma cases (Lee *et al,*1995). LOH on chromosome 10 (Sonoda *et al,*1996) and 13q (Lee *et al,*1995) was found in most anaplastic ependymoma cases.

PTEN abnormalities have been identified in various types of human carcinoma, including gliomas (Li *et al,* 1997; Myers *et al,* 1997; Sakai *et al,* 1998, Hahn *et al,* 1999; Sato *et al,* 2000). Mutations of *PTEN* are restricted to high-grade rather than low-grade gliomas and may be associated with the transition from a low histological grade to anaplasia (Rasheed *et al,* 1997; Maier *et al,* 1998; Davies *et al,* 1999; Hill *et al,* 1999). *PTEN* mutations are preferentially found in glioblastoma multiformes (Rasheed *et al,* 1997). Deletions of the *PTEN* region at 10q23 are also reported as predominant findings in glioblastomas (Watling *et al,* 1995; Li *et al,* 1997; Rasheed *et al* 1997). *PTEN* aberrations are detectable in a low fraction (<10%) of anaplastic astrocytomas and anaplastic oligodendrogliomas and when present, indicate a poor prognosis (Knobbe *et al,* 2002). *PTEN* mutations are either rare or absent in glioneural tumors, WHO grade I and II astrocytic, oligodendroglial and mixed gliomas, as well as low-grade and anaplastic ependymal tumors (Knobbe *et al,*2002).

The present research aimed at detecting allelic losses or LOH at specific loci on chromosomes 10q, 9p, 17p and 13q which might harbor genes or tumor suppressor genes. In addition, this research aimed to identify mutations occurring on exons 5, 6 and 8 of *PTEN* tumor suppressor gene frequently implicated in sporadic brain tumors.

MATERIALS AND METHODS

Specimens and DNA samples

The materials and methods in this study were approved by the Research and Development Committee of the School of Medical Sciences, Universiti Sains Malaysia (USM), Kelantan. Twenty-three malignant glioma specimens collected between 1997 and 2001 were obtained from Malay patients referred to USM Hospital. The tumors were classified histopathologically according to the WHO criteria (Sato *et al*, 2000). The specimens included 7 glioblastoma multiforme, 9 anaplastic astrocytomas, 1 anaplastic pleomorphic xanthoastrocytoma, 4 anaplastic oligodendrogliomas and 2 anaplastic ependymomas. Non-glioma samples that were obtained from tissue samples diagnosed histopathologically as having no tumor cells and from peripheral blood of persons with no major genetic disease were used as normal controls. Genomic DNA was extracted from the tumor and non-glioma tissues for LOH and *PTEN* mutational analysis using standard procedures.

PCR-LOH analysis using microsatellite markers

LOH was detected in the DNA samples using highly polymorphic microsatellite markers on chromosome 10q, D10S532 (10q22), D10S541 (10q23) and D10S216 (10q25); on chromosome 9p21, D9S165 and D9S162; on chromosome 17p13, D17S786 and D17S1176 and on chromosome 13q12, D13S289 and D13S171 (http://www.gdb.org). Thirty cycles of PCR were performed in a reaction mixture of 50μl in an Eppendorf Mastercycler (Eppendorf, GmbH Germany). PCR products were loaded onto a denaturing polyacrylamide gel and electrophoresed. The gel was then visualized by silver staining. All samples, in which two distinct alleles of similar intensity were present in the normal DNA, were considered to be informative. LOH was scored as positive when a clear reduction of signal intensity (or more than 50%) detected in one of the alleles of the tumor DNA compared with the paired normal DNA (Scarisbrick *et al,* 2000). In instances where more than one band was present within each allele, the exact position of each allele was decided by comparing the banding pattern in all samples analyzed with the same marker and selecting the most consistent pattern: the two alleles were identified as two groups of bands of similar number and similar signal intensity. Densitometry was undertaken on samples where the reduction in signal intensity was difficult to quantify visually. All samples showing LOH were subjected to repeat amplification and analysis for confirmation.

PCR-SSCP analysis of *PTEN*

Prescreening for mutations on *PTEN* was carried out by PCR-SSCP analysis of exons 5, 6

and 8 using previously described primers (Sakai *et al,*1998). All PCR reactions were performed with an Eppendorf Gradient Mastercycler (Eppendorf, GmbH Germany) in a 50 μl volume assay containing 100 ng of genomic DNA, 25 pmol of each oligonucleotide primer, 0.2 mM of dNTP mix, 1X PCR buffer containing Tris-HCl (pH 8.8), $(NH_4)_2SO_4$ and 0.1% Tween 20, 0.05 U/μl of Taq DNA Polymerase (Fermentas GmbH, Germany) and 2.0 mM of $MgCl_2$, plus 6% dimethyl sulfoxide for the primer set of exon 5. After an initial denaturation at 94ºC for 3 minutes, 30 cycles of denaturation (94ºC for 1 minute), annealing (55ºC for the primer set of exon 5 and 58ºC for the primer set of exon 6 and 8) and extension (72ºC for 1 minute) were performed. The final extension was performed for 10 minutes. For SSCP analysis, 8 μl of the PCR product were mixed with 4 μl of sequencing gel loading dye (Amresco, USA) and were heat-denatured at 95ºC for 4 minutes. The denatured products were then immediately cooled in ice. Ten μl of samples of exon 6 were loaded onto an MDE gel (FMC Corp, Rockland, ME) containing 10% glycerol, while samples of exons 5 and 8 were analyzed using an 8% polyacrylamide gel containing 10% glycerol. To obtain optimal separation of the single-stranded conformers, the ratio of methylene-bis-acrylamide to acrylamide was 1:19. Gel electrophoresis was performed using DCode™ Universal Mutation Detection System gel apparatus (BioRad Laboratories, UK) at 10 to 15W for 16 hours. The temperature was maintained at 17º-22ºC. The gel was then visualized by silver staining (Echt *et al,*1996; Rahman *et al,*2000) after completion of electrophoresis.

Analysis of purified DNA fragment by sequencing

PCR products of *PTEN* with variant SSCP patterns were purified using PCR Purification Kit (QIAgen GmbH, Germany) before proceeding with direct sequence analysis.

Statistical analysis

To examine the correlative trend between LOH as well as *PTEN* mutations with age and gender of the glioma patients, we performed the chi-square test. A $p < 0.05$ was considered statistically significant.

RESULTS

LOH in 10q, 9p, 17p and 13q

Of a total of 23 malignant glioma specimens available for LOH analysis 12 tumors (52%) were shown to have a loss of one allele and 11 tumors (48%) were classified as having no LOH. Representative results of LOH in 10q and 17p are shown in Figs 1 and 2.

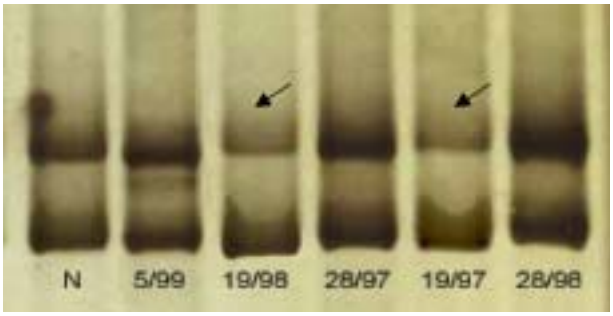

Fig 1–Representative results of LOH on chromosome 10q detected using the microsatellite marker D10S216. The arrows indicate LOH in pleomorphic xanthoastrocytoma (19/98) and glioblastoma multiforme (19/97), with a 50%-90% reduction in signal intensity compared to corresponding normal control.

N = normal control, 5/99 and 19/97 = glioblastoma multiformes, 28/97 = anaplastic astrocytoma, 19/98 = pleomorphic xanthoastrocytoma and 28/98 = anaplastic oligodendroglioma.

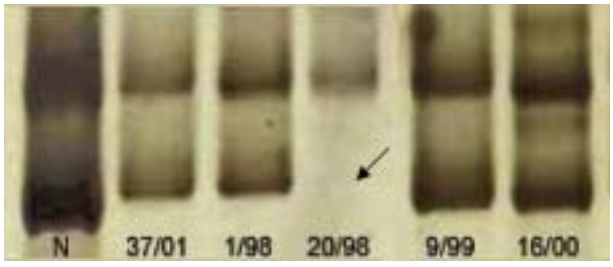

Fig 2–Representative results of LOH on chromosome 17p detected using the microsatellite marker D17S1176. The arrow indicates LOH in glioblastoma multiforme (20/98), with a 50%-90% reduction in signal intensity compared to corresponding normal control.

N = normal control, 37/01 and 20/98 = glioblastoma multiformes, 1/98 = anaplastic oligodendroglioma and 9/99 and 16/00 = anaplastic astrocytomas.

Of 12 cases with allelic losses, 7 (58%) cases demonstrated LOH on chromosome 10q, 3 (25%) cases showed allelic loss on chromosome 9p, 4 (33%) cases exhibited loss of heterozygosity on chromosome 17p and 2 (17%) cases were found to be positive for loss of heterozygosity on chromosome 13q. Four cases showed loss of heterozygosity at more than one locus. The cases with allelic loss included 5 (42%) glioblastoma multiformes (WHO Grade IV), 2 (17%) anaplastic astrocytomas (WHO Grade III), 1 (8%) pleomorphic xanthoastrocytoma (WHO Grade III), 3 (25%) anaplastic oligodendrogliomas (WHO Grade III) and 1 (8%) anaplastic ependymoma (WHO Grade III). The distribution of glioma cases with LOH is summarized in Table 1.

Glioma patients were divided into two age-groups for the evaluation of statistical analysis: patients aged 33 years and below and patients aged above 33 years old. Differences in the frequencies were found for the presence of LOH between both groups of patients. It was observed that the frequency of LOH cases was higher in patients aged above 33 years old (patients aged >33 years old = 58%; patients aged ≤ 33 years old = 42%). However, there is no significant difference between cases with LOH and cases which retain their heterozygosity in the group of patients aged above 33 years old (χ^2 = 0.07; p ≤ 1).

It was observed that the frequency of LOH cases was notably higher in male patients (75%) compared with LOH cases in females (25%). However, there is no significant difference between cases with LOH and cases which retain their heterozygosity in the male patients (χ^2 = 3.49; p ≤ 0.01).

Mutational analysis of *PTEN*

PCR-SSCP analysis of *PTEN* showed aberrant band patterns in 7 of 23 (30%) samples. Sixteen (70%) samples displayed no abnormalities compared to the wild type *PTEN* alleles. Mutations in exon 5 and exon 6 were detected in 5 glioma specimens, with 2 specimens exhibiting mutations of both exons. Representative results of SSCP band aberrations and mutations are shown in Figs 3 and 4.

Of 7 glioma cases with SSCP band alterations, 2 (29%) cases were glioblastoma multiforme (WHO Grade IV), 3 (43%) anaplastic astrocytomas (WHO Grade III), 1 (14%) pleomorphic xanthoastrocytoma (WHO Grade III) and 1 (14%) anaplastic ependymoma (WHO Grade III). Distribution of mutations detected in glioma specimens is listed in Table 2.

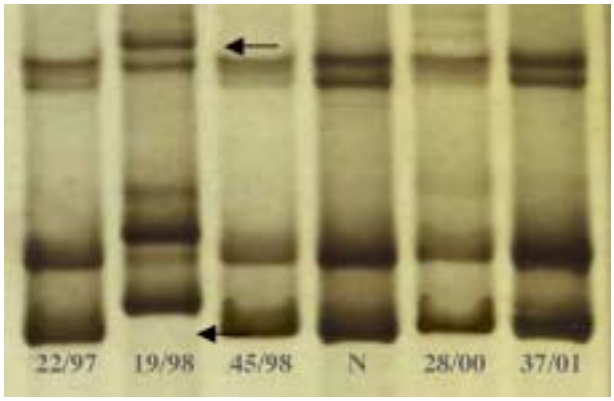

Fig 3–SSCP band aberration of exon 5 of the *PTEN* gene in pleomorphic astrocytoma (19/98). The arrows indicate SSCP band alteration in tumor DNA compared with corresponding normal allele.

N = wild type *PTEN* allele, 22/97 and 19/98 = anaplastic astrocytomas, 45/98 = Grade I astrocytoma, 28/00 = Grade II astrocytoma and 37/01 = glioblastoma multiforme.

DNA sequence analysis of the samples with band alterations revealed point mutations leading to amino acid changes in *PTEN*. Among the base substitutions, 71% were transitions (G:C→A:T, 5 cases) and 29% were transversions (G:C→C:G, 2 cases). Four (57%) of 7 samples revealed missense mutations and 3 (43%) revealed nonsense mutations.

The cases with mutations in exon 5 included 2 anaplastic astrocytoma and 2 glioblastoma multiforme cases. Nonsense mutations were detected in codon 130 in both anaplastic astrocytoma cases whereas in the glioblastoma cases, missense mutations were detected in codons 105 and 124, resulting in the substitution of cysteine for serine. Mutations of exon 6 were detected in 2 anaplastic astrocytoma, 1 anaplastic ependymoma and 2 glioblastoma multiforme cases. Missense mutation In codon 173 in anaplastic astrocytoma and glioblastoma

cases resulted in the substitution of arginine by histidine and arginine by cysteine, respectively. The mutation found in anaplastic ependymoma (codon 171) resulted in the introduction of premature stop codon. Among the glioblastoma multiforme cases with mutations, 2 cases showed aberrant band patterns and mutations in both exons 5 (codon 5) and 6 (codon 173). SSCP band alteration and mutation were not detected in exon 8.

The correlation of glioma cases with mutations with age and gender of patients was evaluated by chi-square test. The mean age of glioma patients in this study was 33.4 ± 17.7 years, with an age range of 3 to 58 years old. There were 13 male and 10 female patients.

The patients were divided into two groups of age for the evaluation of statistical analysis: patients aged 33 years and below and patients aged above 33 years old. Differences in the frequencies were found for the presence of mutations between both groups of patients: 28.6 % in patients aged above 33 years old and 71.4% in patients aged 28 years and below. It was observed that the frequency of mutated cases was significantly higher in patients aged below 33 years old ($\chi^2 = 5.96$; $p \leq 0.025$).

Differences in the frequencies were found for the presence of mutations between male and female patients: 86% in male patients and 14% in female patients. The frequency of mutant cases was notably higher in male patients. However, there is no significant difference between cases with mutations and cases without mutations in the male patients ($\chi^2 = 2.60$; $p \leq 0.20$).

DISCUSSION

The present study is the first to demonstrate that LOH in chromosomes 10q, 9p, 17p and 13q and mutations of the *PTEN* are involved in the progressions of glioma in Malay population. The observed pattern of allelic losses demonstrated that LOH involves several loci and that the particular analyzed regions contain specific tumor suppressor genes, which might be targets for cancer-causing mutations or inactivation (Kleihues *et al,* 2002).

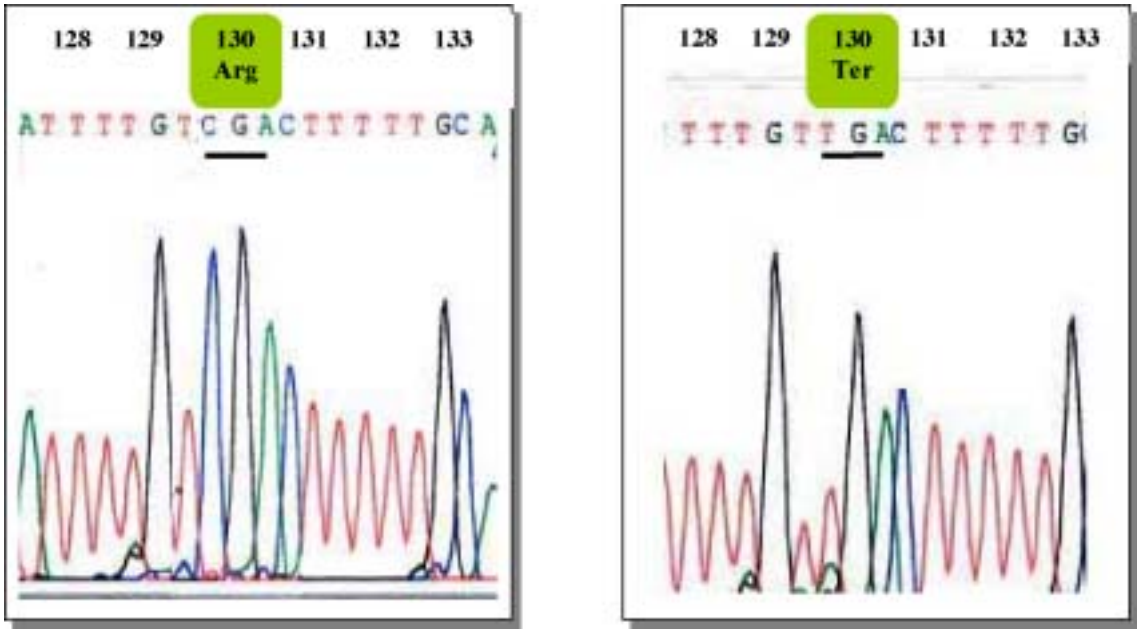

Fig 4–(a) Partial sequence electropherogram of exon 5 of the wild type *PTEN* gene; (b) Partial sequence electropherogram of mutated exon 5 of the *PTEN* gene. The pleomorphic xanthoastrocytoma sample analyzed revealed a transition of C→T at codon 130 resulting in a termination codon (CGA→TGA; Arg→Ter).

Table 1
Distribution of loss of heterozygosity cases on chromosomes 10q, 9p, 17p and 13q in low- and high-grade gliomas according to age, gender and microsatellite markers.

Patient No. (Sex/Age, y)	Diagnosis (WHO Grade)[a]	LOH on 10q			LOH on 9p		LOH on 17p		LOH on 13q	
		D10S532	D10S541	D10S216	D9S165	D9S162	D17S786	D17S1176	D13S289	D13S171
22/97 (M, 32)	AA (III)	LOH	LOH	-	-	-	-	-	-	-
28/97 (F, 56)	AA (III)	-	-	-	-	-	-	-	-	-
26/98 (F, 41)	AA (III)	-	-	-	-	-	-	-	-	-
36/98 (M, 58)	AA (III)	-	-	-	-	-	-	-	-	-
40/98 (M, 36)	AA (III)	-	-	-	-	-	-	-	-	-
42/98 (M, 34)	AA (III)	-	LOH	-	LOH	-	-	-	-	-
9/99 (F, 46)	AA (III)	-	-	-	-	-	-	-	-	-
16/00 (F, 17)	AA (III)	-	-	-	-	-	-	-	-	-
19/00 (M, 12)	AA (III)	-	-	-	-	-	-	-	-	-
19/98 (M, 10)	PXA (III)	LOH	-	LOH	-	-	LOH	-	-	-
1/98 (M, 50)	AO (III)	-	-	-	-	LOH	LOH	-	-	-
8/98 (M, 48)	AO (III)	-	-	-	-	-	-	-	-	LOH
28/98 (F, 18)	AO (III)	-	-	-	-	-	-	-	-	-
4/99 (F, 49)	AO (III)	-	-	-	-	LOH	-	-	-	-
12/98 (F, 3)	AE (III)	-	-	-	-	-	-	-	-	LOH
2/99 (F, 3)	AE (III)	-	-	-	-	-	-	-	-	-
19/97 (M, 55)	GBM (IV)	-	-	LOH	-	-	-	-	-	-
20/97 (M, 8)	GBM (IV)	-	LOH	-	-	-	LOH	-	-	-
20/98 (F, 44)	GBM (IV)	-	-	-	-	-	-	LOH	-	-
27/98 (M, 36)	GBM (IV)	-	-	-	-	-	-	-	-	-
5/99 (M, 27)	GBM (IV)	-	LOH	-	-	-	-	-	-	-
16/99 (M, 40)	GBM (IV)	-	LOH	-	-	-	-	-	-	-
36/01 (F, 46)	GBM (IV)	-	-	-	-	-	-	-	-	-
No. of LOH/total samples (%)			7/23 (30.4%)		3/23 (13.0%)		4/23 (17.4%)		2/23 (8.7%)	
No. of LOH/total cases with LOH (%)			7/12 (58.3%)		3/12 (25.0%)		4/12 (33.3%)		2/12 (16.7%)	

[a]WHO grade: AA = anaplastic astrocytoma, PXA = pleomorphic xanthoastrocytoma, AO = anaplastic oligodendroglioma, AE = anaplastic ependymoma, GBM = glioblastoma multiforme
LOH = case with loss of heterozygosity, - = case which retain heterozygosity

The highest number of cases with LOH was detected on chromosome 10q23.3 harboring *PTEN*. This region was reported to be frequently deleted in LOH studies involving the q arm of chromosome 10 and fine mapping studies of the entire chromosome (Cheng *et al,* 1999; Schmidt *et al,* 1999; Fujisawa *et al,* 2000). LOH was also detected on the regions 10q25.1, 10q22, 9p21 and 13q12.3. These regions have been documented to contain genes or tumor suppressor genes involved in several carcinomas, including malignant gliomas (Schmidt *et al,* 1994; Cheng *et al,* 1999; Lee *et al,* 1995; Walker *et al,* 1995; Watling *et al,* 1995). Less frequent LOH was detected on chromosomes 17p13.1 whereas no allelic loss was detected on the region 13q12.1, suggesting that these regions are not typically involved in the progression of malignant gliomas in Malay patients.

Eleven glioma cases retained their heterozygosity in this study. This presumably occurred because of loss or deletion of both alleles in the tumor DNA so that only the contaminating normal DNA was amplified to produce two alleles with low signal intensity. However, this can only be confirmed by performing a homozygous deletion analysis (Ichimura *et al,*1998). Interestingly, two of the tumors with retention of allele het-

Table 2
Mutations of the *PTEN* gene. 7 of 40 (17.5%) glioma cases showed band alterations in the SSCP analysis of *PTEN* gene. Mutations were detected in exons 5 and 6 and none were detected in exon 8. All aberrations were point mutations, including 4 (57%) missense mutations and 3 (43%) nonsense mutations. Among the base substitutions, 71% were transitions (G:C→A:T, 5 cases) and 29% were transversions (G:C→C:G, 2 cases).

Patient No. (Sex/Age, y)	Diagnosis[a] (WHO Grade)	Exon with SSCP aberration	Codon	Features	Nucleotide change	Amino acid change
22/97 (M, 32)	AA (III)			NA		
28/97 (F, 56)	AA (III)			NA		
26/98 (F, 41)	AA (III)			NA		
36/98 (M, 58)	AA (III)	Exon 6	173	CpG; catalytic domain	C**G**C→C**A**C	Arg→His
40/98 (M, 36)	AA (III)			NA		
42/98 (M, 34)	AA (III)	Exon 6	173	CpG; catalytic domain	C**G**C→C**A**C	Arg→His
19/00 (M, 34)	AA (III)			NA		
9/99 (F, 46)	AA (III)			NA		
16/00 (M, 26)	AA (III)	Exon 5	130	Catalytic core motif; CpG	**C**GA→**T**GA	Arg→stop
19/98 (M, 10)	PXA (III)	Exon 5	130	Catalytic core motif; CpG	**C**GA→**T**GA	Arg→stop
1/98 (M, 50)	AO (III)			NA		
8/98 (M, 48)	AO (III)			NA		
28/98 (F, 18)	AO (III)			NA		
4/99 (F, 49)	AO (III)			NA		
12/98 (F, 3)	AE (III)	Exon 6	171	Catalytic domain	**C**AG→**T**AG	Gln→stop
2/99 (F, 3)	AE (III)			NA		
19/97 (M, 55)	GBM (IV)			NA		
20/97 (M, 8)	GBM (IV)	Exon 5	105	Catalytic domain	T**G**T→T**C**T	Cys→Ser
		Exon 6	173	CpG; catalytic domain	**C**GC→**T**GC	Arg→Cys
20/98 (F, 44)	GBM (IV)			NA		
27/98 (M, 36)	GBM (IV)			NA		
5/99 (M, 27)	GBM (IV)	Exon 5	124	Catalytic core motif	T**G**T→T**C**T	Cys→Ser
		Exon 6	173	CpG; catalytic domain	**C**GC→**T**GC	Arg→Cys
16/99 (M, 40)	GBM (IV)			NA		
36/01 (F, 46)	GBM (IV)			NA		

[a]Diagnosis: AA = anaplastic astrocytoma, PXA = anaplastic pleomorphic xanthoastrocytoma, AO = anaplastic oligodendroglioma, AE = anaplastic ependymoma, GBM = glioblastoma multiforme, M = male, F = female, NA = no abnormalities

erozygosity were glioblastoma multiforme, suggesting a relationship between malignant tumor pathology and more extensive deletions.

Most of *PTEN* mutations detected in the present study were anaplastic astrocytoma and glioblastoma. Five codons containing CpG dinucleotides were found mutated in exons 5 and 6 of *PTEN*, which involved entirely missense and nonsense mutations. The codon most frequently mutated was codon 173 which is conserved in tensin, auxilin and bacterial phosphatase (Ali *et al,* 1999). Mutations of this gene, which lead to amino acid substitutions, may generally affect conserved residues or structurally conserved features of the protein, as demonstrated by Schmidt *et al* (1994). It is believed that the N-terminal half of *PTEN* is functionally more significant for tumor suppression because of homology to tensin, auxilin and phosphatase, regions that may control cell cycle, invasion and metastasis (Ishii *et al,*1999). These findings suggested that mutations of *PTEN* are concentrated

to the N-terminal phosphatase domain with cluster of mutations in the region 5′ to the core phosphatase motif and the 5′-end of exon 6.

Tumors containing *PTEN* mutations also showed loss of heterozygosity in the chromosome 10q23 region flanking the *PTEN* gene. The pattern of allelic losses on 10q23 as well as mutations of the gene itself appear to be associated with the progression of glioma (Ichimura *et al,*1998; Maher *et al,* 2001) and indicated complete loss of the wild-type *PTEN* (Bostrom *et al,*1998). These findings also suggested that *PTEN* gene might be inactivated by point mutations or small deletions (Ichimura *et al,*1998) and that both alleles of the *PTEN* gene were inactivated by a classical two-hit mechanism (Kato *et al,* 2000), therefore confirming the previous idea that *PTEN* acts as a tumor suppressor gene. However, the lack of detectable *PTEN* alterations in a considerable fraction of malignant gliomas with 10q loss suggested that at least one additional tumor suppressor gene responsible in the progression of glioma is located on 10q.

The data obtained on loss of heterozygosity analysis and mutational analysis of the *PTEN* gene have provided relevant information on the presence of putative tumor suppressor genes that might be involved in the pathway of glioma progression in Malay population. In addition, these data provide useful evidence of molecular genetic alterations of malignant glioma in South East Asian patients, particularly in the east coast of Malaysia.

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