J Neurooncol (2007) 82:263–269
DOI 10.1007/s11060-006-9293-z

LABORATORY INVESTIGATION

Impact of novel *PTEN* mutations in Turkish patients with glioblastoma multiforme

Berrin Tunca · Ahmet Bekar · Gulsah Cecener · Unal Egeli · Ozgur Vatan · Sahsine Tolunay · Hasan Kocaeli · Kaya Aksoy

Received: 24 July 2006 / Accepted: 6 November 2006 / Published online: 7 December 2006

Abstract Glioblastoma multiforme (GBM) represents the most common and aggressive type of primary neoplasms of the central nervous system. The *PTEN* (phosphatase, tensin homologue, deleted on chromosome TEN; MIM # 601728) tumor suppressor gene has an essential biological role in the formation of glioblastomas. It is known that there are variations in genetic alterations in tumors that develop in patients with different ethnic backgrounds and because there is no study evaluating *PTEN* mutation in Turkish patients with GBM, we aimed to realize the present study. We investigated 62 GBM tumors for mutations of the *PTEN* gene using single strand conformational polymorphism (SSCP) method followed by DNA sequencing. As a result of our investigation, *PTEN* mutations were detected in 15 of 62 tumors (24.19%). Nine different sequence variants were identified: one novel promoter site mutation (5′UTR –9C → T), one novel intronic mutation (IVS2-2delA), four novel point mutations (61A → G, 105T → G, 248C → G, and 364C → G), two novel frameshift mutations (213delC) and 378delGATA) and one previously reported global exonic transition type mutation (129G → A). Since the majority of *PTEN* mutations identified in the present study are novel, we believe that these alterations may be specific to Turkish population. Furthermore, though no significant correlation was found between *PTEN* mutations and histopathological properties of GBM tumors, our findings indicate that localizations of mutations in *PTEN* gene may have an effect on clinical aggressiveness of GBM tumors.

Keywords Glioblastoma multiforme · *PTEN* · Turkish population · Novel mutations · SSCP · Sequencing

Abbreviations

SSCP	single strand conformational polymorphism
PCR	polymerase chain reaction
LOH	loss of heterozygosity
IVS	intervening sequence

B. Tunca (✉) · G. Cecener · U. Egeli
Department of Medical Biology, School of Medicine, Uludag University, Bursa, Turkey
e-mail: btunca@uludag.edu.tr

A. Bekar · H. Kocaeli
Department of Neurosurgery, School of Medicine, Uludag University, Bursa, Turkey

O. Vatan
Department of Biology, Faculty of Science, Uludag University, Bursa, Turkey

S. Tolunay
Department of Pathology, School of Medicine, Uludag University, Bursa, Turkey

K. Aksoy
Department of Neurosurgery, Acıbadem Hospital, Bursa, Turkey

Introduction

Brain tumors are the eighth most common malignancy in females (representing 3.52% of all neoplasms), and ninth in males (representing 3.40% of all neoplasms) in Turkey. Their incidence in Turkish

women is 1.07/100.000 and in Turkish men is 1.64/100.000 [1]. Glioblastoma multiforme (GBM) is the most common type of primary brain tumor accounting for about 50% of all gliomas and for 12–15% of all intracranial tumors [2]. GBM (WHO grade IV) also represent the most malignant primary neoplasm of the central nervous system [3]. Despite all aggressive treatment regimes, the prognosis remains poor and median survival is generally less than 1 year [4]. A number of studies evaluated genetic alterations associated with the pathogenesis of glioblastomas to identify genetic factors predictive of outcome among patients with GBM [5–10]. The tumorigenesis of GBM has been associated with a number of chromosomal abnormalities such as a gain of chromosome 7 and loss of heterozygosity (LOH) of chromosome 10 and molecular alterations of the *p53, p16* and *PTEN* (phosphatase, tensin homologue, deleted on chromosome TEN; MIM # 601728) tumor suppressor genes [3]. These genetic alterations, particularly the alteration in expression of *PTEN* are correlated with the progression of GBM and a poor patient outcome [11]. Germ-line mutations in the tumor suppressor gene *PTEN*, which encode a lipid phosphatase on 10q23.3 that mediates cell cycle arrest and apoptosis, were first described in Cowden Syndrome in 1997 [12]. In GBM, the prevalence of *PTEN* mutations varies from 14 to 40% [13, 14]. Majority of these studies have been carried out in GBM patients in Europe and the United States. The frequencies and types of genetic alterations may differ in tumors that develop in patients with different ethnic backgrounds. Furthermore, any information on genetic alterations in GBM in Turkish patients was not available until now.

In this study, we aimed to investigate both the types and frequencies of *PTEN* gene alterations in Turkish patients with GBM and determine the relationship between alterations of this gene and the histopathological and clinical properties of patients.

Materials and methods

Patients

A total of 62 GBM patients who were scheduled for surgical treatment, fulfilling all inclusion criteria as assessed by conventional histopathological examination, were evaluated prospectively. Institutional Review Board approval was taken, and written informed consent of the patients was obtained. Clinical and histopathological features of the tumors and oncological outcome were also evaluated with regard to the presence of *PTEN* mutation in the tumors. Morphologic parameters for assessessment included predominant cell types (e.g., fibrillary, gemistocytic, oligodendroglial), cell proliferation, necrosis, microvascular proliferation and sarcomatous growth. The expression of GFAP was also determined. The patients were followed up for at least 24 months or until death.

Mutation detection

Genomic DNA was extracted using the phenol/chloroform method (Amresco® 0883-Q-30ML, Ohio, USA) from each tissue sample taken from the tumors at the time of surgery. PCR primer pairs were designed based on the reported genomic sequence of *PTEN*. Primers were used to amplify the coding regions including exon–intron boundaries (Table 1). Exons 1–9 of *PTEN* gene were subjected to polymerase chain reaction (PCR) analysis. A 25-μl reaction mixture was used for PCR, and this volume contained 0.05 mM of each deoxyribonucleoside triphosphate (dNTP-Sigma® D-7295, Taufkirchen, Germany), 10 pmol of each primer (Table 1), 1 unit of AmpliTaq DNA polymerase (Sigma® D-6677, Taufkirchen Germany), and 100 ng of genomic DNA. The PCR program for amplification of each exon is as follows: 40 cycles of denaturation (94°C for 60 s); then an annealing step (50 or 55°C for 60 s) (Table 1); then an extension step (72°C for 60 s); and finally 7 min of extension at 72°C. PCR products were

Table 1 Primers for *PTEN* SSCP analysis and sequencing

Exon	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	PCR annealing Temperature (°C)
1	CAAGTCCAGAGCCATTTCCAT	AAGAGGAGCAGCCGCAGAAAT	50
2	ATTCACATGTAACTTCTTTTA	CAACATGAATATAAACATCAA	50
3	TAATTTCAAATGTTAGCTCAT	AAGATATTTGCAAGCATACAA	50
4	GTTTGTTAGTATTAGTACTTT	ACAACATAGTACAGTACATTC	50
5	CTAAAGTTACCTACTTGTTAAT	AGGAAAAACATCAAAAAATAAC	50
6	ATATGTTCTTAAATGGCTACG	ACATGGAAGGATGAGAATTTC	55
7	CGTGTATATTGCTGATATTAAT	CTCCCAATGAAAGTAAAGTACA	55
8	ATGTTTAACATAGGTGACAGA	ACACATCACATACATACAAGT	55
9	GTTTAAGATGAGTCATATTTG	TGGTGTTTTATCCCTCTTGAT	55

checked on 2% agarose gel stained with ethidium bromide. The high-quality amplified products were assessed with single strand conformational polymorphism (SSCP) analysis. In order to do this, 7 μl of each PCR product were added on 3 μl of stop solution (95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol) and were heat-denatured at 95°C for 6 min, then tubes were immediately placed on ice and kept there until samples were loaded into the gel. Four microliters of each processed PCR product were electrophoresed on 1× MDE™ gels (FMC Bioproducts, Rockland, Maine, USA). These were run at 600 V for 10 h at room temperature in 0.5× TBE buffer solution (89 mM tris-base, 89 mM boric acid, 2 mM EDTA, pH 8.0). The gels were stained using a non-radioactive silver staining method, and results were visualized and photographed with an imaging analyzer (Vilber Laurmat, Marne La Valle, France). Samples that showed one or two bands separated from the wild-type bands were identified as SSCP-positive. All the samples that contained mutations were subjected to the SSCP analysis procedure at least twice to rule out contamination.

Sequencing analysis

All samples with different SSCP bands were sequenced using the ABI PRISM dye terminator cycle sequencing ready reaction kit (Perkin Elmer, Almeda, USA) and the *PTEN* primers which were used for PCR on an ABI 3700 (Applied Biosystems, Foster City, CA) automated sequencer. Results of sequencing analysis were compared with wild type samples and normal sequencing of *PTEN* gene (MIM# 601728, GenBank accession no. U93051).

Statistical analysis

Chi-squared tests (χ^2) were used for comparing results. Pearson's (χ^2) test and Fisher's exact test were used to compare *PTEN* gene mutations and properties of adverse prognostic factors. Differences with a $P > 0.05$ were accepted as statistically non-significant. Statistical Package for the Social Sciences for Windows, release 11.0.5, was used for all statistical analyses (SPSS, Chicago, IL, USA).

Results

We investigated the coding region of the *PTEN* gene for PCR-SSCP and sequence analysis. using the intronic primer pairs in 62 GBM. The age range of the patients was 25–76 years (mean age ± SEM, 56.24. ± 1.66 years). In total, there were 39 male and 23 female patients. All tumors were de novo gliomas and located supratentorially. In histopathological reevaluation, all of them were diagnosed as GBM according to the WHO criteria for the classification of central nervous system tumors [13]. Histopathological features of the tumors are summarized in Table 2. The mean survival time was 7.73 ± 0.73 months.

The presence of *PTEN* mutations was detected in the tumors of 15 patients (15/62; 24.19%) by SSCP. Mutations were clustered in 5′ UTR (1/62; 1.61%), intron 2 (1/62; 1.61%), exon 5 (4/62; 6.45%), exon 7 (5/62; 8.06%) and exon 9 (3/62; 4.84). As a result of sequencing analysis for SSCP positive samples, nine different gene alterations were determined (Table 3). One of them was previously identified (G → A transition at codon 129). Other eight gene alterations were novel. These alterations were one promoter site point mutation [C → T transition at 5′ UTR site (nine base

Table 2 Histopathological and immunohistochemical features in 62 glioblastomas

	n	%
Predominant cell type		
Small undifferentiated	20	32.2
Fibrillary	21	33.9
Gemistocytic	11	17.7
Giant cell	2	3.2
Spindle cell	0	0
Oligodendroglial	8	12.9
Other major cell types		
Small undifferentiated	4	6.5
Fibrillary	15	24.2
Gemistocytic	9	14.5
Giant cell	23	37.0
Spindle cell	0	0
Oligodendroglial	11	17.7
Not specified	0	0
Necrosis		
Not present	0	0
Present	62	100
Microvascular proliferation		
Not present	3	4.8
Moderate	38	61.3
Extensive	21	33.9
Sarcomatous growth		
Not present	58	93.5
Present	4	6.5
Expression of GFAP		
Not present	0	0
Present	62	100
Cell proliferation (KI 67)		
<2%	15	24.2
2–4%	28	45.2
>4.1%	7	11.3
Not stained	12	19.4

Table 3 Summary of sequence variants determined in this study

Subject No	Exon/ Intron	Localization	Nucleotide transition	Codon transition	Amino acid transition	Mutation type	Previously described
6	5′ UTR site	9 bp upstream of exon 1	C → T	–	–	Promoter site point mutation	–
2	IVS 2	2 bp upstream of exon 3	del A	–	–	Splice site	–
49	Exon 3	Codon 61	A → G	CAT → CGT	His → Arg	Missense	–
15,28,43	Exon 5	Codon 105	T → G	TGT → TGG	Cys → Trp	Missense	–
45	Exon 5	Codon 129	G → A	GGA → AGA	Gly → Arg	Missense	+
52	Exon 7	Codon 213	del C	CCT → CTC	Pro → Leu	Frameshit caused premature stop codon at downstream 7 codon	–
11,36,46,47	Exon 7	Codon 248	C → G	CCT → CGT	Pro → Arg	Missense	–
10,50	Exon 9	Codon 364	C → G	TCT → TGT	Ser → Cys	Missense	–
8	Exon9	Codon 378	del GATA	AGA TAT → ATA CTG	Arg Tyr → Ile Leu	Frameshift caused alienated stop codon site	–

IVS: intervening sequence; del: deletion

pairs upstream of exon 1)], one intronic mutation (a deletion at two base pair upstream of exon 3), four point mutations (61A → G, 105T → G, 248C → G and 364C → G), two frameshift mutations (C deletion at codon 213 and GATA deletion at codon 378) (Table 3, Fig. 1).

The mean age of GBM patients with *PTEN* mutations (55.27 ± 3.78 years) was similar to that of patients without *PTEN* mutations (56.55 ± 1.86 years). The mean survival of GBM patients with one of *PTEN* mutations (7.33 ± 1.72 months) was similar to that of patients without *PTEN* mutations (7.85 ± 0.81 months; $P = 1.000$). However, when the cases that had different *PTEN* mutations were examined individually, certain cases who had mutations such as 5′ UTR –9C → T, 61A → G, 105T → G, 129G → A, 213delC, and 378delGATA had a shorter survival than the others (Table 4). However, due to insufficient number of cases in these groups, statistical analysis was not performed. An association was not determined between

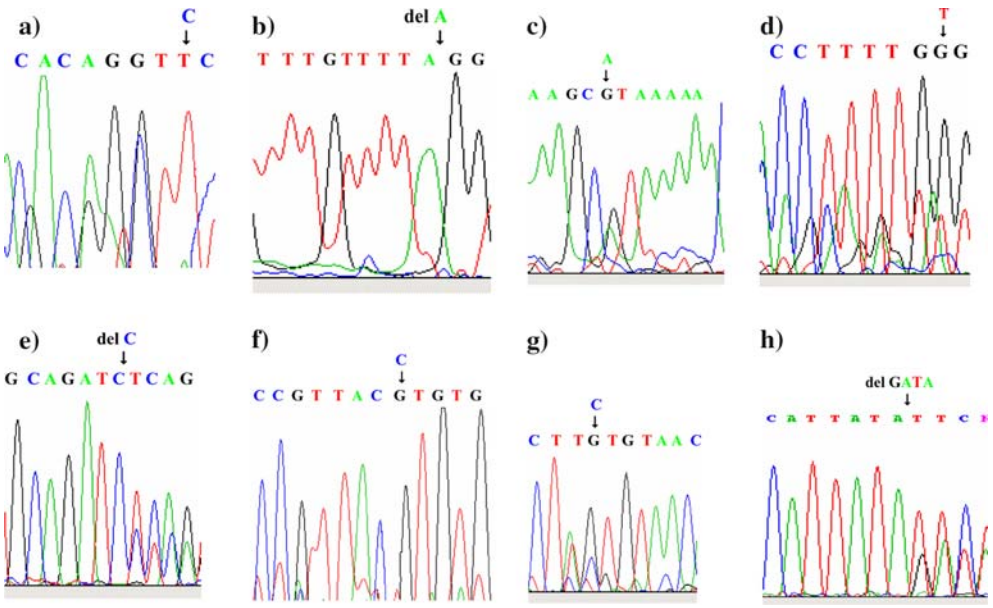

Fig. 1 Novel sequence alterations in *PTEN* gene; (**a**) 5′ UTR –9C → T, (**b**) IVS2 –2 delA, (**c**) 61A → G, (**d**) 105T → G, (**e**) 213delC, (**f**) 248C → G, (**g**) 364C → G, (**h**) 378delGATA

Table 4 Molecular alterations, sex, age, and survival in 62 GBM cases

Gene/ Mutation type	Status	*n*	Age (years)	Sex F/M	Survival (Months)
PTEN	Wt	47	56.55	20/27	7.85
	Mut	15	55.27	3/12	7.33
5′ UTR –9C → T		1	72	0/1	1
IVS –2 del A		1	50	0/1	20
Codon 61 A → G		1	68	0/1	9
Codon 105 T → G		3	47.6	1/2	3.3
Codon 129 G → A		1	74	1/0	1
Codon 213 del C		1	56	0/1	8
Codon 248 C → G		4	47.5	1/3	10.25
Codon 364 C → G		2	55.5	0/2	9.5
Codon 378 del GATA		1	65	0/1	1

PTEN mutations and histopathological parameters such as cell types, cell proliferation, necrosis, microvascular proliferation, sarcomatous growth, and expression of GFAP (Table 2; $P > 0.05$).

Discussion

Glioblastomas are the most frequent and malignant human brain tumors, and despite advances in surgical and clinical neuro-oncology and combined therapy regimes, their prognosis remains poor [5]. In the present study, only a quarter of cases had a survival between 12 and 24 months, the remaining ones had a survival less than 12 months. These rates are in accordance with that of population-based studies [5, 14, 15]. Molecular studies indicates that the variation in expression of *PTEN* which has important roles in the regulation of cell proliferation, apoptosis and tumor invasion is related with the progression of GBM and a poor disease outcome [5, 11]. In GBM, the prevalence of *PTEN* mutations varies from 14 to 40% [13, 14]. The frequencies and types of mutations may vary in tumors of patients in different populations [16]. We evaluated 62 patients with GBM and found mutations in 24.19% of the patients (15/62) by SSCP technique. In spite of incapacity of this method in detection of large deletion to cause loss of exons, SSCP technique is sensitive as 95% as in evaluation of point mutation for especially small amplification products of tumor tissues. This is the first report evaluated *PTEN* mutation in Turkish patients with GBM. We detected nine different gene alterations (Table 3), and only one of them was previously described (G → A transition at codon 129) [12]. Other eight gene alterations were novel. Furthermore, it is meaningful that none of these mutations are previously reported SNPs according to the Enseble site [17]. Novel alterations were identified as one promoter site point mutation [C → T transition at 5′ UTR site (nine base pairs upstream of exon 1)], 1 intronic mutation (A deletion at two base pairs upstream of exon 3), four point mutations (61A → G, 105T → G, 248C → G and 364C → G) and two frameshift mutations (C deletion at codon 213 and GATA deletion at codon 378) (Table 3, Fig. 1). The fact that the majority of these alterations are novel supports the suggestion that there is variability in mutations underlying the tumors of GBM patients in different populations [16]. Moreover, we believe that these mutations may be specific for Turkish population. Further studies using larger GBM tumor samples should contribute to these findings.

The substitution of C with T at position –9 located in the untranslated region of exon 1 was determined in one GBM tumor (1/62, 1.61%). Similarly, Ishihara et al. identified the substitution of C with G at the same position of *PTEN* gene in their study on type 2 diabetes mellitus patients [18]. They determined that this mutation lead to alterations of Akt pathway by occurrence of greater expression of *PTEN*. We do not have any information about role of mutations at this consensus sequence for the initiation of *PTEN* translation in the formation of GBM. Yet, we think that alterations in this important sequence may have a role in the occurrence of glial tumors. As a matter of fact, the patient who had a mutation at this site had a survival of only 1 month and this supports our argument about mutations at this site.

We determined three different alterations localized in the beginning exons of *PTEN* gene (codons 61, 105 and 129). These codons localized in the N-terminal phosphatase domain which has an important biological role in lipid and protein phosphatase activity of PTEN protein. This domain is defined by the first 185 amino acids (exons 1–6) of *PTEN* gene [12]. Although it was previously described as a common mutated codon in tumors, the missense change identified at codon 129 was determined only in one tumor (case#45). Codon 129 encodes amino acid which is an item of CKAGKGR phosphatase catalytic core motive of N-terminal phosphatase domain of *PTEN*. In the literature, approximately 30% of all germ-line and somatic mutations have been determined in this catalytic core. In vitro studies showed that *PTEN* mutations occurred in the catalytic core motive caused loss of lipid phosphatase activity [12].

In addition, we determined an adenine deletion in intron 2 at position –2. Eng declared that the substitution of A with G at the same position was identified in

Cowden Syndrome [12]. Agrawal et al. reviewed that mutations in splice sites may cause to exon skipping [19]. Our mutation point localized on splice site may also cause to skipping of exon 3 and so, this may give rise to a decrease in N-terminal phosphatase domain. Despite the core motive have an essential role in phosphatase activity [20], to carry out this activity; it needs the whole part of N-terminal domain [12]. If our point mutation really caused to skipping of exon 3, it might lead to eradication of phosphatase activities. To find an explanation, the effects of this mutation on the function of PTEN protein must be investigated by in vitro studies.

Approximately 40% of all *PTEN* mutations described in the literature occur in the C terminal C2 domain which is associated with phospholipid binding [12]. Sixty percent of all gene alterations determined in our study (9/15) were also localized at C terminal C2 domain defined by amino acids 186–402. Moreover, two of them were localized in PEST domains which regulate protein stability and important in protein–protein interactions. These PEST domains are defined by amino acids 350–375 and 379–396. Eng stated that in spite of determination of numerous *PTEN* mutations in the area localized upstream in the PEST domains, no mutations have been found in these domains in vivo [12]. So, our study is the first to report mutations in the PEST domains. Although the phosphatase activity of PTEN protein plays an essential role in its tumor suppressor effect, in vitro studies dealing with the structure of PTEN protein showed that the deletion of the PEST sequence in wild type *PTEN* reduced its tumor suppressor activity [21]. Georgescu et al. [22] indicated that mutations in the C-terminal region inactivate the tumor suppressor function of *PTEN* by affecting its intrinsic phosphatase activity most likely as a result of conformational changes. These investigators also were explained that the C-terminal region of *PTEN* contains predicted secondary structure elements that are essential for the tumor suppressor function of protein [22, 23]. The survival in two of three patients who had mutations in the PEST domains of the PTEN protein was only 1 month. This may support the hypothesis that mutations in this region of the protein causes a loss of its tumor suppressor feature and an increase in the aggressiveness of tumor.

Furthermore, we identified a C deletion at codon 213 in exon 7 caused premature stop codon at seven codons downstream of the mutation in one GBM tumor. This pathogenic mutation caused a major part to be lost, including PEST domains of C-terminal domain of PTEN protein; thus, eliminating the tumor suppressor function of the PTEN protein.

In conclusion, we believe that point mutations in *PTEN* gene have a role in formation of GBM tumors due to the high rate of them in advanced brain tumors seen in the present study and other studies, these alterations have demonstrated variability in patients from different ethnical origins, and localizations of alterations in *PTEN* gene affect the malignancy of tumor. We think that this type of studies will be helpful for the identification of different point mutations that have a role in carcinogenesis of glial tumors, and studies evaluating the manifestation of these mutations in the PTEN protein will clarify the role of mutations in the biological mechanism underlying the formation GBM. In connection, we anticipate that such studies will contribute to the detection, prevention and better treatment of GBM.

Acknowledgements We thank to Prizma and Elips Ltds for their support in supplying the experimental equipment.

References

1. Turkish Ministry of Health (2000) Republic of Turkey Health Statistics, Turkish Ministry of Health. www.saglik.gov.tr/istatistikler
2. Lantos PL, Vanderberg SR, Kleihues P (1997) Tumours of the nervous system. In: Graham DI, Lantos PL (eds) Greenfield's neuropathology, 6th edn. Arnold, pp 600–626
3. Zhou XP, Li YJ, Hoang-Xuan K, Laurent-Puig P, Mokhtari K, Longy M, Sanson M, Delattre JY, Thomas G, Hamelin R (1999) Mutational analysis of the *PTEN* gene in gliomas: molecular and pathological correlations. Int J Cancer (Pred. Oncol.) 84:150–154
4. CBTRUS (2000) Statistical report: primary brain tumors in the united states, 1992–1997, published by the central brain tumor registration of the United States
5. Ohgaki H, Dessen P, Jourde B, Hoursmann S, Nishikawa T, Di Patre PL, Burkhard C, Schüler D, Probst-Hensch NM, Maiorka PC, Baeza N, Pisani P, Yonekawa Y, Yasargil MG, Lütolf UM, Kleihues P (2004) Genetic pathways to glioblastoma: a population-based study. Cancer Res 64:6892–6899
6. Ichimura K, Ohgaki H, Kleihues P, Collins VP (2004) Molecular pathogenesis of astrocytic tumours. J Neurooncol 70:137–160
7. Collins VP (2004) Brain tumours: classification and genes. J Neurol Neurosurg Psychiatry 75(suppl 2):2–11
8. Batchelor TT, Betensky RA, Esposito JM, Pham LD, Dorfman MV, Piscatelli N, Jhung S, Rhee D, Louis DN (2004) Age-dependent prognostic effects of genetic alterations in glioblastoma. Clin Cancer Res 10:228–233
9. Hill C, Hunter SB, Brat DJ (2003) Genetic markers in glioblastoma: prognostic significance and future therapeutic implications. Adv Anat Pathol 10:212–17
10. Ohgaki H, Kleihues P (2005) Population-based studies on incidence, survival rates, and genetic alterations in astrocytic and oligodendroglial gliomas. J Neuropathol Exp Neurol 64:479–489
11. Zhou YH, Tan F, Hess KR, Yung WKA (2003) The expression of *PAX6, PTEN*, vascular endothelial growth factor, and epidermal growth factor reseptor in gliomas:

relationship to tumor grade and survival. Clin Cancer Res 9:3369–3375
12. Eng C (2003) *PTEN*: one gene, many syndromes. Human Mutat 22:183–198
13. Kleihues P, Burger PC, Scheithauer BW (1993) Histological typing of tumors of the central nervous system. In: Kleihues P, Burger PC, Scheithaver BW (eds) International histological classification of tumors. World Health Organization, Geneva
14. Kraus JA, Glesmann N, Beck M, Krex D, Klockgether T, Schackert G, Schlegel U (2000) Molecular analysis of the *PTEN, TP53*, and *CDKN2A* tumor suppressor genes in long-term survivors of glioblastoma multiforme. J Neuroncol 48:89–94
15. Knobbe CB, Merlo A, Reifenberger F (2002) PTEN signaling in gliomas. Neurooncol 4:196–211
16. Fukushima T, Favereaux A, Huang H, Shimizu T, Yonekawa Y, Nakazato Y, Ohagki H (2006) Genetic alterations in primary glioblastomas in Japan. J Neuropathol Exp Neurol 65(1): 12–18
17. www.ensembl.org/Homo_sapiens/protview?db=core;peptide=ENSP00000361021
18. Ishihara H, Sasaoka T, Kagawa S, Murakami S, Fukui K, Kawagishi Y, Yamazaki K, Sato A, Iwata M, Urakaze M, Ishiki M, Wada T, Yaguchi S, Tsuneki H, Kimura I, Kobayashi M (2003) Association of the polymorphisms in the 5′-untranslated region of *PTEN* gene with type 2 diabetes in a Japanase population. FEBS Lett 554:450–454
19. Agrawal S, Pilarski R, Eng C (2005) Different splicing defects lead to differential effects downstream of the lipid and protein phosphatase activities of PTEN. Human Mol Genet 14(16):2459–2468
20. Furnari FB, Lin H, Huang HJS, Cavenee WK (1997) Growth suppression of glioma cells by *PTEN* requires a functional phosphatase catalytic domain. Proc Natl Acad Sci USA 94:12479–12484
21. Georgescu MM, Kirsch KH, Kaloudis P, Yang H, Pavletich NP, Hanafusa H (2000) Stabilization and productive positioning roles of the C2 domain of *PTEN* tumor suppressor. Cancer Res 60:7033–7038
22. Georgescu MM, Kirsch KH, Akagi T, Shishido T, Hanafusa H (1999) The tumor-suppressor activity of *PTEN* is regulated by its carboxyl-terminal region. Proc Natl Acad Sci USA 96: 10182–10187
23. Kato H, Kato S, Kumabe T, Sonoda Y, Yoshimoto T, Kato S, Han SY, Suzuki T, Shibata H, Kanamaru R, Ishioka C (2000) Functional evaluation of p53 and *PTEN* gene mutations in Gliomas. Clin Cancer Res 6:3937–3943