# Mutations of *PTEN* Gene in Gliomas Correlate to Tumor Differentiation and Short-term Survival Rate

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**Abstract.** *The present study determined mutations of phosphatase and tensin homolog (PTEN) gene in patients suffering from high-grade gliomas (WHO grades III and IV) and further investigated the mutations in correlation to patients' histopathological classification and short-term survival. Total RNA and genomic DNA were extracted from tumor tissues. Full-length PTEN cDNA sequences were amplified by polymerase chain reaction (PCR). The PCR products were directly sequenced, and the PTEN mutations were analyzed. It demonstrated that the incidence of PTEN mutations was 8/22 in these patients: one patient with WHO grade III glioma (1/11) and 7 patients with WHO grade IV glioma (7/11). Most patients had three or more mutations in the PTEN gene, with exons 2, 3, 4, 5, 6 and 7 as hot mutation regions, with mutation incidence from 62.5% to 75%. About 68.4% of mutations were missense, 26.3% same-sense and 5.3% nonsense mutations. The median survival times of the WHO grade III and IV groups were 250 and 53 weeks after surgery, respectively (p=0.016). The 36-week survival rate of patients with and without PTEN mutations was 62.5% and 92.9% (p=0.038, odds ratio=7.80), respectively. The present study suggests that PTEN mutations are late events in the malignant progression of glioma and the occurrence of PTEN mutations are significantly correlated to patients' short-term survival.*

Glioma is the most common neoplasm of the central nervous system, accounting for about 40%-50% of brain tumors (1). It is suggested that WHO high-grade gliomas, including anaplastic astrocytoma (AA) and glioblastoma multiform (GBM), have poor prognosis and high mortality. Despite the advances in diagnostics and treatments, such as modern diagnostic procedures, novel surgical techniques, and effective radiation therapy as well as chemotherapy, the prognosis of patients suffered from high-grade gliomas remains dismal. The majority of patients with GBM die within one year of diagnosis (2).

It has been suggested that genetic alterations of certain genes are critical events behind the pathogenesis of gliomas. Several investigations demonstrated that the deletions and/or mutations of tumor suppressor genes might play an important role. Phosphatase and tensin homolog (*PTEN*), a tumor suppressor gene, located on chromosome 10q23.3, encodes a 403 amino acid, which is a dual-specificity phosphatase with homology both to the protein tyrosine phosphatase (PTP) family and to the cytoskeletal proteins, *i.e.* tensin and auxilin (3-5). In general, *PTEN* negatively regulates the anti-apoptotic action of akt phosphorylation (6). It has been reported that mutations of *PTEN* have been implicated in the malignant progression of astrocytic gliomas, as these alterations are most frequently observed in GBM, less commonly found in AA, and very rare in the lower grade astrocytoma (7-8).

We previously examined *PTEN* expression in glioma tissue, tumor-adjacent normal brain tissues and benign brain tumor tissues, and found that mRNA levels of *PTEN* were much lower in the glioma tissues. *PTEN* mRNA levels were significantly different between the highly differentiated gliomas (WHO grades I-II) and high-grade gliomas (WHO grades III-IV). As mutations of *PTEN* are very rare in highly differentiated gliomas (9), in the present study, we investigated the mutation types and frequencies of *PTEN* alterations in 22 patients suffering from high-grade glioma and investigated the correlation between these alterations and the patients' histopathological classification as well as their short-term survival.

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*Table I. Characteristics of the patients.*

| Case | Gender/age (years) | WHO classification | *PTEN* gene alteration | Survival time (weeks) |
|---|---|---|---|---|
| 1 | F/48 | III | + | 323 |
| 2 | F/37 | IV | + | 16 |
| 3 | M/50 | IV | + | 366 |
| 4 | M/41 | IV | + | 77 |
| 5 | M/36 | IV | + | 57 |
| 6 | M/52 | IV | – | 53 |
| 7 | M/49 | III | – | 340+ |
| 8 | M/32 | III | – | 195 |
| 9 | M/63 | III | – | 21 |
| 10 | M/59 | IV | – | 47 |
| 11 | M/56 | IV | – | 61 |
| 12 | M/54 | IV | + | 324 |
| 13 | M/25 | III | – | 381+ |
| 14 | F/61 | IV | – | 38 |
| 15 | M/67 | III | – | 84 |
| 16 | M/62 | IV | + | 19 |
| 17 | F/38 | III | – | 48 |
| 18 | M/72 | III | – | 114 |
| 19 | F/36 | III | – | 366+ |
| 20 | M/47 | IV | + | 30 |
| 21 | M/69 | III | – | 357+ |
| 22 | M/52 | III | – | 250 |

+Patients still alive at the time of writing.

## Patients and Methods

*Patients and tissue samples*. A total of 22 patients (17 males and 5 females, 25- to 72-years-old; median age 51 years) suffering from gliomas and hospitalized in our hospital from 2000 to 2002 were surgical treated and followed up in the present study. All tumor samples were quickly frozen in liquid nitrogen after resection and were kept at –80˚C until nucleotide extraction. All samples were examined histopathologically and were classified according to the WHO classification of tumors in the central nervous system (10). There were 11 cases of grade III and 11 cases of grade IV that were all defined as high-grade gliomas. None of the patients received chemotherapy or radiotherapy before surgery. The characteristics of the patients are listed in Table I.

*Nucleotide extraction and polymerase chain reaction (PCR)*. Total RNA and genomic DNA (for amplifying the first and last exon of *PTEN*) were extracted by using the total RNA isolation classic kit (SNBC, Shanghai, P. R. China) and UIIQ-10 column blood genomic DNA isolation kit (Sangon, Shanghai, P. R. China), respectively. The quality of the RNA samples was determined by absorbance measurements at 260/280 nm. Before RNA extraction, frozen sections of the specimens were examined histologically to ensure that they were representative of the tumor tissues. First-strand cDNAs were synthesized by using a RevertAid first-strand cDNA synthesis kit (MBI Fermentas Inc., Canada). According to the mRNA (HSU93051) and DNA (AC063965) sequences of *PTEN* in the GenBank, four PCR primer sets were designed respectively (as shown in Table II) to amplify four fragments

Table II. *Primers of human PTEN gene.*

| | Sequence | Length (bp) |
|---|---|---|
| Fragment 1 | | |
| Forward | 5’ ATCAAAGAGATCGTTAGCAGAAAC 3’ | 747 |
| Reverse | 5’ GATATCACCACACACAGGTAACG 3’ | |
| Fragment 2 | | |
| Forward | 5’ ACACGACGGGAAGACAAGTT 3’ | 516 |
| Reverse | 5’ GACTTTTGTAATTTGTGTATGCTGAT 3’ | |
| Fragment 3 | | |
| Forward | 5’ GACATTTTCGCATCCGTCTACTCC 3’ | 430 |
| Reverse | 5’ AGCCGTTCGGAGGATTATTCGT 3’ | |
| Fragment 4 | | |
| Forward | 5’ GCTACACAACCTTTTTTCAACTGG 3’ | 362 |
| Reverse | 5’ GGAGCCGTCAAATCCAGAGG 3’ | |

(747 bp, 516 bp, 430 bp and 362 bp, respectively) which can be joined to form a full-length cDNA sequence (1212 bp). The PCR reaction for each fragment was performed in a 50 μl volume, containing 0.2 μl 100 mM primers and probes, 2 μl cDNA, 5 μl 10x buffer, 3 μl $MgCl_2$ (25 mM), 1 μl dNTP (10 mmol/l) and 1 μl Taq DNA polymerase. The thermal cycling conditions included the following steps: 1 min at 95˚C to activate Taq polymerase, then a 40-cycle 3-step PCR was performed consisting of 30 s at 95˚C, 30 s at 60˚C (56˚C and 58˚C when 747 bp and 516 bp fragments were amplified, respectively) and 60 s at 72˚C. All PCRs were performed on a GeneAmp PCR system 9700 (Perkin-Elmer Applied Biosystems, USA)

*Sequencing of PCR products and analyses of PTEN mutations*. The PCR products were directly sequenced on an automatic sequencer from Applied Biosystems, model 3730 (Invitrogen, Shanghai, P. R. China). After sequencing, four fragments were spliced and joined to obtain full length *PTEN* cDNA of 1212 bp. Vector NTI advance 10 software package (Invitrogen Corporation, CA, USA) was used to analyze the *PTEN* mutations.

*Statistic analyses*. All data were statistically analyzed by Prism version 4 (GraphPad Software, Inc. CA, USA). Comparisons between these with and without mutations of *PTEN* in the two groups (WHO grade III and WHO grade IV) were statistically evaluated by the Chi-square test. A *p*-value less than 0.05 was considered as significant. Correlation of patients’ life span with tumor grades and mutations of *PTEN* were analyzed by the Kaplan-Meier curve.

## Results

*Frequency and genotype of PTEN mutations*. Eight out of a total 22 cases were found to have mutations in their *PTEN* cDNA. Among these mutations, there was only one case in the WHO grade III patient group, which represents mutation incidence of 1/11 and 7 cases in the WHO grade IV patient group (7/11). The mutation distribution is shown in Table III. The frequency of mutation was similar for each exon, but there were only two cases with mutation of exon

Table III. *PTEN mutations in the WHO high-grade gliomas.*

| Exon | Codon number | nt substitution | Predicted effect* | Case no. (n=8) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 4 | 5 | 12 | 16 | 20 |
| 2 | 30 | CCA/CTA | P/L | + | + | + | | + | + | + | |
| 3 | 68 | TAC/CAC | Y/H | + | + | + | | | + | + | |
| 4 | 81 | TTT/TCT | F/S | + | + | + | | | + | + | |
| | 83 | TGC/TAC | C/Y | + | + | + | | | + | + | |
| 5 | 86 | GCA/GCG | A/A | + | + | + | | + | + | + | |
| | 115 | GAT/AAT | D/N | | | | | + | | | |
| | 133 | GTA/ATA | V/I | + | + | + | | | + | + | |
| | 135 | ATA/ATT | I/I | + | + | + | | | + | + | |
| | 136 | TGT/TAT | C/Y | + | + | + | | | + | + | |
| 6 | 171 | CAG/CGG | Q/R | | | | | | | | + |
| | 177 | TAT/TAC | Y/Y | + | + | + | | | + | + | |
| | 182 | TTA/GTA | L/V | + | + | + | | | + | + | |
| | 186 | CTG/GTG | L/V | + | | + | | | + | + | |
| 7 | 224 | ATA/ATG | I/M | + | + | + | | | + | + | |
| | 234 | CGG/TGG | R/W | + | + | + | | | + | + | |
| | 235 | GAA/GAG | E/E | + | + | + | | | | + | |
| | 240 | TAC/TAT | Y/Y | + | + | + | | | | + | |
| 8 | 335 | CGA/TGA | R/– | | | | + | | | | |
| | 341 | TTT/GTT | F/V | | + | | | | | | |

*Indicates amino acid changes.

8 and none for mutation of exon 1 and 9. Most patients had three or more mutations of their *PTEN* (Table III). Exons 2, 3, 4, 5, 6 and 7 are hot mutation regions, with a mutation incidence from 5/8 to 6/8 (Table III). In the present study, there were 19 different mutations found in these patients (Table III). There were 13/19 mutations which represented missense mutation, 5/19 as same-sense mutation and 1/19 as nonsense mutation.

*PTEN mutations in correlation to tumor histopathological classification and to patients' short-term survival.* The incidence of *PTEN* mutations was significantly different between WHO grade III and WHO grade IV (Table IV) ($p$=0.024). The risk rate of histopathological diagnosis of WHO grade IV was 3.06-fold for a patient with mutation of *PTEN* than for those without (95% confidence interval, CI: 1.284-7.302). The median survival time of WHO grade III and IV groups were 250 and 53 weeks, respectively (Figure 1A) ($p$=0.016). At the time of writing, there are four patients still alive in the WHO grade III group, however, all patients in the WHO grade IV group have died. As shown in Figure 1B, the median survival period of patients with and without *PTEN* mutations were 67 and 99 weeks ($p$=0.292), respectively. However, as shown in Table V, the 36-week survival rates of these two group patients were 62.5% and 92.9% respectively, which is statistically significant ($p$=0.038, odds ratio, *OR*=7.80, one-sided test).

Table IV. *Association between mutation of PTEN and histopathological classification.*

| | Grade IV | Grade III | Total |
|---|---|---|---|
| Mutation | 7* | 1 | 8 |
| Non-mutation | 4 | 10 | 14 |
| Total | 11 | 11 | 22 |

*$p$=0.024 *vs*. grade III

Table V. *Comparison of 36-week survival rate.*

| | With mutation | Without mutation | Total |
|---|---|---|---|
| Death | 3* | 1 | 4 |
| Survival | 5 | 13 | 18 |
| Total | 8 | 14 | 22 |

*$p$=0.038 *vs*. Without mutation (one-sided test).

## Discussion

In the present study, we analyzed *PTEN* mutations in WHO high-grade gliomas by direct sequencing of full-length *PTEN* cDNA. Only one case had *PTEN* mutation in WHO grade III patients (1/11), whereas there were seven cases with *PTEN* mutations in the WHO grade IV patients, which

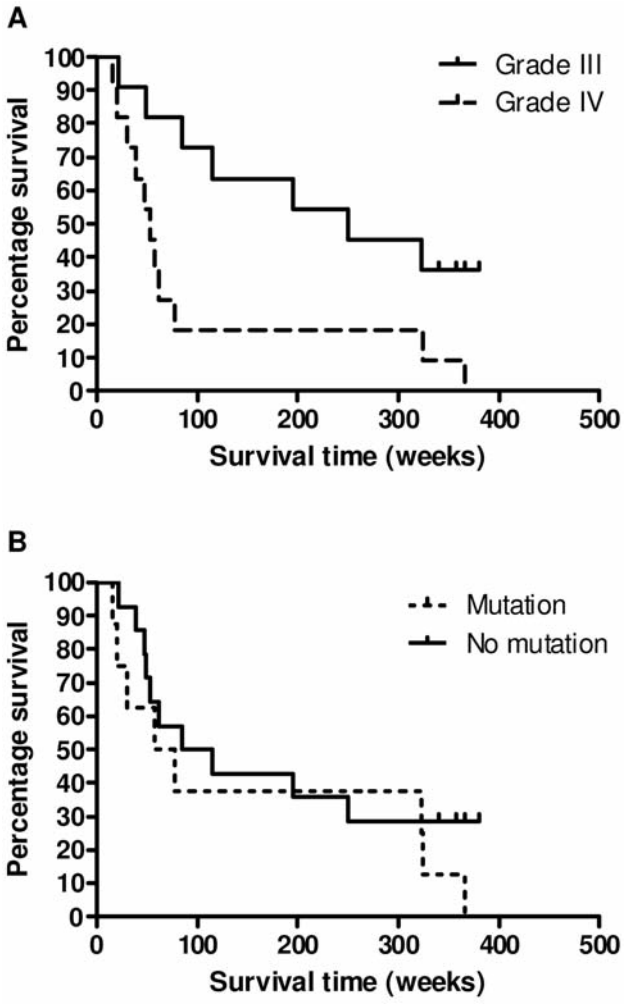


Figure 1. *Comparison of survival curves. A: Histopathological classification; B: PTEN gene alteration.*

revealed that mutation of *PTEN* appears to be a late event in the malignant progression of gliomas. Although the *PTEN* gene contains 9 exons and encodes a 403-amino acid protein, we found *PTEN* mutations mainly occurred with high frequency in the region encoding the first 240 amino acids, which suggests that the latter part of the *PTEN* gene is a relative conserved region.

The present study demonstrated that the median survival time of patients with WHO grade III was significantly longer than these with WHO grade IV, which suggests that there is a strong correlation between tumor histopathological classifications and patients' prognoses (11, 12). According to the literature, it is still controversial whether *PTEN* mutation is correlated to prognosis in patients with WHO high-grade glioma. Several studies have addressed whether *PTEN* gene aberrations could be considered as a potential prognostic index for patients suffered from malignant glioma. Sasaki *et al*. (13) investigated 47 patients with anaplastic oligodendroglioma and demonstrated that the presence of *PTEN* aberrations was significantly associated with shorter survival time in these patients. The median survival time was only 14.8 months in patients with *PTEN* aberration, however, in patients without *PTEN* aberration, the median survival time was extended to 123.4 months. This suggested that *PTEN* gene alterations might be regarded as one of the key roles in the pathogenesis of certain anaplastic oligodendrogliomas and may be associated with an aggressive tumor phenotype regardless of chemosensitivity. In another study of 62 anaplastic astrocytomas, Smith *et al*. (14) reported that *PTEN* mutations were detected in 11 out of 62 cases, with median survival times of 4.4 months and 34.4 months in patients with and without *PTEN* mutations, respectively. This indicated that *PTEN* mutation may be a powerful independent index for reduced survival time in patients suffering from anaplastic astrocytomas (WHO grade III). In the case of glioblastoma, however, Zhou *et al*. (15) analyzed the correlation between clinical data and *PTEN* mutations, and showed there was no significant correlation between *PTEN* mutation and patients' survival time; the median survival time was 11 and 12 months in patients with and without *PTEN*-mutated tumors, respectively. A similar study revealed that mutations in the *PTEN* gene lacked prognostic significance for overall survival time in GBM patients (16). In addition, another two independent studies suggested that loss of heterozygosity at microsatellite markers flanking the *PTEN* gene locus on 10q23.3 might be a significant predictor of the shorter survival time among glioblastoma patients (17-18).

In order to elucidate whether *PTEN* mutation is correlated to patient prognosis in WHO high-grade gliomas, we therefore assessed *PTEN* mutations in corresponding cDNA obtained from patients with WHO grade III and IV gliomas. Our data demonstrated that the median survival time of patients with *PTEN* mutations was only 67 weeks, while it was 99 weeks in the patients without *PTEN* mutation, but the survival analysis indicated that the difference of the median survival time between these two groups did not reach the significance level. However, the 36-week survival rate was statistically different between patients with and without *PTEN* mutations, which may suggest that *PTEN* mutation may only be correlated to patients' short-term survival.

## Conclusion

*PTEN* mutations mainly occurred with high frequency in the first part of the *PTEN* gene. These mutations appear to be late events in the malignant progression of glioma, as well as being significantly correlated to patients' short-term survival.

## References


1 Wrensch M, Minn Y, Chew T, Bondy M and Berger MS: Epidemiology of primary brain tumors: current concepts and review of the literature. Neuro Oncol *4*: 278-299, 2002.

2 Leenstra S, Oskam NT, Bijleveld EH, Bosch DA, Troost D and Hulsebos TJ: Genetic sub-types of human malignant astrocytoma correlate with survival. Int J Cancer *79*: 159-165, 1998.

3 Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliaresis C, Rodgers L, McCombie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH and Parsons R: *PTEN*, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science *275*: 1943-1947, 1997.

4 Steck PA, Pershouse MA, Jasser SA, Yung WK, Lin H, Ligon AH, Langford LA, Baumgard ML, Hattier T, Davis T, Frye C, Hu R, Swedlund B, Teng DH and Tavtigian SV: Identification of a candidate tumour suppressor gene, *MMAC1*, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat Genet *15*: 356-362, 1997.

5 Myers MP, Stolarov JP, Eng C, Li J, Wang SI, Wigler MH, Parsons R and Tonks NK: *P-TEN*, the tumor suppressor from human chromosome 10q23, is a dual-specificity phosphatase. Proc Natl Acad Sci USA *94*: 9052-9057, 1997.

6 Stambolic V, Suzuki A, de la Pompa JL, Brothers GM, Mirtsos C, Sasaki T, Ruland J, Penninger JM, Siderovski DP and Mak TW: Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. Cell *95*: 29-39, 1998.

7 Rasheed BK, Stenzel TT, McLendon RE, Parsons R, Friedman AH, Friedman HS, Bigner DD and Bigner SH: *PTEN* gene mutations are seen in high-grade but not in low-grade gliomas. Cancer Res *57*: 4187-4190, 1997.

8 Kitange GJ, Templeton KL and Jenkins RB: Recent advances in the molecular genetics of primary gliomas. Curr Opin Oncol 15: 197-203, 2003.

9 Yang Y, Shao N, Luo G, Li L, Nilsson-Ehle P and Xu N: Relationship between *PTEN* gene expression and differentiation of human glioma. Scand J Clin Lab Invest *66*: 469-475, 2006.

10 Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, Scheithauer BW and Kleihues P: The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol *114*: 97-109, 2007.

11 Tihan T, Zhou T, Holmes E, Burger PC, Ozuysal S and Rushing EJ: The prognostic value of histological grading of posterior fossa ependymomas in children: a Children's Oncology Group study and a review of prognostic factors. Mod Pathol *21*: 165-177, 2008.

12 Burnet NG, Lynch AG, Jefferies SJ, Price SJ, Jones PH, Antoun NM, Xuereb JH and Pohl U: High-grade glioma: imaging combined with pathological grade defines management and predicts prognosis. Radiother Oncol *85*: 371-378, 2007.

13 Sasaki H, Zlatescu MC, Betensky RA, Ino Y, Cairncross JG and Louis DN: *PTEN* is a target of chromosome 10q loss in anaplastic oligodendrogliomas and *PTEN* alterations are associated with poor prognosis. Am J Pathol *159*: 359-367, 2001.

14 Smith JS, Tachibana I, Passe SM, Huntley BK, Borell TJ, Iturria N, O'Fallon JR, Schaefer PL, Scheithauer BW, James CD, Buckner JC and Jenkins RB: *PTEN* mutation, *EGFR* amplification, and outcome in patients with anaplastic astrocytoma and glioblastoma multiforme. J Natl Cancer Inst *93*: 1246-1256, 2001.

15 Zhou XP, Li YJ, Hoang-Xuan K, Laurent-Puig P, Mokhtari K, Longy M, Sanson M, Delattre JY, Thomas G and Hamelin R: Mutational analysis of the *PTEN* gene in gliomas: molecular and pathological correlations. Int J Cancer *84*: 150-154, 1999.

16 Kraus JA, Glesmann N, Beck M, Krex D, Klockgether T, Schackert G and Schlegel U: Molecular analysis of the *PTEN, TP53* and *CDKN2A* tumor suppressor genes in long-term survivors of glioblastoma multiforme. J Neurooncol *48*: 89-94, 2000.

17 Lin H, Bondy ML, Langford LA, Hess KR, Delclos GL, Wu X, Chan W, Pershouse MA, Yung WK and Steck PA: Allelic deletion analyses of *MMAC/PTEN* and *DMBT1* loci in gliomas: relationship to prognostic significance. Clin Cancer Res *4*: 2447-2454, 1998.

18 Tada K, Shiraishi S, Kamiryo T, Nakamura H, Hirano H, Kuratsu J, Kochi M, Saya H and Ushio Y: Analysis of loss of heterozygosity on chromosome 10 in patients with malignant astrocytic tumors: correlation with patient age and survival. J Neurosurg *95*: 651-659, 2001.


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