# DELETION AND 5'CPG ISLAND METHYLATION OF p15 GENE IN BRAIN GLIOMA

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## ABSTRACT

**Objective: To investigate the abnormality of p15 gene in brain glioma and the correlation of it with occurrence or malignant progression of brain glioma. Methods: Deletion and 5'CPG island methylation of p15 gene were detected by the methods of PCR and PCR-based methylation in 56 cases of brain glioma. Results: Out of 43 cases of high grade glioma, 14 cases were found to have homozygous deletion of p15E1, while none of the 13 cases of low grade glioma was found to have deletion of p15E1 ($P<0.05$). Methylation of 5'CPG Island of p15 gene was found only in four cases of glioma. Conclusion: Abnormality of p15 gene may involved in the occurrence and malignant progression of brain glioma. Homozygous deletion of gene is the major mechanism of inactivation for p15 gene in brain glioma.**

**Key words: p15, Brain glioma, Methylation, PCR**

P15 gene is an another tumor suppressor gene, which is located on 9p21 and adjacent to p16 gene. It encodes p15 protein that has biochemical functions similar to those of p16 protein. There have been reports about abnormality of p15 gene in brain glioma.[1-3] In this study, we detected deletion and 5'CPG island methylation of p15 gene in 56 cases of brain glioma by the methods of PCR and PCR-based methylation to investigate the correlation between abnormality of p15 gene and occurrence or malignant progression of brain glioma.

## MATERIALS AND METHODS

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### Specimens

Fifty-six fresh specimens of brain glioma were obtained from patients undergoing operation from January, 1997 to July, 1998 at the First and Second Affiliated Hospital of Hubei Medical university, Wuhan, China. Tumor samples without blood and normal brain tissue were snap frozen in liquid nitrogen, and then stored at -70°C. According to Kernohan's rule, there were 5 grade I, 8 grade II, 23 grade III and 20 grade IV of the 56 cases of glioma which were diagnosed by a neuropathologist. Five normal brain tissues were used as control.

### DNA Extraction

Frozen tumor samples were crushed to a fine powder. DNA was isolated by proteinase k digestion, followed by extraction with phenol: chloroform: isoamyl alcohol.

### Detection of Deletion of p15 Gene

The primer set used for amplifying exonl of p15 was designed according to Jen's report.[3] The primer set of glyceraldehyde phosphate dehydrogenase (GAPDH) gene served as internal control. The primer sets are detailed in Table 1. PCR was performed in a final volume of 20 μl containing 0.2 μg of genomic DNA, 1.5 mmol/L $MgCl_2$, 50 mmol/L KC1, 10 mmol/L Tris. HC1 (pH 8.3), 0.2 mmol/L deoxyribonucleotide triphosphates, 20 mmol/L of each primer. Cycling was performed under the following conditions: Initial denatural at 95°C for 5 min and adding 1.25 unit of Taq polymerase in the reaction system were following by 30 cycles of PCR: 95°C 30", 50°C 30", 72°C 60". At last cycle, another extension of 5 min at 72°C was added. The products of PCR reactions were electrophoresed on 2% agarose gels for 1 hour at 80 volt voltage and stained with ethidium bromide. Then the gels were either observed or photographed under ultraviolate light (Figure 1).

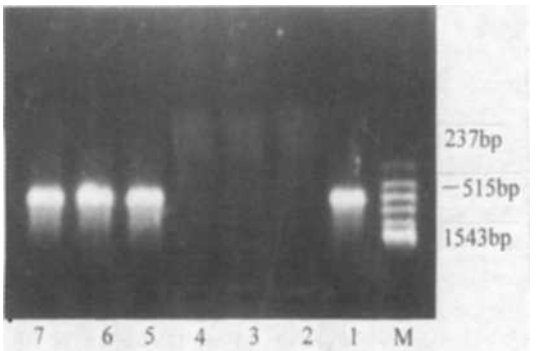


Fig. 1. M represented PCR marker; lane 1,5,6,7 had positive amplification of p15E1 while lane 2,3,4 had no amplification of p15E1

### PCR-based Methylation Assay

A PCR assay relying on the inability of some restriction enzymes to cut methylated sequences was used to analyze the methylation status of the first exon of p15. The primer set used for methylation analysis of p15 exonl is detailed in Table 1. There are five HpaII sites on the amplifying location of p15E1. DNA digests were performed according to the manufacturer's directions. 1 μg DNA was digested with 20 unit enzyme and 20 μl digestion buffers (Boehringer Mannheim Indianapolis, IN) at 37°C overnight. 0.2 μg digested DNA were amplified with primer set of p15E1. PCR reaction system and condition detailed above. An undigested DNA control and a MspI-digested DNA control were included for HpaII site examined. To rule out the possibility of incomplete digestion, all samples were digested twice with each of the enzymes in independent experiments. The products of PCR reaction were electrophoresed on 2% agarose gels for 1 hour at 80 volt voltage and stained with ethidium bromide. Then the gels were either observed or photographed under ultraviolate light (Figure 2).

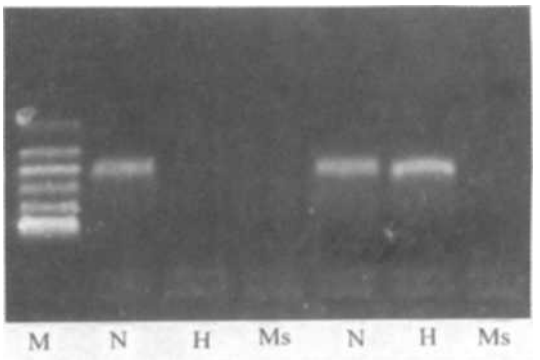


Fig 2. N, H, Ms represented PCR amplification results of undigested, HapII digested and MsPI digested DNA respectively.

*Table 1 Sequence of p15E1 and GAPDH gene*

| Gene | Sequence of primer | Annealing temperature | Size |
|---|---|---|---|
| P15E1 | 5'-CCAGAAGCAGTCCTGGCGCG-3' | 50°C | 532bp |
| | 5'-AATGCACACACCTCGCCAAGC-3' | | |
| GAPDH | 5'-GTGAAGGTCGGAGTCAAC-3' | 52°C | 356BP |
| | 5'-GAGATGATGACCCTTTTGGC-3' | | |

## RESULTS

### Deletion of p15E1

Of the 56 brain gliomas 14 high grade cases were found to have deletion of p15E1, while deletion of p15E1 wasn't found in any of the low grade gliomas (Table 2).

*Table 2. Deletion of p15E1 in 56 brain gliomas*

| Grade | Number | Negative |
|---|---|---|
| I | 5 | 0 |
| II | 8 | 0 |
| III | 23 | 5 (21.8%) |
| IV | 20 | 9 (45%) |

Test of exact probability: Comparison of grade I and II with grade III and IV, $P<0.05$

### 5'CPG Island Methylation of P15E1

5'CPG island methylation of p15E1 were demonstrated in 4 cases of the 42 gliomas without deletion of p15E1 demonstrated by PCR (Table 3).

*Table 3. 5'CPG island methylation of p15E1 in 42 brain gliomas*

| Grade | Number | 5'CPG island methylation |
|---|---|---|
| I | 5 | 0 |
| II | 8 | 1 (12.5%) |
| III | 18 | 1 (5.6%) |
| IV | 11 | 2 (18.2%) |

## DISCUSSION

P15 gene which is also called multiple tumor suppressor 2 (MTS2) is located on 9p21, about 30kb upstream of p16 gene. It has two exons. 93% sequence of the exon2 is homologous to exon2 of p16. The product of p15 gene can specifically inhibit the function of cyclin-dependent kinase CDK4/CDK6 which is involved in regulating progresssion of cell cycle, so it indirectly prevents cells from entering into S phase through G1 phase and suppresses growing of cell.[4] P15 is up-regulated by TGF-β.[5] Leukemia cell lines with inactivation of p15 resist to growth suppression by TGF-β.[6]

Jen, et al.[3] found that p15 gene was often homozygously deleted in glioblastoma multiform, so they suggested that deletion is a more efficient mechanism for inactivation of p15 gene. Sonoda, et al[2] reported losses of one allele of p15 gene in 12 of 27 malignant gliomas and one of 10 less malignant gliomas, and homozygous deletion of p15 gene in three gliomas which were all glioblastomas. They suggested those p15 gene functions as tumor suppressor genes in human gliomas and homozygous deletion of p15 gene is associated with the tumorigenesis of some gliblastomas. In this study, we found that 14 of the 43 malignant gliomas had homozygous deletion of p15E1, while deletion of p15E1 wasn't found in any of the 13 cases of low grade glioma ($P<0.05$). The results indicated that homozygous deletion of p15 gene might involve in tumorigenesis and malignant progression of brain glioma.

CPG Island is G+C rich region. It shows a higher frequency of CPG dinucleotides than normally seen in the vertebrate genome. CPG islands are usually unmethylated in normal somatic cells. In contrast, widespread methylation of CPG islands occurs on autosomal genes during oncogenic transformation. In this study, we found only four gliomas with methylation of 5'CPG Island of p15 gene, which included one grade II, one grade III and two grades IV. The results indicated that 5'CPG island methylation is another mechanism of p15 gene inactivation.

So we concluded that abnormality of p15 gene might involved in tumorigenesis and malignant progression of brain glioma. Homozygous deletion is the major mechanism of inactivation for p15 gene in brain glioma. 5'CPG island methylation is another mechanism for p15 gene inactivation in brain glioma, but it happens less frequently.

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