P53 Gene Mutation and Expression of MDM2, P53, P16 Protein and their Relationship in Human Glioma

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Summary: To investigate the effect of P53 protein accumulation and p53 gene mutation in the pathogenesis of gliomas and to study the role of MDM2, P53 and P16 protein in glioma formation and progression and their relationship with each other, the LSAB immunohistochemical staining method and non-isotopic PCR-SSCP techniques were used to detect the expression of MDM2, P53 and P16 protein and p53 gene mutation in 48 cases of gliomas. The results showed that the positive expression rate of MDM2, P53 and the negative rate of P16 was 22.9%, 41.7% and 60.4%, respectively. The latter two in high grade (grade II, III) gliomas had a significantly higher rate than in the low grade (grade I) gliomas. Moreover, the co-expression of MDM2 and P53 protein was confirmed in only 1 of 48 cases. No significant difference was found in the rate of the expression of MDM2 between high grade and low grade gliomas (P>0.1). PCR-SSCP results showed that mutation of 5–8 exons of p53 gene was detected in 17 out of 48 cases (35.42%). Mutation was detected in 16 of 20 cases of positive p53 expression, and another one was detected in 28 cases of negative expression cases. The correlation between p53 mutation and p53 immunopositivity was observed in 89.6% of the cases. P53 gene mutation and the level of MDM2, P53 and P16 protein were not related to age, gender of the patients, tumor location and size. It is concluded that the mutation of p53 and deletion of p16 might play important roles in the tumorigenesis of gliomas and it was significantly associated with the grade of tumor differentiation. P53 protein accumulation can indirectly reflect p53 mutation. MDM2 amplification and overexpression might be an early event in the growth of human gliomas.

Key words: gliomas; gene mutation; gene product

Glioma is a common aggressive intracranial tumor and presents a formidable challenge to clinicians. Molecular genetic studies have demonstrated that MDM2, p53 and p16 genes are implicated in the pathogenesis of glioma and the findings of the studies were conflicting. The aims of this study were to investigate the roles of oncogene MDM2 and tumor suppressor gene p53, p16 and their relationship by using immunohistochemical techniques. Meanwhile, we evaluated the correlation between p53 mutation and p53 immunopositivity by single-strand conformation polymorphism analysis of polymerase chain reaction (PCR-SSCP analysis) and immunohistochemistry.

1 MATERIALS AND METHODS

1.1 Samples

All 48 tumor specimens were obtained from patients (31 males and 17 females, with age ranging 6–70 and an average of 36.9 years) undergoing therapeutic operation for brain tumors at the Affiliated Hospital of Jining Medical University from 1997 to 2001. These samples were paraffin-embedded and selected on the basis of the adequacy for the molecular biological and immunohistochemical studies.

Of the 48 cases, the consensus histological diagnosis was astrocytomas in 23 (grade 2), oligodendrogliomas (grade 2) in 4, ependymal tumor (grade 2) in 1, mixed oligoastrocytoma in 1 (grade 2), anaplastic astrocytomas in 15 (grade 3), anaplastic oligodendrogliomas (grade 3) in 1 and glioblastomas (grade 4) in 3 on WHO classification of brain tumors (2002).

1.2 Methods

1.2.1 Immunohistochemistry

Paraffin-embedded tissue slices, 6 μm in thickness, were stained by the labeled streptavidin biotin (LSAB) method. The sections were incubated respectively with monoclonal antibodies for P53, MDM2 and polyclonal antibodies for P16 (Dako, USA) using routine LSAB methods[1]. The immunohistochemical results were evaluated semi-quantitatively by comparing the staining intensity of tumor cells with that of the adjacent, non-stained cells. A brown-yellow color in the nucleus was deemed as positive cells, Breast cancer and normal brain tissues served as positive and negative controls, respectively.

The positive staining (+) was defined as nuclear staining of 25% of the cells; strong positive staining (++) as nuclear staining of 25% to 50% of the cells, and very strongly staining (+++) as nu-
clear staining of over 50% of the cells.

1.2.2 PCR-SSCP Analysis for P53 Mutations
The primers of exon 5–8 were synthesized by the Department of Pathology, Health Science Center, Peking University. DNA was extracted from all paraffin sections and polymorphism analysis was performed by using stabilizing PCR method. Subsequently, the resulting fragments were electrophoresed on 8% non-denaturing polyacrylamide gels containing 5% glycerol for 12–24 h. SSCP gels were stained with a silver staining kit.

1.3 Statistical Analysis
The chi-square and student’s t-test were used to evaluate the results of immunohistochemistry and PCR-SSCP. P<0.05 was considered to be statistically significant.

2 RESULTS
Exon 5–8 of p53 of all 48 formalin-fixed paraffin-embedded tumor specimens were detected by SSCP gel electrophoresis. The results showed that p53 mutations (aberrant band) were detected in 17 out of 48 cases (35.42%). In 7 cases (41.22%) the mutations were located in exon 5 (fig. 1A), 1 (5.9%) in exon 6, 4 (23.5%) in exon 7 (fig. 1B) and 5 (29.4%) in exon 8, respectively (fig. 1). p53 mutation was observed in 6 out of 29 cases (20.7%) of grade II gliomas, and in 11 out of 19 (57.9%) of grade III and IV tumors.

P53 protein was expressed in 41.7% (20/48) of glioma samples (fig. 2A). Immunoreactivity to the p53 gene product was observed in 27.6% (8/29) grade II gliomas and in 31.6% (6/19) grade III and IV gliomas, respectively. There was a significant difference in rate of P53 protein expression between low grade gliomas and their high grade counterparts (P<0.1). Moreover, no significant difference existed in the expression between them (P>0.05).

Significant electrophoretic mobility shifts were detected in 16 out of 20 gliomas which expressed P53 protein, and in 1 out of 28 cases of negative P53 protein expression. The correlative results were observed in 21 out of 48 gliomas between p53 mutation by PCR-SSCP and p53 immunoreactivity. The correlation rate of two methods was 89.6% (43/48). The sensitivity of immunohistochemical method was 80% (16/20), and the rate of false negative results was 3.6% (1/28).

MDM2 was expressed in 22.9% (11/48) of the cases (fig. 2B). Immunoreactivity to the MDM2 gene product was observed in 17.2% (5/29) grade II gliomas and in 31.6% (6/19) grade III and IV ones respectively. There was no significant difference in rate of MDM2 protein expression between low grade gliomas and their high grade counterparts (P>0.1). Moreover, no significant difference existed in the expression between them (P<0.05).

P16 protein was detected in 39.6% (19/48) of the cases. The rate of p16 expression deletion was 60.4% (29/48). The immunoreactivity to the p53 gene product was not observed in 44.8% (13/29) of the cases of low grade gliomas and in 84.2% (16/19) of the high grade cases.

The concordance rate of P16 and P53 protein expression (P16 positive/P53 negative and P16 negative/P53 positive) was 45.9% (22/48) in 48 gliomas. The abnormal cases (P16 negative and