P27/Kip1 Expression in Gliomas and Its Clinical Significance

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Abstract
Objective: To study p27/Kip1 expression in gliomas and its application value. Methods: Immunohistochemical technique was used to detect the expression of p27/Kip1 gene in 48 different malignant grade human brain glioma tissues categorized according to WHO classification and 12 normal human brain tissues, which were analyzed quantitatively by using the image system and compared retrospectively with the patients’ clinical characteristics. Results: In this series, the immunohistochemical reaction for p27/Kip1 was confined to the nuclei. The abnormal positive expression rate of p27/Kip1 in gliomas was found to be higher than that in normal tissues (P<0.05). The positive nuclei expression of p27/Kip1 decreased in number and staining intensity with the increasing degree of histological malignancy (P<0.05). Lower expression of p27/Kip1 was associated with poor prognosis (P<0.05). P27/Kip1 expression could be regarded as an independent prognostic factor. Conclusion: The abnormal expression of p27/Kip1 may be closely related to the occurrence and development of gliomas, and also can be used to evaluate the prognosis independently.

Key words: glioma; p27/Kip1; immunohistochemistry; prognosis

The occurrence and development of malignant tumors are closely related to the abnormality of cell cycle regulation. The aberrant expression of p27/Kip1 was involved in the development and progression of many malignant tumors, but there were few studies about the expression of p27/Kip1 in malignant gliomas⁴¹. In order to study the roles of p27/Kip1 in malignant gliomas, the expression of p27/Kip1 in 48 cases of malignant gliomas and 12 normal tissues was detected by immunohistochemical technique.

Materials and methods

Tissues and patients
Sixty paraffin-embedded specimens were used in this experiment from our hospital and Xi Jing Hospital of the Forth Military Medical University, including 48 malignant glioma tissues and 12 normal brain tissues. Twenty-six were male and twenty-two were female in 48 malignant glioma patients. Their ages ranged from 24 to 68 (average 50) years. The specimens were histologically graded by using WHO grading system, including 27 cases in stage I–II, 21 cases in stage III–IV. None of the patients received radiotherapy or chemotherapy before surgery. Long-term follow-up observation after operation was carried out in 48 patients for 2.6–5.4 years (average 3.2 years).

Immunohistochemical methods
Monoclonal mouse anti-p27/Kip1 antibody (R-124) was bought from Santa Crus Co. (USA) and Histostain™Mouse SP Kit from Zymed Co. (USA). The dilutions of anti-p27/Kip1 antibody was 1:30. Histological sections (4 µm) were dewaxed, dehydrated and incubated in 3% hydrogen peroxide. Following antigen retrieval by microwave, biotinylated secondary antibody and SP compound were added successively. DAB-H2O2 was used for color development of the complexes of the antigens and antibodies of p27/Kip1. Specimens were considered positive when the percentage of positive cells is not less than 5% of tumor cells under 10×20 multiple microscope. The staining intensity was analyzed quantitatively by using the image system.

Statistical analysis
The chi-square test and t-test were used for the valuation of the expression of p27/Kip1. P value less than 0.05 was considered to be significant in difference.
The expression of p27/Kip1 in gliomas and normal brain tissues (Table 1, 2)

The brown granules were seen in nuclei and cytoplasm of p27/Kip1 positive cells, but mainly in the nuclei. There was no significant difference in expression of p27/Kip1 in sex and age (P>0.05). There were 29 positive staining cases of p27/Kip1 in 48 glioma cases, but only 2 cases in 12 normal human brain tissues. The number and staining intensity of p27/Kip1 positive nuclei in gliomas were remarkably stronger than those in the normal brain tissues (P<0.05).

P27/Kip1 expression in different histological malignant glioma tissues (Table 1, 2)

The positive rate of p27/Kip1 expression in low malignant cases (74.07%) was higher than that in high malignant cases (42.86%). The p27/Kip1 expression in gliomas decreased in number and staining intensity with the increasing histological malignancy (P<0.05). That is, the abnormal expression in gliomas of p27/Kip1 is closely correlated to histological malignant grade.

The relationship between abnormal expression of p27/Kip1 in glioma tissues and prognosis (Table 1, 2)

The p27/Kip1 positive expression rate in 27 cases with under 5-year survival (37.04%) was lower than that of 21 cases with over 5-year survival (90.48%). The number and staining intensity of p27/Kip1 expression in 27 cases with under 5-year survival was lower than that in 21 cases with over 5-year survival (P<0.05). Lower expression of p27 was associated with poor prognosis (P<0.05).

Results

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Discussion

P27/Kip1 gene is located on chromosome 12q13[2-4]. And it is a new tumor suppressor gene and could inhibit tumor growth. The cell cycle regulatory protein p27, an inhibitor of cyclin-dependent kinases (CDK), regulates the G1 phase to S phase transition of the cell cycle by inhibiting cyclinD-CDK4, cyclinE-CDK2 and cyclinA-CDK2[5, 6]. Modulation of p27/Kip1 cellular abundance occurs mainly at post-translational level by the ubiquitin-proteasome proteolysis.

Although rearrangements and mutations of p27/Kip1 are extremely rare events[3, 4], there are abnormal expression of p27/Kip1 in many human carcinomas and it is associated with a poor prognosis in many human carcinomas[7-10]. In this study, the positive expression rate and staining intensity of p27/Kip1 in gliomas were strongly higher than those in normal human brain tissues. The positive expression rate and staining intensity of p27/Kip1 in gliomas of lower histological malignant grade were higher than those in gliomas of higher histological grade. The data was analyzed by \( \chi^2 \) test and t-test and the results showed that the positive expression of p27/Kip1 decreased in number and staining intensity with histological malignancy of gliomas (P<0.05). After analyzing the correlation between the 5-year survival of past operation and the abnormal expression of p27/Kip1 in gliomas, we found that the expression of p27/Kip1 in 21 cases with more than 5-year survival was higher than that in 27 cases with less than 5-year survival. Lower expression of p27/Kip1 was associated with poor prognosis (P<0.05). The results of our research were similar to those of other researches on gliomas and other carcinomas.

In conclusion, the cell cycle regulatory protein p27/Kip1 may play an important role in the occurrence and development of human brain gliomas. The abnormal expression of p27/Kip1 in gliomas is closely associated to the histological malignancy of gliomas and prognosis. The abnormal expression levels of p27/Kip1 in gliomas may be helpful to prognosis evaluation.