IMMUNOHISTOCHEMICAL DETECTION OF P73 PRODUCT IN BRAIN GLIOMAS

ZHAI Guang 翟广 [1], YUAN Xian-hou 袁先厚 [1], PAN Hui-jin 潘惠锦 [2]
QIU Shang-ming 邱尚明 [2], ZHOU Ming-yong 周明勇 [2]

[1]*Department of Neurosurgery, Second Affiliated Hospital, Hubei Medical University, Wuhan 430071, China*
[2]*Chun Shan Medical College, Taichung, Taiwan, China*

Abstract

Objective: To elucidate the role of p73 in the genesis or development of glioma. Methods: P73 and p53 expression of 63 gliomas were detected by immunohistochemistry. Results: Out of the 63 gliomas, 17 cases appeared p73 positive. The positive-rate in high grade gliomas was higher than that in low grade gliomas (x^2=4.75, P<0.05). Among the 17 cases with p73-positive gliomas, 12 cases overexpressed p53 protein. Conclusion: Overexpression of wild p73 may involve in the genesis or development of glioma.

Key words: p73, p53, Brain glioma, Immunohistochemistry.

Genetic changes of several tumor suppressor genes such as p53 and p16 are involved in the genesis or development of glioma.[1,2] Recently, a novel gene which encodes a protein with similarity to p53 throughout its DNA-binding, transactivation, and oligomerization domains, called p73, was identified. P73 maps to 1p36, 33 region with the function of activating transcription of p53-responsive gene and inhibiting cell growth in a p53-like manner by apoptosis.[3,4] P73 was found that it is singularly expressed in neuroblastoma cell lines as well as in normal human peripheral blood cells and frequently deleted in neuroblastoma and lung cancer.[3,5] Based on the observations, p73 was regarded as a tumor suppressor gene at 1p36, which may play an important role in tumorigenesis if deregulated. Because there was cytogenetic evidence of chromosome 1p abnormality in some brain tumors, in this study we detected the protein expression of p73 in 63 cases of brain gliomas by immunohistochemistry to investigate if alteration of p73 is involved in the pathogenesis of brain glioma.

Accepted for publication: December 21, 1998
Correspondence to: ZHAI Guang; Department of Neurosurgery, Second Affiliated Hospital, Hubei Medical University, No. 39, Donghu Road, Wuchang District, Wuhan 430071, China; Phone: (0086-27)-87898366

MATERIALS AND METHODS

Specimens

Sixty-three paraffin-embedded samples were archival brain gliomas which were obtained from Department of Pathology of Second Affiliated Hospital of Hubei Medical University from 1993 to 1996. Among the gliomas, there were 8 grade I, 21 grade II, 24 grade III and 10 grade IV (according to WHO classification).

Immunohistochemistry

Five μm thick sections were normally deparaffinated and rehydrated. After antigen retrieval (microwave), the sections were blocked with normal serum (10%), then the sections were incubated with p73α polyclonal antibody (2 μg/ml) or p53 monoclonal antibody (1:300) (Santa Cruz.) at 4°C overnight (negative control was done with blocking serum only). Subsequent steps for p73 were performed according to Vectastain ABC-AP Kit (Vector). The slides were visualized with Vector Blue Alkaline Phosphatase Substrate Kit III (Vector) and counterstained with Fast Red (Vector). For p53, SABC Kit (bo shi de biologic corporation, Wuhan, China) was applied. The slides were visualized with 0.06% diamino-benzidine (Sigma) and counterstained with hematoxylin. All the slides were examined under light microscope. P73-positive cell appeared blue staining in nucleus while p53-positive cells had yellow-brown nuclei (Figure 1, 2).

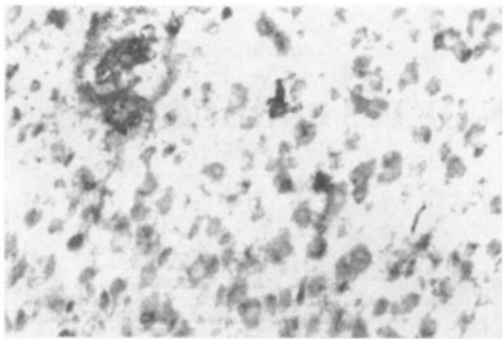

Fig. 1. P73-positive expression: nuclei of tumor cells were dyed blue. 400×

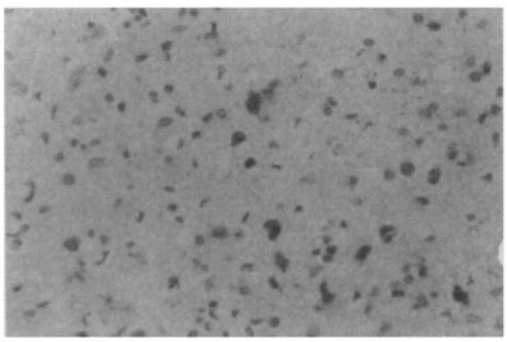

Fig. 2. P53 overexpression: nuclei of tumor cells were dyed yellow-brown. 400×

RESULTS

Seventeen cases of gliomas were found p73-positive, most of which were high grade gliomas (Table 1). Positive cell appeared blue staining in nucleus. Positive cells were all tumor cells. Section was scored as positive if positive cells were over 10%. Twenty-six cases of the 63 gliomas showed over-expression of p53 protein (the percentage of positive cells throughout a slide was over 25%). Among the 17 cases of p73-positive gliomas, 12 cases overexpressed p53 protein.

Table 1. Results of immunohistochemical detection of p73 and p53

Grade	Number	Positive	Negative
I	8	0	8
II	21	4	17
III	24	10	14
IV	10	3	7

Chi-square test: Comparison of grade I, II and III, IV: x^2=4.75, P<0.05.

DISCUSSION

P73 gene is a recently identified gene with similarity to p53, and presumed as a tumor suppressor gene. Abnormality of p73 was reported to be involved in the pathogenesis of neuroblastoma and lung cancer.[3,5] We firstly detected the expression of p73 protein in 63 cases of brain gliomas by immuno-histochemistry. P73-positive expression was found in 17 cases out of the 63 gliomas (about 27%). P73-positive rate in high grade gliomas (grade III and IV, about 38.2%) was significantly higher than that in low grade ones (grade I and II, about 13.8%) (x^2=4.75, P<0.05). The results indicated that expression of p73 correlated with the pathological grade and increased with elevation of grade of glioma. Kaghad et al.[3] also reported that the majority of nonneuroblastoma cell lines which had mutations in p53 gene expressed high level of wild type p73 while most normal tissues showed low levels of p73 transcript and protein. They concluded that elevated p73 in tumor cell lines may be due to the disruption of normal p53 function which in turn results in compensatory or deleterious upregulation of p73 expression. In our experiment, we found that 12 cases out of the 17 p73-positive gliomas had overexpression of p53. The results seemed to agree with Kaghad's conclusion, but we couldn't explain the other 5 cases. Recently, Mai et al.[6] investigated p73 expression in 21 pairs of lung cancers and matched normal lung tissues by semi-quantitative RT-PCR. They also found that p73 expression was higher in tumor samples than in normal tissues. Mutation analysis revealed no tumor-specific mutations, but allele-specific expression analysis in 5 heterozygous samples by SANUPE method revealed that 5 tumors exclusively expressed both alleles while matched normal tissues expressed monoallelically. Based on these finding, they suggested that p73 may play an important role in lung tumorigenesis through activation of a silent allele which results in overexpression of wild-type p73.

Based on our experimental results and the previous discussion, we suggested that overexpression of p73 may be involved in genesis or development of glioma, and that overexpression of p73 may be due to mutation of p53 which results in upregulation of p73 expression or activation of a silent allele of p73. Nevertheless, further studies to explain p73 function, mechanism of p73 activation or inactivation are necessary.

REFERENCES

[1] Walker DG, Duan W, Popovic EA, et al. Homozygous of the multiple tumor suppressor gene 1 in the progression of human astrocytomas. Cancer Res 1995; 55:20.

[2] Frankel RH, Bayona W, Koslow M, et al. P53 mutation in human malignant gliomas: comparison of

heterozygosity with mutation frequency. Cancer Res 1992; 52:1427.
[3] Kaghad M, Bonnet H, Yang A, et al. Monoallelically expressed gene related to p53 at 1p36, a region frequently deleted in neuroblastoma and other human cancers. Cell 1997; 90:809.
[4] Jost CA, Marin MC, Kaelin WC, et al. P73 is a human p53-related protein that can induce apoptosis. Nature (Lond) 1997; 389:191.
[5] Nomoto S, Haruki N, Kondo M, et al. Search for mutation and examination of allelic expression imbalance of the p73 gene at 1p36, 33 in human lung cancer. Cancer Res 1998; 58:1380.
[6] Mai M, Yokomizo A, Qian C, et al. Activation of p73 silent allele in lung cancer. Cancer Res 1998; 58: 2347.

FAMILIAL ADENOMATOUS POLYPOSIS

XU Ning 徐宁,[1] DING Yan-qing 丁彦青 [1], XU Li 徐莉 [2]

[1]*Department of pathology, First Military Medical University,* [2]*Depatment of Parasite, First Military Medical University, Guangzhou 510515, China*

Clinical History

A 41-year-old female was admitted into Nan Fang Hospital for severe abdominal pain with bloody-mucoid stool for a month. The symptoms started a year ago without obvious causes and she did not have any systemic treatment. The patient felt fatigue and loss of weight for the last three months and increased frequency of bloody-mucoid discharge from 2–4 times/day to 10 times/day for the last month. Two weeks ago the patient had a proctoscope with biopsy in Pan Yu people's Hospital. The pathological diagnosis was rectal villous adenoma with focal malignant changes. Rectal examination in this hospital found a rectal mass, 4 cm from the anus, longitudinal growing and occupying a quarter of the circumference. Further colono-fiberscope diagnosis was familial polyposis of colon. Family history showed that her father died of lung cancer, her mother died of colonic cancer and her brother and sister were healthy. A total colo-rectectomy with ileostomy was performed.

Pathology Report

The specimen was a segment of colon measuring 79 cm in length with multiple polypus varying in size, from 0.1 mm to 30 mm in diameter. Sitnated at 15 mm from the pectinate line there was a polypoid mass, 45×40×18 mm, sitnated at 75 mm and 140 mm from the pectinate line there are two ulcerated masses measuring 40×30 mm and 30×25 mm respectively. The surrounding mucosa was elevated. The pathological diagnoses was polyposis; moderately colo-rectal multiple polyps (>100) consistent with familial differentiated adeno-carcinoma infiltrating through full thickness of the bowel wall; sigmoid flexure tubulovillous adenoma with superficial malignant changes; lymph node metastasis carcinoma (6/33).

Discussion

Familial adenomatous polyposis (FAP) also called familial adenomatous is an autosomal dominant genetic disease. About 1/10,000 population carries this abnor-mal gene. Polypus is its surface-type mark. The muta-tion of APC is relation with FAP tumorigenesis. APC is a suppressor gene, it is reported the APC germ-line mutation may be response to 67% FAP. Theoretically, if a patent carries this gene, the half of the children can be involved. In fact, only 8% clinically present with FAP. Pathologically, the entire colonic mucosa can be involved by multiple, various polypus from 150 to 5000, or even more. Most patients have 500–2500, average 1000 polypus. Usually the minimal number to diagnose FAP is 100 polypus; if less 100, it is called multiple adenoma. FAP involves the rectum the most, but never involves the small bowel. Small polypus in FAP is just mucosa millet papules. FAP has a tendency of malignant changes. Usually, 2/3 of the patients are associated with carcinoma in their the first visit. Malignancy always starts from adenoma (polyp) itself but not the mucosa in between. It takes about 10 year from adenoma to develop carcinoma. Cautery or surgery is the treatment. FAP with malignant changes should be treated as carcinoma.

Accepted for publication: Novermber 9, 1998