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Effect of HCMV IE86 on the Expression of p21 in Malignant Glioma Cells

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Abstract: Human cytomegalovirus (HCMV) can induce malignant transformation of tumor cells and inhibit tumor cell apoptosis, but whether the key immediate regulatory protein IE86 encoded by HCMV plays a key role in this process remains unknown. The purpose of this study was to investigate the effect of IE86 on p21 expression of glioblastoma cells in genetically modified glioblastoma mice. The expression of IE86 in genetically modified mice was identified by PCR. Transgenic mice were tumor-bearing by glioma cell line GL261. The expression of p21 protein was detected by Western blot. The results showed that the IE86 gene-modified mouse model was successfully constructed. Compared with the IE86-negative group, the expression level of p21 was decreased in the IE86-positive group. The above results indicate that IE86 is continuously expressed in genetically modified mice, but the indicator p21 of p53 transcriptional activity is downregulated, and this suggests that IE86 can increase the anti-apoptotic ability of malignant glioma cells.

Keywords: Human cytomegalovirus; Transgenic Mice; IE86; p21

Introduction

Human cytomegalovirus (HCMV) is a linear double-stranded DNA virus belonging to the beta herpesvirus subfamily [1, 2]. HCMV infection is quite common, the infection rate of the general population is over 80% in China, and it can reach 100% in some special areas or populations [3, 4].The IE86 gene-modified mouse model uses the DNA pronuclear microinjection method to integrate the IE86 gene into one of the chromosomes of the mouse, thereby obtaining a hemizygous first-generation (F0) transgenic mouse, and only a part of the offspring have integrated genes. In theory, 50% of F1 mice are transgenic heterozygous mice, and 50% are wild-type mice, which need to be screened and identified. After making the same batch of transgenic mice, some of them were crossed with normal wild-type mice, and then mated with male and female positive mice of the same litter. Under the homologous recombination of the genes, homozygous mice were finally obtained [5].

IE86 is an important transactivator that plays a key role in HCMV replication and is involved in the pathogenesis of many diseases [6, 7]. Previous studies have shown that IE86 regulates host gene expression in host cells and affects cell proliferation and apoptosis by interacting directly with cellular genes or proteins [8]. The study found that HCMV infection is closely related to malignant glioma, and the expression of its gene increases with the degree of malignancy [9]. However, the relationship between IE86 and the development of malignant glioma is unclear.

p21, one of the key regulators of cell cycle progression at the G1/S transition, serves a crucial role in tumorigenesis [10-12]. p21 is widely accepted as a tumor suppressive protein and a negative regulator in the G1/S transition [13]. A number of studies have reported that downregulation of p21 expression is involved in various human cancers and is correlated with cell growth [14, 15]. p21 may acts independently as well as with other cell cycle regulators, such as p53 [16]. Actually, the p53 protein can activate the p21 protein, which acts as a mediator and inhibits cell differentiation and proliferation [17]. Therefore, it is speculated that IE86 protein can affect the apoptosis of malignant glioma cells through p21. This paper aims to overcome the specificity of species and establish an organic internal environment by using a genetically modified mouse model that can stably and stably express IE86. So, we can further explore the role of IE86 protein and p21 in vivo, and its effect on glioma cell apoptosis. The aim is to elucidate the role of IE86 protein in maintaining viral infection and provide a theoretical basis for the molecular mechanisms involved in apoptosis of malignant glioma cells.

MATERIALS AND METHODS

Animals and cells

Twenty four IE86 genetically modified mice (six weeks old) and twenty four wild mice (six weeks old) were obtained from Laboratory of pathogenic biology of Qingdao University. All animal experiments were authorized by the Animal Experiments Committee of Qingdao University. UL261 cell line purchased from

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Saiqi Huadong cell bank. The UL261 cells were subcultured, 0.1 mL of logarithmic growth phase (about 3×10^6 viable cells) was selected, and 12 IE86 gene-modified mice and 12 wild mice were taken, then the cells were slowly injected into the right inferior temporal skin. After tumor transplantation, the time, tumor size, texture and other changes of the tumor implantation site were observed. The vernier caliper measures the longest diameter (a) and the shortest diameter (b) of the tumor every 2 days, and the tumor volume formula is V (mm^3) = $a \times b^2 \times 0.5$. Two sets of tumor growth curves were plotted as a function of tumor volume (ordinate) versus time (abscissa). The terminal tumor weight was measured when the experiment was terminated.

Extraction of DNA and PCR

The PCR was executed according to the manufacturer's protocol after DNA was extracted
using a DNeasy tissue Kit (CWBIO). The cycling condition details as follows: Pre-denaturation at 95°C for 5 min; denaturation at 95°C for 30 sec; annealing at 55°C for 35 sec; extension at 72°C for 30 sec and further extension at 72°C for 10 min. This PCR cycle was performed for 35 times. The primers' sequences were 5'-3: CAGTCCGCCCTGAGCAAAGA (Forward) and 5'-3': TATGAACAAACGACCCAACACCC (Reverse). An agarose gel was prepared, Super GelRed (US Everbright Inc.) was added, and the reaction product was subjected to agarose gel electrophoresis to observe a DNA band of interest.

Extraction of Protine and Western-blot

After the tumor tissue was fully ground, 501 μL of lysate (RIPA: PMSF=500:1) was added, and the supernatant was collected by centrifugation to obtain total protein. The extracted protein was added to 5×SDS loading buffer at a ratio of 1:4, boiled for 5 min, and after SDS-PAGE gel electrophoresis, the protein was transferred from the gel to the PVDF membrane with a constant current of 300 mA. 5 % skim milk powder prepared with TBST was blocked for 2 h, p21 (diluted 1:1000) was incubated overnight at 4 °C, secondary antibody (diluted 1:2000) was incubated for 2 h, TBST was washed 3 times for 10 min, and ECL was added. The color developing solution (solution A: solution B = 1:1) was stored in the dark for 5 min and then developed to detect the expression of the target protein.

RESULTS

Identification of IE86 in genetically modified mice

DNA was extracted from the tip of the mouse. IE86 positive and negative mice were identified by PCR. The results showed that IE86 was significantly expressed in genetically modified mice, and IE86 was not expressed in wild mice. The results showed that the IE86 gene-modified mouse model was successfully constructed (Figure 1). Mice were divided into an experimental group and a control group according to whether IE86 was expressed or not.

Growth of glioma in the tumor-bearing mice

Two weeks after the UL261 cell suspension was implanted subcutaneously, the tumor-bearing mice began to appear fatigue and lack of weight. On the 6th day of tumor implantation, the size of the bean granules was observed under the armpit of the tumor-bearing mice. At 17 days after inoculation, the tumor of the tumor-bearing mice showed near-exponential growth, and the tumor volume increased significantly with time (Figure 2A). The average survival time of tumor-bearing mice was 17 to 25 days, and the tumorigenic rate was 100%. The anatomy showed that the tumor was oval or irregular, and the boundary was clear. The anatomical surface of the tumor was solid, white and fishy, and hemorrhagic necrosis was seen in the tumor (Figure 2B). At the end of the experiment, the tumor weight of IE86-positive tumor tissue was significantly higher than that of IE86-negative group, and the difference was significant, $P<0.01$ (Figure 2C).

IE86 downregulates p21 protein expression in gliomas

The tumor tissues were taken for Western-blot to detect the expression of p21 protein. The results of Western-blot showed that the positive staining of p21 in tumor tissues was significantly stronger than normal brain tissues. In tumor tissues, the expression of p21 protein in IE86-positive group was significantly lower than that in the negative group, which was also observed in normal brain tissue, $P<0.01$ (Figure 3).

Discussion

There is a close relationship between HCMV infection and malignant glioma, in which IE protein is the most active cell regulatory protein after HCMV infection. Studies have shown that IE protein can transactivate a variety of cellular genes and affect cell proliferation and apoptosis [18], of which IE86 is the most important functional protein [19]. IE86 affects cell growth and function by regulating important genes involved in cell cycle and apoptosis, which is an important step in the intervention of host cells after HCMV infection. However, there is no consistent conclusion on the relationship between IE86 and p21.

The IE86 gene-modified mouse model used in this study was to integrate the IE86 gene into one of the chromosomes of mice using a DNA pronuclear microinjection method to obtain a hemizygous first-generation (F0) transgenic mouse. After making the same batch of transgenic mice, some of them were crossed with normal wild-type mice, and then IE86-positive mice were identified by PCR screening for subsequent experiments.

The p21 gene belongs to the CIP/Kip family and is a cell cycle-dependent protein kinase inhibitor downstream of the p53 gene, which binds to and inhibits the activity of the cyclin-dependent kinase (CDK)/cyclin complex. These complexes are closely related to p53-mediated G1/S phase arrest [20]. The

p53 gene can induce transcription of its downstream gene p21, thereby increasing the synthesis of p21 protein [21]. Studies have shown that p21 is involved in tumor differentiation, invasion, proliferation and metastasis [22]. Early studies showed that HPV16 E5 can inhibit the activity of p21 gene promoter and downregulate its expression level. P21 is a key protein regulating cell cycle progression, and its downregulation eventually leads to accelerated cell growth and malignant proliferation [23]. However, studies on the effects of HCMV IE86 on p53 and its downstream gene p21 have been rarely reported.

This study found that HCMV IE86 can induce malignant transformation of tumor cells and inhibit tumor cell apoptosis. The results of gene and protein assay showed that there was a significant positive correlation between the expression of p53 and the expression of IE86 in malignant tumors. To further demonstrate the effect of IE86 on the transcriptional activity of p53 in malignant glioma, immunohistochemistry was used to detect the expression of p21 protein downstream of p53 in tumor tissues, and the apoptosis of tumor tissue was detected by TUNEL method. The results showed that IE86 can downregulate p53 transcriptional activity and increase the anti-apoptotic ability of malignant glioma cells. The IE86 gene-modified mouse model established in this study is helpful to further explore the role of HCMV IE86 in the development of malignant glioma.

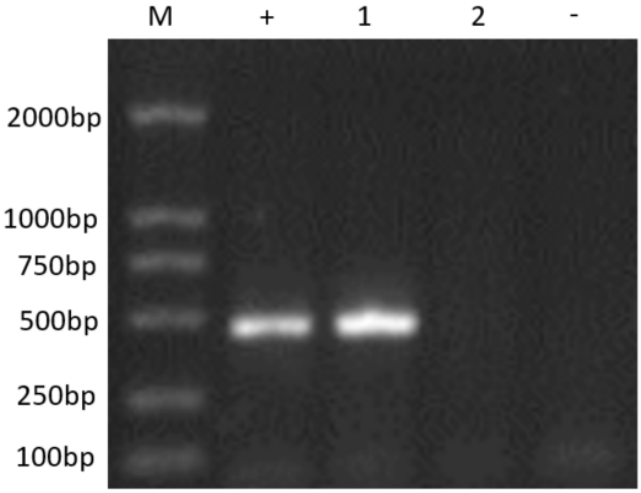

Figure 1 Lane M: DNA molecular marker; lane +: positive control; Lane 1: positive band; Lane 2: negative band; lane -: water control. The PCR product band size was 335bp.

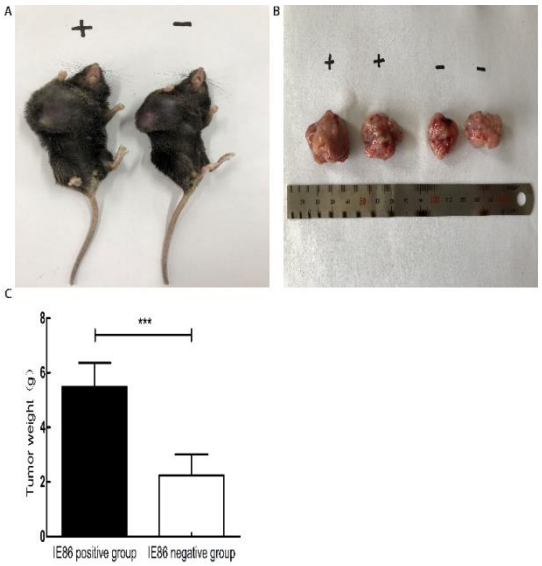

Figure 2 Effect of IE86 on tumor-related morphological changes in tumor-bearing mice. (A, B) tumor-bearing condition in mice (a, b are negative and positive mice, respectively). C. Terminal tumor weight, and compared with the control group, the tumor weight of the experimental group were significantly increased, $P<0.01$.

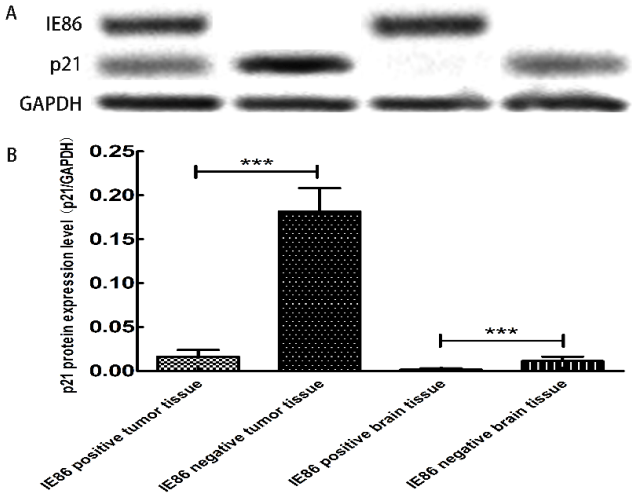

Figure 3 Western blot detection of p21 protein expression in tissue. A. Strips exposed in a protein gel imaging system. B. The relative band intensity was calculated by the intensity of the p21 protein band against the intensity of GAPDH protein, P＜0.01.

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