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Effect of ATF5 in Mice Transfected with Human Cytomegalovirus IE2 Gene

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Abstract: To investigate the qualitative and quantitative detection of ATF5 activated transcription factors in tumor tissue by using the method of transfecting IE2 mouse tumor to explore the effect of HCMV immediate early protein IE86 on ATF5 function. Transfected mice were treated with GL261 mouse glioma cells. The expression of transcription factor ATF5 in the tumor tissues of tumor-bearing mice was detected by PCR and Western-blotting methods. The experimental results showed that the expression of ATF5 was higher in IE2 transgenic mice than in normal mice. ATF5 is highly expressed in IE2 transgenic mice. ATF5 is a transcription factor closely related to apoptosis and plays an important role in apoptosis, differentiation and development.

Keywords: Human Cytomegalovirus, IE86, Mouse Glioma Cell, ATF5

Introduction

HCMV belongs to the herpes virus family and causes widespread and persistent infections in the human population. The brain is a preferred site for HCMV infection, leading to dysfunction such as mental disorders or epilepsy [1-2]. HCMV regulates the malignant phenotype of the tumor by affecting cell survival, cell cycle and invasion potential. The IE2 gene mouse was established by DNA pronuclear microinjection method[3]. The target gene transferred is only integrated into one of the chromosomes of the diploid animal, so it is a hemizygous, and its offspring only have a part of the individual. There are integrated genes, some have no target genes, so transgenic mice require a fairly long screening and purification process to obtain homozygous transgenic mice. The first viral gene after HCMV infection is an immediate early gene, and the most abundantly expressed product in the gene is called immediate early 1 and 2 proteins [4] (IE72 and IE86). The IE86 protein is a potent transactivator that interacts with factors of the underlying transcriptional machinery. The IE86 protein involved in the transactivation of viral and cellular promoters is unique in viral regulatory proteins because it regulates both viral and cellular promoters both negatively and positively [5]. There is increasing evidence that ATF5 is a factor closely related to apoptosis. According to experiments, the interference inhibiting the function of ATF5 in the murine glioma model causes specific apoptosis of glioma cells, but does not have such apoptotic effect on normal cells surrounding glioma cells[6]. High expression of ATF5 has also been found in many tumor tissues, for example, interference in the inhibition of ATF5 expression in breast cancer cells can also cause apoptosis in cells [7]. ATF5 is a transcription factor closely related to apoptosis and plays an important role in physiological processes such as apoptosis, differentiation and development.

MATERIALS AND METHODS

Animals and Cell culture

Thirty C57BL/6 mice, female, body weight 18-20g, 6-8 weeks old; GL261 mouse glioma cells were cultured in DMEM medium containing 10% fetal calf serum containing penicillin and streptomycin, cultured in 37 ° C, 5% CO_2 incubator, subcultured every three days for logarithmic growth phase The cells were subjected to the following experiments. When the cells were grown to 90% confluence in the culture flask, the cells were digested with trypsin containing EDTA, and the cells were digested into 2 ml of 1×10^7/ml suspension cells; the mice of the three experimental groups were given the right side of the experimental group by syringe. Under the injection, the control group was injected with the same dose of suspension cells at the same position.

RNA extraction of tumor tissue and RT-PCR identification of ATF5

RNA was extracted from each tissue of thirty transgenic mice using RNApure tissue Cell Kit (CWBIO, China). And 1 μg RNA was reverse-transcribed using the Transcriptor First Strand cDNAs Synthesis Kit (Roche, Germany) to produce cDNAs according to the manufacturer's instructions. Primers ATF5-F: ACCTCCACCACCAGCAGCAG

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and ATF5-R: AGCCAGCAGGTCCAAGGTATCC were used to amplify the ATF5 gene with 225 bp product. The band intensity was obtained using Image J software.

Protein extraction of tumor tissue and quantitative detection of ATF5 by Western-blot

Weigh the tumor tissue with an electronic balance and weigh 20-30 mg into a 1.5 ml autoclaved centrifuge tube, grind thoroughly with a sterile grind, add 501 μL of lysate (RIPA:PMSF=500:1), and finally on ice. The cells were lysed for 30 min, centrifuged at 13000 r/min for 4 min at 4 ° C, and the supernatant was collected for total protein. The extracted protein was added to 5×SDS loading buffer at a ratio of 1:4, boiled for 5 min, and subjected to SDS-PAGE gel electrophoresis. After SDS-PAGE gel electrophoresis, the protein was transferred from the gel to the PVDF membrane with a constant current of 300 mA. On the top, 5% skim milk powder prepared with TBST was blocked for 2 h, primary antibody (1:2000 dilution) was incubated at 4 °C overnight, secondary antibody (1:2000 dilution) was incubated for 2 h, TBST was washed 3 times for 10 min, and ECL was added. The solution (Form A: B solution = 1:1) was stored in the dark for 5 min and then developed to detect the expression of the target protein.

RESULTS

PCR qualitative identification of ATF5

The 30 standardized mice of the experiment were identified by tail tail, and the identified negative mice and positive mice were grouped for subsequent experiments. The results showed that the expression of ATF5 in 5 pairs of negative mice and positive mice. Significant differences can be observed by electrophoresis strips (Figure 1).

Tumor status after tumor-bearing in mice

For the selected tumor-bearing mice, the body weight of the mice was no longer increased, and the mice were sacrificed by cervical dislocation and dissected (Figure 2A). The tumor was stripped and a distinct state difference was observed (Figure 2B). The positive tumor-bearing mice had significant volume difference compared with the negative tumor-bearing mice, and there was a significant difference compared with the control group, which was statistically significant ($P<0.0001$).

Western blot analysis of quantitative detection of ATF5 expression levels

Protein expression of tumor-bearing mice was followed by Western blotting to detect the expression of ATF5. Western blot was used to detect the quantitative relationship between ATF5 and IE86 protein (Figure 3A). The experimental group was positive tumor-bearing mice ATF5. The mean value of expression, the control group was the mean value of ATF5 expression in the negative tumor-bearing mice (Figure 3B), and there was a significant difference compared with the control group, which was statistically significant ($P<0.05$).

Discussion

Human cytomegalovirus (HCMV) belongs to the beta herpesvirus subfamily and is the largest group of human herpesviruses [8, 9], with an infection rate as high as 100%. The HCMV genome is a double-stranded DNA of more than 240 kb in length, and the entire genome contains 250 open reading frames (ORFs). In the process of viral infection, the expression of HCMV gene shows a certain sequence, which can be divided into early stage (IE), early (E) and late (L) genes [10-12]. After the virus penetrates into the cell, the IE gene is activated by the host cytokine and is expressed first, encoding two important regulatory proteins, IE1 (IE72) and IE2 (IE86). Among them, IE2 (IE86) protein is an important transactivator and plays an important role in HCMV infection [13]. In recent years, it has been confirmed that IE2 (IE86) regulates many factors related to the control of cell cycle.

The IE2 gene mouse used in this experiment is a micronucleus injection method of DNA, and the target gene transferred is only integrated into one of the chromosomes of the diploid animal, so it is a hemizygous, its In the offspring, only a part of individuals have integrated genes, and some have no target genes [14]. Therefore, transgenic mice require a relatively long screening and purification process in order to obtain homozygous transgenic mice. Activating transcription factor 5 (ATF5) was originally isolated from human T lymphocytes and plays an important role in tumor development [15]. ATF5 is a member of the ATF/CREB family of basic leucine zipper (bZIP) domains. Due to the leucine zipper motif and the basic region rich in lysine and arginine residues, ATF5 is involved in DNA binding and protein-protein interactions and has anti-apoptotic effects [16]. The study found that the anti-apoptotic function of ATF5 is selective and has no anti-apoptotic function in the process of apoptosis induced by DNA damage agents. ATF5 is a transcription factor closely related to apoptosis and plays an important role in physiological processes such as apoptosis, differentiation and development [17].

The innovation of this study is to establish animal model of glioma cells under the skin of mice with transgenic mice as an entry point, focusing on the relationship between expression and distribution between IE86 and ATF5 at the molecular level. The results showed that activating transcription factor 5 (ATF5) showed a high expression status in the transgenic IE2 mice, and there was a significant quantitative relationship with it [18-20]. The results of this study are important for the treatment of viral infections and the expansion of new ideas for the treatment and prevention of cancer.

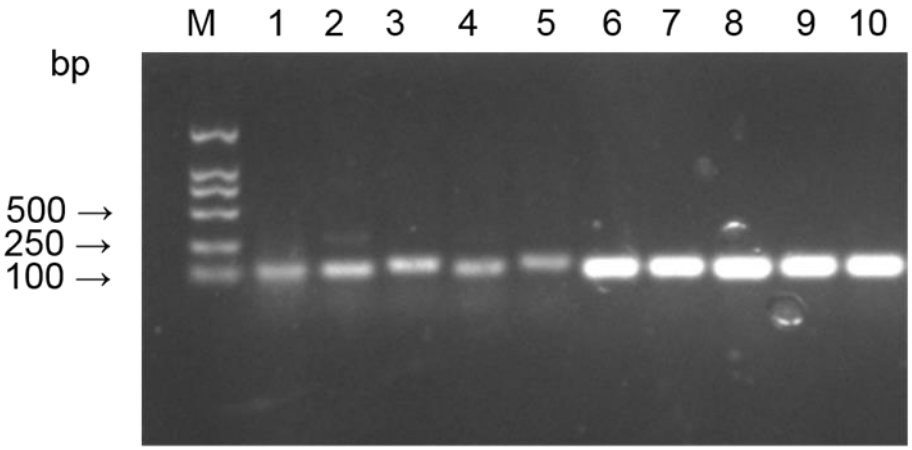

Figure 1 M: DNA marker; 1-5: expression of ATF5 in negative mice, 6-10: Expression of ATF5 in positive mice

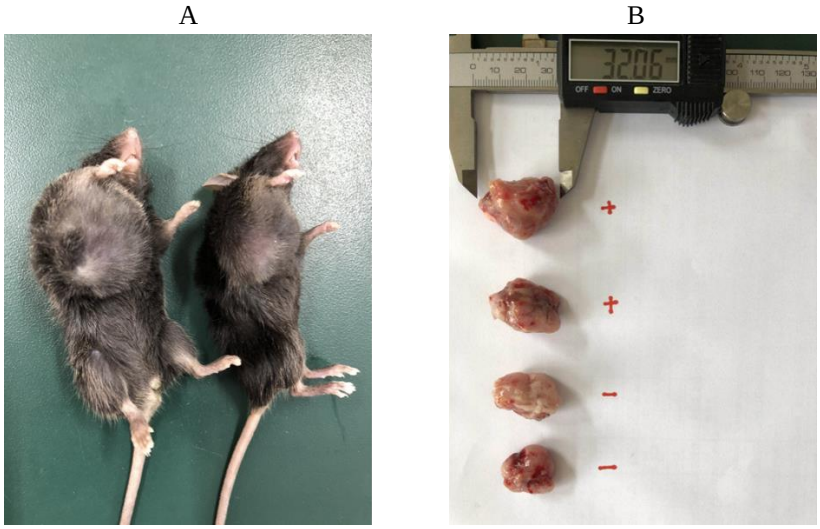

Figure 2 Morphological changes of GL261 murine glioma cells in tumor-bearing mice. In vivo morphological changes (A) and in vitro morphological changes (B).

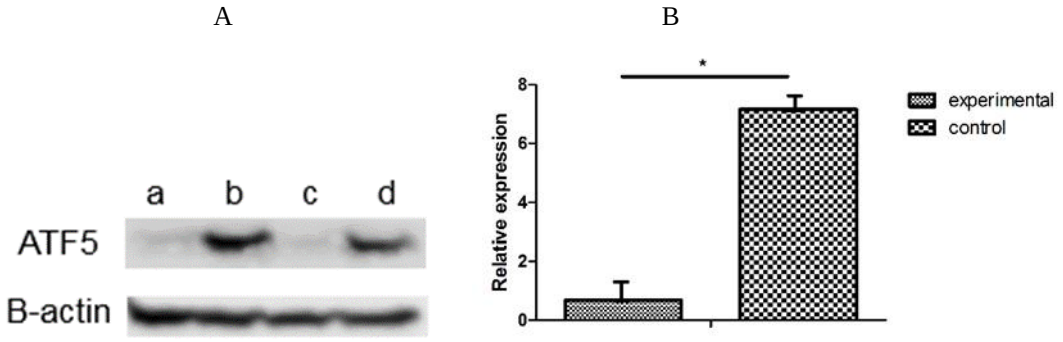

Figure 3 A: The quantitative relationship between ATF5 and IE2 was detected from the protein level by Western blot; B: a, c is the expression level of ATF5 in negativity mice, b, d is the expression level of ATF5 in positive mice, * P ＜0.05

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