Effects of siRNA Targeting Interference of APE1 Gene on Proliferation and Apoptosis of Glioma Cells

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ABSTRACT

This paper explored the application of RNA in cancer gene therapy. Second, the effect of the recombinant plasmid APE1 siRNA on the expression of APE1 protein was observed. In addition, the proliferation of cells in apoptosis and the radiosensitivity of human glioma U251 cells were also discussed. U251 cells were divided into control group, negative group and APE1siRNA group for culturing. There were two holes in each group, and independent experiments were repeated three times. The expression of APE1 protein in U251 cells was detected by Western blot experiments for siRNA transfection. The siRNA cells were transfected, while another group of FAM fluorescent labeled non-sense sequence siRNA was transfected to determine the transfection rate and extract the total protein of the cell. BCA protein assay kit was used for determining the concentration. Meanwhile, 50g sample and SDS-polypropylene diamine gel were prepared. Coomassie blue staining was used to observe the protein electrophoresis, after which the protein on the gel was transferred to the PVDF membrane and then developed in a chemiluminescent detection system with specific antibodies. The β -action test was used as an internal controller, while the optical density scanner was used to detect the gray value of the target strip. The relative expression of target protein was equal to the target band gray value or the same sample reference value of gray. MTT method was used to detect the cell growth curve experiments, while AO or EB method was used to detect the apoptosis rate of glioma cells. SPSS17.0 was used for statistical analysis. The results show that the APE1 siRNA expression plasmid was successfully introduced into human glioma cells by RNA interference, which can reduce the expression of APE1 in cells. It was confirmed that the reduction of APE1 protein could inhibit proliferation and promote the apoptosis of glioma cells. Third, APE1 targeting interference may be an effective gene therapy strategy for human glioma, which can provide an experimental basis for the clinical gene therapy of glioma.

Key Words: APE1 Targeting Interference, MTT Method, Apoptosis Rate

DOI Number: 10.14704/nq.2018.16.5.1413 **NeuroQuantology 2018; 16(5):44-52**

Introduction

RNA interference was a typical gene expression induced by double stranded RNA, which specifically inhibits the corresponding complementary bases. It is characterized by high specificity, small molecular and transitivity. Now, this technology has become an important tool in gene research, and been gradually applied to cancer gene therapy and other fields (Montaldi *et al*., 2015). Gene delivery system was the key to RNA, the application of gene therapy and RNA interference. There were four main methods for foreign genes to enter cells, such as electric shock, calcium phosphate, liposome mediated and virus mediated. The constructed recombinant plasmid APE1 siRNA expression vector was introduced into human glioma U251 cells (Hou *et al*., 2015).

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
Received: 9 April 2018; **Accepted:** 8 May 2018

In addition, its effects on the expression of APE1 protein, cell proliferation and apoptosis in human glioma cells were observed. At the same time, the changes of radiosensitivity were observed, and the possible mechanism was analyzed, which can provide experimental bases for further gene therapy of brain glioma (Zhu *et al.*, 2015).

Methods

Experimental materials

(1) Experimental subjects

Glioblastoma multiforme *in vitro* cell line; U251 cell.

(2) Main reagents

DMEM medium (produced by USA Gibco Company) used in cell culture; Fetal bovine serum(produced by Sijiqing Biological Engineering Company in Hangzhou); Trypsin (produced by Sigma Company); Liposomes LIPOFECTAMINE 2000 (produced by Invitrogene Company); Opti-MEMLOW serum medium(produced by Invitrogene Company); Mouse anti human APE 1 polyclonal antibody (produced by Invitrogene Company); Rabbit anti mouse polyclonal antibody marked by Hrp (produced by Invitrogene Company); Protein extraction reagent (produced by USA novogen Company); Protein quantification reagent (produced by USA pierce Company); MTT (produced by Ruibo biological); AO/EB (produced by Sigma Company).

(3) Main instruments

Fluorescence phase contrast inverted microscope IX-71 (produced by Japan Olympus Company); Ordinary electrophoresis instrument (produced by Beijing 61 factory); Optical microscope (produced by Japan Olympus Company); ND-1000 spectrophotometer (produced by USA Nano Drop Company); Hermle (produced by German Heraeus Company); Constant temperature water bath box (produced by USA Pharmacia Company); CO_2 Incubator (produced by German Heraeus Company); Western Blot Instrument (produced by USA BioRad Company); Chemiluminescence detection system (produced by USA pierce Company); Biorad gel imaging system (produced by Alphainnotech Company).

Experimental methods

(1) Experimental grouping

All the indexes of the experiment were grouped into the following categories: control group (transfected empty plasmid), negative group (without transfection of any plasmid) and APE1 siRNA group (transfected with APE1siRNA plasmid). Cells were cultured in 6-hole culture plates. There were two holes in each group, and the experiment was repeated three times independently.

(2) Resuscitation, culture, passage and cryopreservation of U251 cell

a. U251 cell resuscitation. First, frozen cell tubes stored in liquid nitrogen tanks were removed. Second, the frozen tubes were quickly placed in a water bath of 37°C and they were constantly shaken to thaw as soon as possible. Third, 70% alcohol was used for cleaning and disinfecting. The cryopreservation tube was moved to the ultra-clean table and the cell suspension was sucked into the culture bottle. Every 1ml cell solution was supplemented with 9ml medium containing 10% fetal bovine serum and cultured in an incubator of 37°C. After 24 hours, the survival rate of the cells was observed and the culture was continued after the liquid change.

b. U251 cell passage. First, the old culture solutions were carefully sucked, and 5 ml sterilized PBS solution was added to the surface of the cell and continued to shake. Second, 2ml warm trypsin was digested for l-2min until the cell was fully digested. Third, the digestive juices were carefully absorbed, while the complete medium containing 10% fetal calf serum and two resistance were added. Fourth, culture fluid was absorbed by straw, and the bottle wall cells were blown repeatedly and gently. The uniform cell suspension was made. Fifth, suspension cells were divided into culture flasks and cultured in a CO_2 incubator of 37℃ according to the proportion of 1: 3. Finally, U251 cells were passaged and amplified for 3-5 generations. Parts of them were used in the experiment, and the remaining cells were cryopreserved.

c. U251 cell cryopreservation. First, cells in the logarithmic phase were selected and the solution was replaced one day before the cryopreservation. Second, the cell culture solutions in the bottle were carefully sucked and the PBS solution was rinsed gently twice. Third, 0.25% trypsin was used for digestion, which was completely aspirated until the cells were fully digested. A small amount of culture medium containing 10% fetal bovine serum by digestion was terminated. Pipetting was repeatedly added into the cell suspension. Fourth, 2000r/min

centrifugation was performed for 10 minutes. Fifth, the supernatant was removed, and the amount of dilution of the cryopreserved fluid was added. These cells were uniform, and concentration was raised to 3×106-1×107/ml. In addition, the cells were packed in the frozen tubes according to l ml/tube, while the lid was tightly sealed and marked. Finally, medical cotton pad was used to wrap the frozen tube, which was stored in a freezer of 80°C for about 6 months.

d. Cell preparation. U251 cell in the exponential growth phase was digested by trypsin, and it was made into cell suspension while the number of viable cells was calculated. The 10 μl cell suspension and 0.4% trypan blue solution were selected and mixed thoroughly, and the number of cells was counted after 5 minutes. The dead cells were colored, while the living cells were unstained. The concentration of U251 cell suspension was adjusted to 1×105/ml.

(3) Transfection of siRNA

a. siRNA dissolution. siRNA (10D) was placed in the EP tube for a brief centrifugation. 150 μl RNase deionized water was added, and the final concentration of siRNA was 2 μM/L. In order to avoid degradation during the storage of siRNA, 20 μM/L could be prepared and preserved at -80℃ for reserving, which can avoid repeated freezing and thawing.

b. U251 cells in logarithmic phase were digested with trypsin and inoculated in 6 cell culture plates.

c. After about 24 h (50-80% fusion), the complete medium was carefully removed by using a straw. No serum DMEM was used to clean twice. 0.8 ml/bore DMEM medium without serum and antibiotics was added at 37℃ and 5% CO_2 was starved in culture for 1h.

d. 3 μl lipofectamine and 15 μl serum free DMEM were mixed and stored at room temperature for 5min.

e. 10μl siRNA solution and 175μ1 serum free DMEM were mixed.

f. The solutions in the two steps were mixed and collocated at room temperature for 30min, then the siRNA transfection solution was obtained.

g. siRNA transfection solution was moved to 6 well plates that has been trained well and was gently piprmixed by pipetting. It was continued to develop for 4-6 h, and the complete medium containing 10% FBS was replaced to continue the culture.

h. At the same time, a group of FAM fluorescent labeled nonsense sequence siRNA was transfected to determine the transfection rate.

The expression of APE1 protein in U251 cells by western blot tests

(1) Extraction of total cell protein

First, after transfection of 48 hours, the cells were collected. 1000 μl total cell protein extracts were added, which was placed on ice for fully cracking. Scraping rods were used to scrape cells, while lysis and cell debris were transferred to the 1.5mlEP tube. The whole journey was carried out on ice. Second, 12000r/min was centrifuged for 10 minutes. Third, the supernatant was absorbed in the new EP tube by a pipette while a suitable amount of sample buffer was added.

(2) Sample concentration determination

BCA protein concentration kit was used to determine the concentration. The UV lamp of the spectrophotometer was preheated for 30 minutes in advance and the wavelength was adjusted to 570nm.The blank space of the solvent was set to zero. The standard 1.2 ml protein preparation was added according to the tube protein standard (30 mg, BSA), which could be fully dissolved and formulated into a 25mg/ml protein standard solution as it was kept at 20°C. 20 μl 25 mg/ml protein standard solution was added to the 980 μl dilution, which was formulated to 0.5mg/ml protein standard solution. 4 ml kit and A80μl reagent B were mixed to prepare 4.08 ml BCA working fluid. 0.5mg/ml protein standard solution was added to the standard 96-hole orifice by 0,1, 2,4,8,12,16,20 μl standard. Standard diluent was up to 20 μl. 2μl tested cell protein solution was added to the sample hole, and the standard diluent was up to 20 μl. 200 μl BCA solution was added to the wells, which was placed for 30 minutes under the temperature of 37℃. The A570 of each hole was measured. Sample concentration was obtained according to the standard curve.

(3) Sample preparation

The extracted protein solution was dissolved in the sample buffer. The final sample buffer was 100 mmol/LDTT (prepared by l mol/L storage liquid, placed at -20°C), 2% SDS, 10% glycerol, 0.01% phenol blue and 60 mmol/L Tris (ph 6.8). The sample volume was 50 μg.

(4) SDS-PAGE

SDS- polypropylene diamine gel was used to

install the mould for filling glue, so that it does not leak glue all around. Preparation: s mol/L TrisHCI (ph 8.8), TEMED, 10% SDS, 10% ammonium persulfate, double distilled water and 30% acrylamide. The solution was shaken immediately after the addition of TEMED. It was made into 10% separate gel. The gel solution was slowly poured between the glass sheets and no bubbles were left. A small amount of water was injected on the surface of the gel solution to accelerate solidification and remove the residual bubbles.

The solution was placed vertically at room temperature for 30-60min, which allows the gel to solidify and absorb the upper solution. Deionized water was used for washing several times to remove the less polymerized polypropylene diamine. The liquid on the gel should be drained as much as possible. Filter paper could be used to suck the residual liquid carefully.

a. 4% concentrated gel was prepared with 10% SDS, 10% persulfate, 30% acryl amide and TEMED.

b. 1.0 mol/L TrisHCI (pH 6.8) and double distilled water were used to produce 4% concentrated gum, which would be shaked immediately after entering TEMED.

c. The concentrated gel was poured directly into the laminate and the separation gel until the gel level reached the top of the front glass plate.

d. The 1.5mm comb was slowly inserted horizontally into the vertical glass plate filled with concentrated glue, leaving no bubbles at the end of the comb. It was placed vertically at room temperature for 30min until the gel was fully polymerized, and the comb was gradually pulled out by vertical upward.

e. The gel was placed in the electrophoresis, whose bath was filled with buffer. The upper and lower ends of the gel were soaked in buffer. 20 pl prepared protein samples (about 107 cells/sample holes) were used for centrifugation and sampling. 100V constant pressure electrophoresis was adopted, and the time period was 100 minutes until the dye reached the separation of rubber.

(5) Use of Coomassie brilliant blue staining

a. Preparation of storage solution: The preparation of Coomassie brilliant blue dye. 1g R-250 Coomassie brilliant blue was added to 450ml PBS, while 450ml methanol and 100ml acetic acid were added. The magnetic stirrer was used for mixing and filtering. Coomassie brilliant blue decoloring liquid could be obtained by adding 100 ml methanol and acetic acid into 800ml PBS.

b. Dyeing: The gel was carefully placed in a 15cm glass dish and 40ml Coomassie blue staining solution was added and placed in a sealed container. It was placed in a horizontal shaker and slowly vibrated for 1h. PBS was used for cleaning three times. 40ml Coomassie brilliant blue decoloring liquid was added, and the container was sealed to continue to shock. The bleaching liquid was changed every 10 minutes. About half an hour later, the band of protein appears. After replacing the bleaching liquid, the solution needs to be shaken slowly until it was completely decolorized.

c. Electrical transfer: The protein on the gel after electrophoresis was transferred to the PVDF film by Mini TRANS-BLOT (BI0-RAD). The electrophoretic gel was immersed in the electrophoretic fluid transferred and balanced for about 20min. Meanwhile, 2 PVDF films with equal gel size and 3 filter papers were prepared. After soaking the PVDF film for 15 seconds, the PVDF film was rapidly placed in DDW and soaked within 10min. The PVDF film and 3 filter papers were soaked in the electric liquid. The gel holder was opened and leveled, and the impregnated sponge plate was placed on the cathode plate, on which a filter paper was spread to remove the bubbles. The gel, the PVDF film, the filter paper and the soaked sponge plate were sequentially laid on the cathode plate, and the bubbles were removed. The anode plate was closed and the bracket was clamped. The stand was vertically inserted into the electric groove and was vertically placed into the transfer box along with an ice trough. The precooling electric fluid was added so that it does not exceed the upper edge of the rotor. 100V was transferred for 20min at 4°C or ice bath. After electrophoresis, Ponceau-R staining PVDF membrane was carried out for 5min. 2 DDW were used for repeated irrigation until a protein band and the electrical rotation were observed. DDW was used to rinse the PVDF membrane until the protein band disappeared. Then PVDF films were immersed in the methanol for 15 seconds, and 2 filter papers were used to clamp the PVDF film and to dry at room temperature to fix the protein for 30min.

(6) Protein immunoassay

a. Antibodies and antigen binding PVDF membranes were immersed in the methanol. After 10 seconds, the substance was placed in PBS

immediately and soaked for 5min.The closed liquid was placed at 4℃. A large amount of PBST was washed for three times at 10min/ times. At room temperature, rabbit anti human APE1-Anti (1:500) was placed at the horizontal table for 1h. PBST washing method was the same as above. Alkaline phosphatase labeled anti rabbit two anti (1:1000) was placed at the horizontal table for 1h at room temperature. PBST flush method was the same as above.

b. Development. The experiment adopted a chemiluminescence detection system. The PVDF film faced up to a flat tile dish, and the chemical configured substrate to the PVDF film, which was placed at dark incubation for 5min. Light bands could be seen, and light substrate was abandoned. The PVDF film was wrapped by Parafilm and placed between several prepared X films of equal size. It was attached to the cassette screens placed in an exposure for about l-3min. X ray film was selected in every other minute, which was developed and fixed for 5min. In addition, it would be dry after cleaning for 10min.

c. The reuse of PVDF membrane and the two hybridizations. The developed PVDF film was soaked in 10ml elution buffer and incubated at 70 ℃ bath for 30min, while the Washbuffer (1 * PBS, 0.l% Tween-20) was used to wash the solution tube three times at 5min/ times. By the same method, the β-actin test was used as the internal controller.

(7) Result analysis

An optical density scanner was used to detect the gray value of the target strip. There is the method for calculating the relative expression of target strips: the relative expression of the target protein band was equal to the gray value of gray / the same sample reference value.

MTT assay for cell growth curve test

(1) The principle of MTT method: The living cells can metabolize thiazole blue (MTT) and produce formazan (FM) in the cell. FM was soluble in two methyl sulfoxide (DMSO) and the color was blue. Its color can reflect the survival and proliferation of the cells.

(2) U251 cells in the logarithmic growth phase were made into single cell suspension by cell culture medium. They were inoculated in the cell culture plate by I × 104 / pore cells and cultured in 37.5% CO_2 incubator.

(3) After transfection 0, 24, 48, 72, 96h, MTT method was used to detect the growth of the three groups of cells. A piece of MTT medium plate was removed and added to freshly prepared (5 mg/ml) l/20 hole every day. Then it was mixed evenly and incubated for 4 hours after the termination of training. The supernatant was removed and 150 μl/DMSO was added to the hole and shaken for 10 minutes. When the crystal was fully dissolved, the optical density value was detected by enzyme-linked immunosorbent assay (570nm) wavelength. The OD value indicated the extent of cell proliferation.

(4) The corrected /W can be obtained by subtracting the A5- of the reagent group from the measured A5- in each group. The average of each group was calculated and the growth curve was plotted.

AO/EB assay for detecting the apoptotic rate of glioma cells

After the transfection of 48h, each group of cells was collected. 1500 r/min centrifugation was performed within 5min, and the supernatant was discarded. PBS solution was added and washed for ten times. Suspension cultures were added to the cell suspension, and proper amount of culture medium was added. The concentration of suspension cell reached 109/L. 100 μl cell suspension was selected, and 4μ1 AO/EB dye was added, which was mixed thoroughly on a slide. A drop of the suspension was mixed with the drops on the glass slide covered by glass. 200 cells were counted under fluorescence microscope. The nucleus DNA of living cell (VN) was orange or yellow with uniform fluorescence, and RNA of cytoplasm was green fluorescence. In early apoptotic cells (VA), the nucleus chromatin was yellow green. In late apoptotic cells (NVA), the chromatin of the nucleus was orange red in a state of pyknosis or rupture. Nonapoptotic death cells (NVN) all showed orange-red fluorescence. The count was carried out by 2 persons to reduce subjective errors. The average apoptotic rate and the apoptosis rate were calculated according to the following formula:

Apoptosis rate (%) =
(VA+NVA)/(AVA+NVA+IN+NVN) ×100%

Statistical analysis

The quantitative data were expressed as: mean±standard deviation (x±s). SPSS17.0 statistical software was used to analyze the variance analysis (F test) results of the single

factor and the multi-level random design, and the value was significant (p<0.05).

Results

Determination of transfection rate of siRNA

U251 cells were transfected according to the Oligofectamine manual. In figure 1, A was green fluorescent with transfection positive cells under fluorescent microscopy, and B was seen in the same field under phase contrast microscopy, which was convenient to see all the cells. The transfection rate was over 71%, which met the requirements of this experiment.

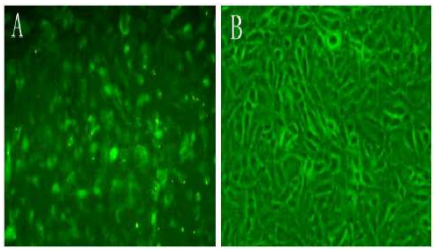

Figure1. Transfection rate detection (×200)

The changes of APE1 protein before and after transfection of 2 and APE1 siRNA U251 by western blot

APE1, western blot, U251 hybridization results in the control group, the negative group and the APEl siRNA group were shown in figure 2.

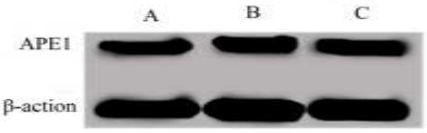

Figure 2. Results of western blot hybridization

The results showed that after transfection, the expression of APE1 protein in the APE1 siRNA group was significantly lower than that in the control group and the negative group (see Figure 3 and Table 1). It was proved that APE1 siRNA expression vector can transfect U251 cells effectively and reduce the expression of APE1 protein in U251 cells successfully.

Experimental results of MTT method

After the addition of thiazole blue, the living cells in each group produced blue violet needle like formazan crystals. As the DMSO was dissolved, OD value was measured by enzyme labeling instrument (A570). Compared with the control group and the negative group, the cell growth, light absorption value and 24h of the, 24, 48, 72 and 96h in the APE1 siRNA group were significantly different (see Table 2). There was no significant difference between the control group and the negative group (P>0.05).

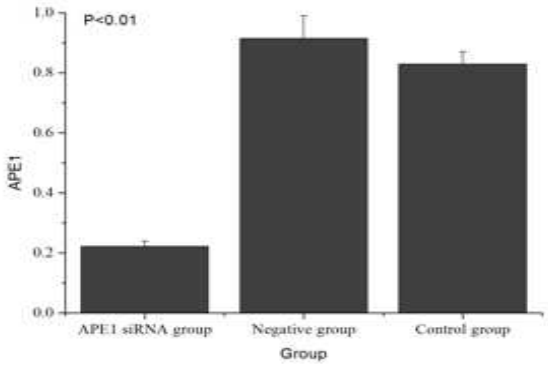

Figure 3. Expression of APE1 protein in U251 cells before and after transfection

Table 1. Effects of APE1 siRNA liposome transfection on the expression of APK1 protein in U251 cells

Group	A_{APE1}
APE1 siRNA group	0.222±0.028[a]
Negative group	0.915±0.065[a]
Control group	0.829±0.037[a]

Compared with the APE1 siRNA group, A_{APE1} was the gray value (P<0.01).

The time was set to the horizontal axis, and the average value of A570 in each group was set to the vertical axis so that the growth curve of U251 cells after transfection was plotted (see Figure 4). The curve showed that the proliferation of U251 cells was inhibited by APE1 siRNA expression vector, and the inhibition was more obvious with the increase of transfection time.

Table 2. Proliferation of U251 cells after transfection

Group			A570		
	0h	24h	48h	72h	96h
APE1siRNA	0.447± 0.007	0.527± 0.051	0.667± 0.069	0.879± 0.053	1.125± 0.066
Control	0.443± 0.061	0.721± 0.029	1.089± 0.071	1.518± 0.062	1.729± 0.040
Negtive*	0.450± 0.004	0.712± 0.005	1.102± 0.007	1.539± 0.004	1.783± 0.003

**Compared with the control group and the negative group, it can be concluded that P<0.05.*

Detection for apoptosis rate in brain glioma after AO/EB staining

After AO/EB staining, the morphology of the cells in each group was observed, and the rate of apoptosis in the experimental cells was estimated. As shown in table 3, at least 200 cells were counted in each group, and the apoptotic rate of each group was calculated, among which the

apoptosis rate of APE1 siRNA group was significantly higher than that of the negative group and of the control group (F=71.056, P<0.05).

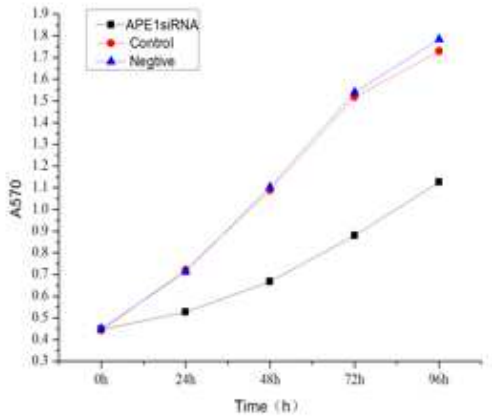

Figure 4. Growth curve of U251 cells in each group

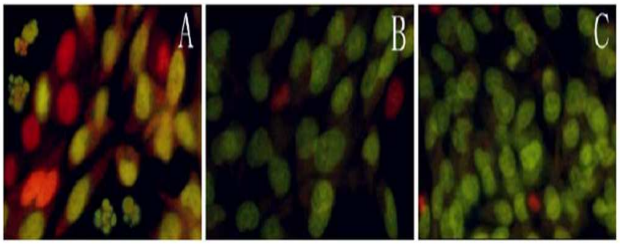

Figure 5. AO/EB staining (×400)
Note: A showed the rate of APE1 siRNA group, B showed the rate of the control group, and C showed the rate of the negative group

Table 3. Apoptosis rate of U251 cells in different groups by using AO/EB method

Group	N	NVN	VA	NVA	Total	Apoptotic rate (%)	*P* value
APE1siRNA	17	21	46	16	200	29	
Negative	91	5	2	2	200	3	0.05
Control	77	9	7	7	200	6	0.05

Note: The P values were compared with those of the other two groups; the units of NA, NVN, VA and NVA were all /200 cells

Discussion

Glioma was the most common intracranial malignant tumor in human's central nervous system. Although the incidence varies with time and regions, it still accounts for more than half of the incidence of intracranial tumors (Wei *et al*., 2016). Astrocytoma was the most common seen tumor among them. Gliomas were characterized by diffusible and invasive growth (Shrotriya *et al.*, 2010). There was no clear boundary between normal brain tissues around the brain (Sakai *et al.*, 2015). They often invaded important nerve tissues and were difficult to be completely removed. Moreover, chemotherapy and radiation therapy can not kill them or kill glioma cells in particular, and the curative effects were very poor (Yuki *et al.,* 2011). It may also produce toxic effects that the systemic or central nervous system cannot tolerate (Jiang *et al.,* 2015). Therefore, the prognosis of glioma was poor while the survival time was short, and the recurrence rate as well as the fatality rate was high (Montaldi *et al.,* 2015). Among all systemic malignancies, 5-year mortality ranked third beinding lung cancer and pancreatic cancer (Fleming *et al*., 2017).

APE1 (apurinic/apyrimidinic, endonuclease, 1) was one of the most important biological and functional molecules found in recent years (Cheng *et al.*, 2015). The APE1 related gene was located at human chromosome fourteenth and it encoded a protein of about 37 KD, which contains 318 amino acid residues. In the study of nuclease activity, Robson found a purine / pyrimidine endonuclease (APE1) (Nagoya *et al*., 2014). In addition, some researchers isolated and purified Hela cells and found the intracellular reducing factor of AP-1, which was also named redox factor-1 (Ref-l) (Alagoz *et al*., 2014). Later tests confirmed that Ref-1 was APE1, which consisted of two relatively independent functional regions, DNA repair and redox respectively (Kievit *et al.,* 2015; Nagoya *et al.*, 2014). As a speed limiting enzyme in the DNA excision repair (base, excision, repair, BER) pathway, APE1 was an extremely important repair factor for genotoxic drugs (such as alkylating agents) and radiation damage of cells. In addition, APE1 still has redox (reduction-oxidation) function (Riemenschneider *et al.*, 2016). Through the regulation of dependent and non-dependent redox, it can be used to maintain the organization including unique transcription factors (such as p53, Egr-1, AP-1), tissue-specific transcription factors (such as TTF-1, PAX-8, PEBP-2), and transcription factor activation reduced state. It was also involved in the regulation of many important cellular responses, such as the control and apoptosis of the cell cycle, and the regulation of transferred factors, oxidative stress, etc. (Kerklaan *et al.,* 2016). The mice died during the embryonic phase after that APE1 was knocked out. This suggested that APE1 was the key gene determining cell survival (Abbotts *et al.*, 2014; He *et al.,* 2015).

At present, it can be found that the regulation of APE1 on apoptosis may result in two opposite effects according to different functional

status of cells. During normal cell division or in the internal environment, APE1 was shown to inhibit oxidative stress, which thereby can protect cells and inhibit apoptosis (Wang *et al*., 2016). Studies have shown that over-expression of APE1 can inhibit apoptosis of tumor necrosis factor or hypoxia (Liu *et al*., 2017). In vivo experiments and cultured cells, it also can be found that the expression of APE1 was negatively related to apoptosis, and the decrease in APE1 expression was always preceded by apoptotic cells. This phenomenon can be repaired by APK1 with DNA so that the reduction of expression can lead to a new DNA damage which cannot be repaired and the apoptosis was inevitable to explain. In addition, studies have shown that APE1 can also play a role in promoting apoptosis by using AP21 mediated pathway. The activation of AP21 was positively related to the apoptosis of endothelial cells, which could be increased by over-expression. In tumor tissues such as cervical cancer, rhabdomyosarcoma, non-small cell lung cancer and head and neck squamous cell carcinoma, the expression of APE1 could be detected by quantitative studies (Chen *et al.*, 2013). The expression level of APE1 in tumor cells was obviously higher. Some studies have shown that approximately 72% of human osteosarcoma tissues have been over-expressed in APE1, suggesting that their over expression of APE1 may be related to the release, chemotherapy tolerance and prognosis of osteosarcoma (Roychoudhury *et al.,* 2017). It was generally accepted that the expression level of APE1 and its imbalance in the distribution of cells could be used as an indicator of sensitivity to tumor radiotherapy and chemotherapy.

It has been found that the expression of APE1 was significantly increased in tumor tissues of different types and grades of gliomas, and the expression level was positively correlated to the degree of malignancy (Fang *et al.*, 2015), which indicated that the increase of APE1 expression was closely related to the occurrence, development and treatment sensitivity of brain glioma. It was considered that the clinical prognosis of glioma can be indirectly understood by measuring the level of APE1. This may be related to the ability of APE1 of repairing DNA damage caused by radiotherapy and chemotherapy.

In order to determine that the blocking U251 cell APE1 expression can influence cell proliferation, apoptosis and sensitivity to radiotherapy, this paper applied the constructed APEl siRNA expression vector to U251 cells by silencing the expression of APE1 related genes so as to reduce the level of APE1 cells objective. The proliferation and apoptosis of U251 cells and its sensitivity to radiotherapy were detected before and after the transfection. The results showed that the expression of APE1 in APE1siRNA group was significantly lower than that in the other two groups after that APE1 siRNA expression vector was introduced to the cells, and the difference was significant. There was no significant difference between the control group and the negative group. MTT showed that the proliferation ability of APE1 siRNA group was significantly lower than that before transfection. After A0/EB staining, more apoptotic cells were found in the transfected cells. It is believed that APE1 siRNA can induce apoptosis of glioma cells very effectively.

In conclusion, APE1 targeting interference with RNA was the expression of APE1 by inhibiting human glioma cells (U251). It effectively inhibited the proliferation and induced apoptosis of tumor cells. These studies have shown that APE1 targeting interference may serve as an effective strategy for human glioma gene therapy by providing an experimental basis for clinical gene therapy of glioma.

The results indicated that based on the RNA interference technology, APE1 siRNA expression plasmid was successfully introduced to human glioma cells, which can reduce the expression of APE1 in cells. Also, the experimental results confirmed that the reduction of APE1 protein could inhibit the proliferation of glioma cells and promote apoptosis.

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