J Huazhong Univ Sci Technol［Med Sci］
DOI 10.1007/s11596-011-0157-1
31(1):94-99,2011

PPM1D Silencing by Lentiviral-mediated RNA Interference Inhibits Proliferation and Invasion of Human Glioma Cells*

Peng WANG (王 鹏)[1, 2], Jing RAO (饶 竞)[2] , Haifeng YANG (杨海峰)[2], Hongyang ZHAO (赵洪洋)[2], Lin YANG (杨 林)[2#]
[1]*Department of Neurosurgery, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou 510080, China*
[2]*Department of Neurosurgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China*

Summary: To construct a lentiviral shRNA vector targeting human protein phosphatase 1D magnesium-dependent (PPM1D) gene and detect its effectiveness of gene silencing in human gliomas, specific siRNA targets with short hairpin frame were designed and synthesized. DNA oligo was cloned into the pFU-GW-iRNA lentiviral expression vector, and then PCR and sequencing analyses were conducted to verify the constructs. After the verified plasmids were transfected into 293T cells, the lentivirus was produced and the titer of virus was determined. Real-time quantitative PCR and Western blot were performed to detect the PPM1D expression level in the infected glioma cells. PCR and Western blot analyses revealed the optimal interfering target, and the virus with a titer of 6×10^8 TU/mL was successfully packaged. The PPM1D expression in human glioma cells was knocked down at both mRNA and protein levels by virus infection. The expression of PPM1D mRNA and protein was decreased by 76.3% and 87.0% respectively as compared with control group. The multiple functions of human glioma cells after PPM1D RNA interference were detected by flow cytometry and cell counting kit-8 (CCK-8). Efficient down-regulation of PPM1D resulted in significantly increased cell apoptosis and reduced cell proliferation and invasion potential in U87-MG cells. We have successfully constructed the lentiviral shRNA expression vector capable of stable PPM1D gene silencing at both mRNA and protein levels in glioma cells. And our data gave evidence that the reduced cell growth observed after PPM1D silencing in glioma cells was at least partly due to increased apoptotic cell death.
Key words: PPM1D; glioma; RNA interference; lentivirus; apoptosis

Gliomas are the most common primary intracranial tumors. Glioblastoma multiforme, the most aggressive and malignant of this type (WHO grade Ⅳ), grows fast and invades quickly nearby normal brain tissue. Although many efforts have been made toward the therapy, the median survival time of patients with glioblastoma is only about 1 year from diagnosis. One of the reasons is contributed to the fact that glioma cells possess high proliferation and invasion potentials[1]. Novel strategies for controlling the growth of gliomas are of urgent needs[2].

The protein phosphatase 1D magnesium-dependent (PPM1D) is initially identified as a p53-regulated allele located on 17q23–24[3]. PPM1D belongs to the Ser/Thr PP2C family of phosphatases and, like many PP2C phosphatases, depends on mechanisms for its activation [4]. It was reported that PPM1D cooperated with RAS and MYC to transform primary mouse fibroblasts and over-expression of PPM1D suppressed cell senescence and apoptosis mediated by RAS[5]. PPM1D over-expression inhibits p53 functions and reduces selection for *TP53* mutations during cancer progression[6, 7], while the p53 signal pathway plays a crucial regulatory role in cell proliferation, apoptosis, and DNA repair[8]. And it was reported that PPM1D was amplified or overexpressed in several human tumor types, including breast cancers, ovarian cancers, and medulloblastomas[9-11]. Such PPM1D may have oncogenic function[12, 13].

To investigate the roles that PPM1D plays in the growth and invasion of glioma, we used the RNA interference (RNAi) technology to knock down the PPM1D gene in glioma cells and analyzed its effect on the growth and invasion behavior of glioma cells. We established a stable PPM1D silencing glioma cell line with lentiviral vector-mediated RNA interference and found that silencing of the PPM1D gene significantly suppressed the proliferation and invasion behavior of glioma cells. Our results demonstrated that activation of the PPM1D was crucial for proliferation and invasion behavior of glioma and lentivirus-mediated targeting human PPM1D gene might offer a novel strategy for controlling the growth behavior of gliomas.

1 MATERIALS AND METHODS

1.1 Cell Line and Reagents

Human glioma cell line U87-MG was obtained from the China Center for Type Culture Collection (CCTCC, Shanghai, China). Dulbecco's modified Eagle's medium

Peng WANG, E-mail: wangpeng_82@sina.com
#Corresponding author, E-mail: linyang@hust.edu.cn
*This project was supported by a grant from National Natural Sciences Foundation of China (No. 30772240).

(DMEM), fetal bovine serum (FBS), L-glutamine, penicillin G and streptomycin were all purchased from Gibco-Invitrogen (USA). pFU-GW-iRNA vector was purchased from Genechem Co. (China). Trizol was purchased from Invitrogen (USA). DNase Ⅰ was from Takara (Japan). Reverse transcription and real-time qPCR were performed with a ReverTra Ace-α-^{TM} kit and real-time qPCR kit (Toyobo, Japan). Primary antibodies included Wip1 polyclone antibody (Santa Cruz Biotechnology, USA) and β-actin monoclonal antibody (Sigma, USA). Apoptosis detection kit and matrigel were from BD Biosciences (USA). M-PER mammalian protein extraction reagent, BCA assay kit and enhanced chemiluminescence kit were from Pierce (USA). CCK-8 kit was from Dojindo (Japan). Matrigel Invasion Chambers were from Costa (Germany).

1.2 Methods

1.2.1 Cell Culture U87-MG cells were cultured in complete medium consisting with DMEM containing high glucose and pyruvate, supplemented with 10% FBS, 2 mmol/L L-glutamine, 100 U/mL penicillin G and 100 ng/mL streptomycin. Cells were maintained at 37°C in a humidified 5% CO_2 atmosphere.

1.2.2 Design and Cloning of Lentiviral shRNA Vectors The target shRNAs against human PPM1D gene (GenBank accession NM_003620) for RNAi were designed as: shRNA1: 5′-GGAAAGAGAACGAATCGAA-3′, shRNA 2: 5′-GTGATGGACTTTGGAATAT-3′; and shRNA3: 5′-GCATAGACGAAATGGCTTA-3′. The sequence 5′-TTC TCCGAACGTGTCACGT-3′, which had no significant homology to any known human or mouse genes, was used as a negative control[14]. Oligonucleotides were synthesized according to the sequences and annealed into double-strands by the DNA synthesis company (Shanghai Genechem Co., China). Lentiviral frame plasmids were double digested and connected with the double-stranded DNA. They were then transformed into *E. coli* and positive recombinant clones were selected by using PCR, using primers 5′-GCCCCGGTTAATTTG CATAT-3′ and 5′-GAGGC CAGATCTTGGGTG-3′. Recombinant lentivirus vectors were produced by co-transfecting 293T cells with the lentivirus expression plasmid and packaging plasmids (pHelper 1.0 including gag/pol and pHelper 2.0 including VSVG) using the calcium phosphate method. Infectious lentivirus vectors were harvested at 48 h post-transfection and then concentrated. The infectious titer was determined by real-time quantitative PCR to determine GFP-tagged positive rate in 293 cells.

1.2.3 Lentiviral Vector Infection Cells were divided into 3 groups: control (infection with PBS alone), RNAi-NC (infection with negative control lentiviral vector) and RNAi-PPM1D (infection with RNAi-PPM1D lentiviral vector). According to different shRNA sequences, the RNAi-PPM1D group was divided into 3 subgroups: RNAi-R1, RNAi-R2 and RNAi-R3. Cells were subcultured at a density of 2×10^5 cells per well into 6-well tissue culture plates. After 48 h of culture, the cells were infected with specific or negative control lentiviral vectors, at the multiplicity of infection (MOI) 1.5, according to the pre-experimental results. The final volume of culture medium was 1 mL per well. The culture medium with 10% FBS was then added, and the cells were incubated for desired durations. Then the cells were observed under the fluorescence microscopy and harvested at 4th, 7th and 10th day after infection. The knockdown of PPM1D was analyzed by real-time quantitative PCR and Western blot.

1.2.4 Real-time Quantitative PCR (RT-qPCR) After infection with lentiviruses for 4 days, total RNA was extracted from cells with Trizol, according to the manufacturer's instructions. DNase I was used to treat the total RNA extraction following the protocols. Reverse transcription was performed with a ReverTra Ace-α-^{TM} kit following the instructions. This cDNA was used for PCR reactions containing 2 μL of each cDNA dilution and 7.2 μL of water. A total of 20 μL of a solution containing 10 μL of SYBR Green Real-time PCR Master Mix, and 0.2 μmol/L sense and antisense primers. Primer sequences used to amplify Wip1 mRNA for RT-qPCR were as follows: forward 5′-GTTCGTAGCAATGCCTTCTCA-3′ and reverse 5′-CACTTTCTTGGGCTTTCATTTG-3′; these amplified a 262-bp product. Primer sequences of the control amplification of β-actin mRNA were as follows: forward 5′-GTTGCGTTACACCCTTTCTTG-3′ and reverse 5′-CTGCTGTCACCTTCACCGTT-3′; these amplified a fragment of 155 bp. Serially diluted plasmids containing Wip1 cDNA were utilized to construct a standard curve. The cutoff point (Ct) of each sample was plotted on the standard curve and the mRNA copy numbers were calculated. The β-actin gene was used as an endogenous control. The relative Wip1 mRNA levels were expressed as a ratio of Wip1 to β-actin.

1.2.5 Western Blot Analysis After infection with lentiviruses for 7 days, the cells were lysed using M-PER lysis buffer. Protein was extracted and quantified using a BCA assay kit. A total of 30 μg of each sample was heated at 95°C for 10 min and loaded into 10% gel. Samples were electrophoresed at 110 V for 60−90 min and then transferred to PVDF membranes at 200 mA for 1 h using a semi-dry transfer apparatus. Membranes were blocked in 5% non-fat dry milk for 2 h, incubated with primary antibodies overnight, washed with TBS containing 0.05% Triton-X 100 (TBST) followed by an incubation of 1 h in goat anti-rabbit secondary antibody conjugated with HRP. After final washing with TBST, the membranes were developed using chemiluminescence and exposed to X-ray films. The immunoblots were quantified with software quantity one version 4.6.2. The expression of PPM1D in each sample was internally normalized to β-actin and levels were given relative to expression in control groups.

1.2.6 Cell Proliferation Assay Cell proliferation assay was performed with CCK-8, according to the manufacturer's instructions. In brief, the U87-MG cells and cells infected with lentiviruses were seeded in a 96-well plate and then incubated for 1, 2, 3 and 4 days. Then 10 μL of the CCK-8 solution was added to each well of the plate. The absorbance (*A*) at 450 nm was measured using a microplate reader after the plate was incubated. Then a calibration curve was prepared using the data obtained from the wells that contained known numbers of viable cells. Experiments were performed three times with representative data presented.

1.2.7 Apoptosis Assay A total of 375 000 cells per well

were seeded into 6-well cell culture plates and at the 4th and 7th day after infection with lentiviral vectors, detached and adherent cells were collected. The cell aliquots were combined, washed with ice-cold PBS, resuspended in 1×binding buffer, and stained using the Annexin Ⅴ apoptosis detection kit following the protocols. The fractions of annexin Ⅴ APC and iodide PI staining cells were quantified by using a Becton Dickinson FACScan (USA) and were classified as early apoptotic (annexin Ⅴ-positive, PI-negative cells), late apoptotic (annexin Ⅴ positive, PI positive cells), or dead (annexin Ⅴ negative, PI positive cells), according to the manufacturer's instructions.

1.2.8 Transwell Invasion Assay Cell invasion assay was performed in Matrigel Invasion Chambers in 24-well culture plates. NIH3T3 cells were cultured with serum-free medium over 2 days and the supernatant was centrifuged and collected. 100 μL of the matrigel diluted in serum-free cold medium (1 mg/mL) was put into upper chamber of 24-well Transwell and incubated at 37°C at least 4 to 5 h for gelling. Cells were trypsined, washed and resuspended in media containing 1% FBS at a density of 10^6 cells/mL. 100 μL of the cell suspension was put onto the matrigel and the lower chamber of the transwell was filled with 500 μL of culture media containing 50% of the NIH3T3 supernatant, as an adhesive substrate. After 24 h of incubation, noninvasive cells were removed with a cotton swab and the Transwell was stained with 0.1% viola crystallina. For quantification, the cells were counted under a microscope (×200).

1.2.9 Statistical Analysis Data were expressed as $\bar{x}\pm s$. Statistic analysis was performed using SPSS software version 13.0. Data were analyzed statistically by one-way analysis of variance (ANOVA). A value of $P<005$ was considered significant.

2 RESULTS

2.1 Construction and Package of Lentiviral Vector

The target shRNAs against human PPM1D for RNAi were constructed and connected with lentiviral frame plasmids. Positive recombinant clones were selected and identified with PCR. The sizes of PCR amplified fragments of recombinant clones and unconnected shRNA empty vectors were 343 bp and 299 bp, respectively (fig. 1). Sequence analysis confirmed that the inserted PPM1D shRNA sequences were correct. The recombinant lentiviral vectors were applied to co-transfect 293T cells and virus titers were determined as 3×10^8 (RNAi-R1), 5×10^8 (RNAi-R2) and 6×10^8 (RNAi-R3) TU/ml, respectively.

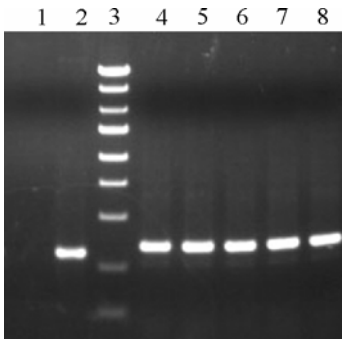

Fig. 1 Identification of recombinant lentiviral vectors by PCR
Lane 1: Negative control; Lane 2: Recombinant negative lentiviral Vector (343 bp); Lane 3: Marker ladder (5000, 3000, 2000, 1500, 1000, 750, 500, 250, 100 bp); Lanes 4−8: Recombinant positive lentiviral vector with PPM1D shRNA (343 bp)

2.2 Infection Efficiency of Human U87-MG Cells with Lentivirus

Assessment of infection rate was accomplished with the determination of the positive expression rate of GFP under the fluorescent microscopy 96 h after infection. As shown in fig. 2, infection efficiency of lentiviral RNAi vectors of PPM1D (RNAi-PPM1D) and the negative control (RNAi-NC) in U87-MG cells was >90%.

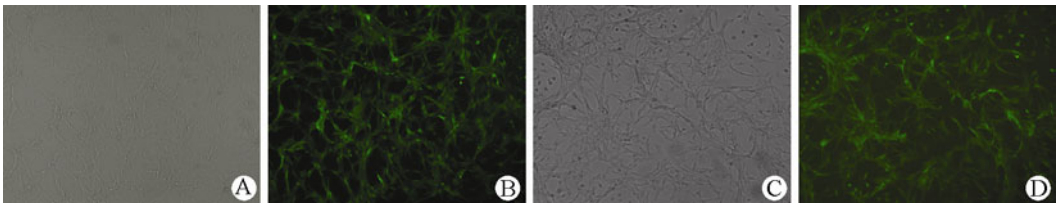

Fig. 2 Fluorescence images of U87-MG cells after lentiviral vector infection (×100)
A and B: Negative control (U87-MG+lentiveral vector without special sequence); C and D: RNAi-PPM1D (U87-MG+ lentiviral vector with shRNA of PPM1D)

2.3 Silencing Efficacy of PPM1D shRNA Lentivirus

The silencing efficacy of PPM1D shRNA lentivirus at mRNA and protein levels in glioma cells was determined by RT-qPCR 4 days and Western blot 7 days in control group, RNAi-NC group and RNAi-PPM1D group (RNAi-R1−RNAi-R3 subgroups), respectively. RT-qPCR showed that the expression levels of PPM1D in RNAi-R1−RNAi-R3 subgroups were remarkably reduced by 51.2%, 63.3% and 76.3%, respectively (fig. 3A). And Western blot revealed that the protein expression levels of PPM1D in RNAi-R1−RNAi-R3 subgroups were 152%, 14.3% and 23.0% of those in control group (fig. 3B and 3C). Both RT-qPCR and Western blot demonstrated that lentiviral vectors were effective for PPM1D silencing and that RNAi-R3 was the most effective construct. Stable infection was performed with RNAi-R3 construct.

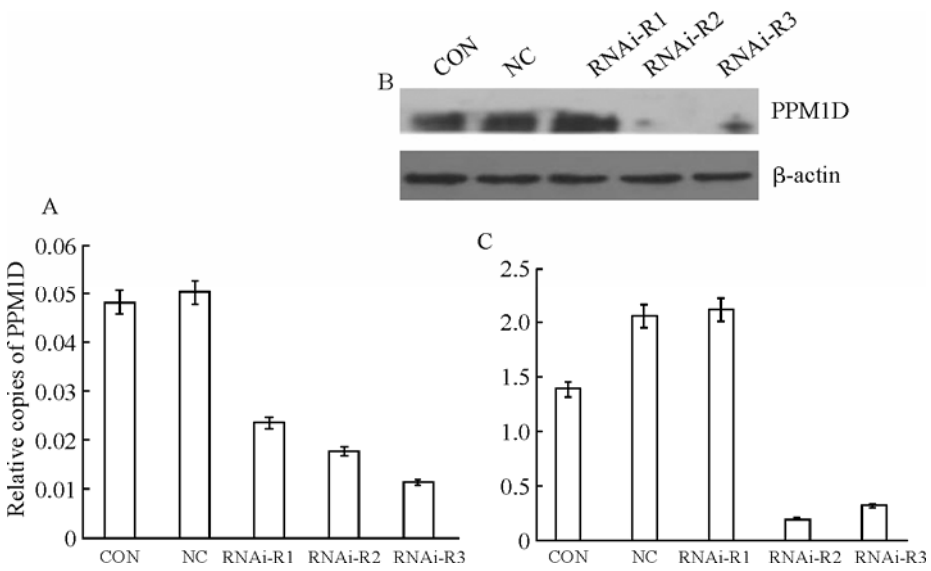

Fig. 3 Expression of PPM1D mRNA in U87-MG cells after infected with lentivirus

A: The relative copies of PPM1D mRNA in human glioma U87-MG cells after infected with lentivirus; B: PPM1D in U87-MG cells 7 days after infection with lentivirus; C: The immunoblots of PPM1D in U87-MG cells 7 days after infection with lentivirus were quantified.

2.4 PPM1D Gene Silencing Suppresses Cell Proliferation and Induces Apoptosis

The effect of PPM1D silencing on the proliferation of glioma cells was determined by CCK-8. As shown in fig. 4, though U87-MG/mock cells (U87-MG transfected with negative control lentivirus) showed proliferation ability approximating that of U87-MG cells (non-transfected), U87-MG/PPM1D– (PPM1D shRNA lentivirus R3) had reduced proliferation ability compared to negative control cells. Therefore, cell proliferation ability correlates with the PPM1D expression. To explore whether reduced proliferation ability was related with the apoptotic status of glioma cells, we subjected the PPM1D specific and negative control cells to apoptosis analyses. PPM1D silencing did not induce any changes in the quantity of apoptotic cells in U87-MG cells at the 4th day (data not shown), but significantly increased the quantity of apoptotic (including early apoptotic and apoptotic or necrotic cells) U87-MG cells at the 7th day (fig. 4).

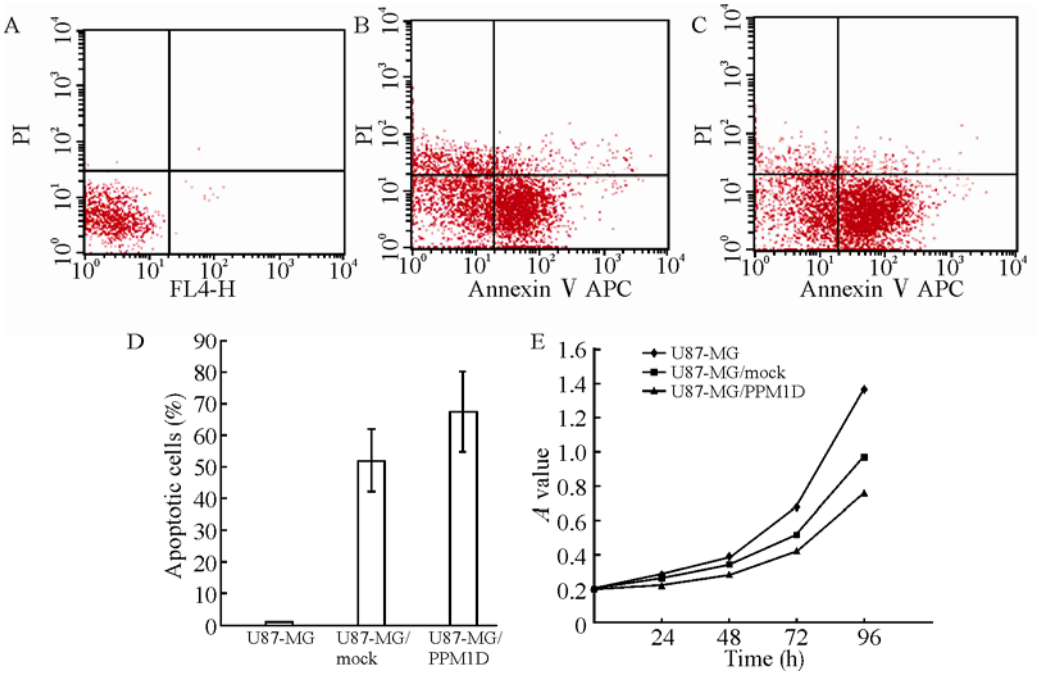

Fig. 4 Influence of PPM1D silencing on proliferation and apoptosis of U87-MG cells

The number of apoptotic cells was determined at 7th day after infection with specific or negative control lentiviral vectors by using flow cytometry. A: Un-infected U87-MG cells; B: U87-MG/mock cells; C: U87-MG/PPM1D cells; D: Summary of the experiment illustrated in A–C. Error bars indicate $\bar{x}\pm s$ of three replicates. The proliferation curve of U87-MG cell is also shown in E.

2.5 PPM1D Gene Silencing Suppresses Cell Invasion

To evaluate the effect of PPM1D gene silencing on the invasion of U87-MG cells, Transwell was performed as described above. When compared with negative control cells, U87-MG/PPM1D cells showed a substantial reduction in invasive capacity. The number of invasive U87-MG/PPM1D– cells was reduced to 47.0% of the negative control group, suggesting the significantly decreased invasion potential of U87-MG/PPM1D– cells (fig. 5).

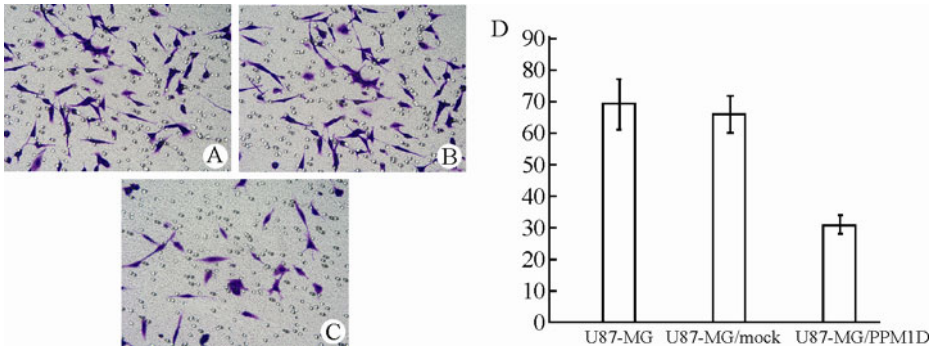

Fig. 5 Transwell invasion of U87-MG cells is inhibited by PPM1D silencing

Cell invasion assay was performed in Matrigel invasion chambers in 24-well culture plates. After 24 h of incubation, the Transwell was stained with 0.1% viola crystallina. For quantification, the cells were counted under a microscope (×200). A: Un-infected U87-MG cells; B: U87-MG/mock cells; C: U87-MG/PPM1D– cells; D: Summary of the experiment illustrated in A–C. Data were presented as $\bar{x}\pm s$ of three Transwell filters from a single experiment.

3 DISCUSSION

PPM1D is a growth-promoting phosphatase which can inhibit p53 signaling and abrogate Ras-induced senescence of primary cells and prevent apoptosis induced by serum starvation[5, 14-16]. PPM1D is overexpressed in many cancers and is associated with poor prognosis and low *TP53* mutation rate in these cancers examined[10]. Therefore PPM1D may have oncogenic function[12]. However the roles and mechanisms of PPM1D gene in gliomas have not been reported yet. To investigate the functional significance of PPM1D specifically, we used RNAi to inhibit PPM1D expression in U87-MG cells, and detected the influences induced by decreased expression of PPM1D.

In the past few years, shRNA have been widely used to silence the expression of many target genes because of its high specificity and apparent nontoxicity[17, 18]. Based on the high affinity characterization of lentiviral high-expressed vector to the target cells of nervous system[19, 20], we constructed lentiviral shRNA vector of PPM1D gene which could express GFP in infected glioma cells. Our results showed that the constructed lentiviral shRNA vector possessed high infection efficiency to U87-MG cells and could silence the expression of PPM1D gene in U87-MG cells effectively. These results indicated that an effective shRNA vector targeting human PPM1D gene had been designed and constructed successfully. For lentiviral vector-mediated RNAi could create stable and effective effect of gene silencing, it provided substantial work in studies of the function of PPM1D gene in glioma cells.

Studies have demonstrated that PPM1D gene played an important role in the proliferation and apoptosis in breast cancer cell lines by inhibiting p53 kinase or cooperating with other oncogenes such as Ras, Myc and Neu[15, 21]. Also it has been confirmed that PPM1D played an important role in DNA damage response[22, 23]. To determine the role of PPM1D gene in the malignant proliferation of gliomas, we detected the proliferation of U87-MG cells after PPM1D silencing. Our data revealed that PPM1D silencing had a strong antiproliferative effect in U87-MG cells. When we subjected the PPM1D specific and negative control cells to apoptosis analyses, we detected that PPM1D silencing in U87-MG cells increased the quantity of apoptotic cells. It may be coincident with the fact that PPM1D can prevent apoptosis induced by serum starvation[5]. Our results that PPM1D silencing by RNAi inhibited proliferation and induced apoptosis in glioma cells indicated PPM1D may serve as an oncogene in gliomas. It is known that the overexpression of Wip1 is likely to be oncogenic given its interaction with p53 pathway, but the pathogenetic mechanism of the PPM1D gene expression in U87-MG glioma cells remains to be elucidated.

Glioblastoma multiforme is the most aggressive and malignant primary intracranial tumor with high invasion potential. It was reported that overexpressed PPM1D in pancreatic adenocarcinoma and breast cancer was associated with poor prognosis[24, 25]. So we presumed that PPM1D might be concerned with the high invasion potential of glioma cells. And we performed Transwell to detect the invasion capacity caused by PPM1D expression. Our experiments showed that PPM1D silencing in U87-MG glioma cells reduced their capacity to invade ECMatrix-coated membranes. It provides the cue that PPM1D played a role in the invasion potential of malignant gliomas.

Taken together, our results indicated that PPM1D expression silencing by RNAi suppressed the proliferation and invasion potential of glioma cells. This is due at least in part to increased apoptotic cell death. Our results provide a promising target for therapeutic intervention of glioma.

REFERENCES

1 Dehdashti AR, Hegi ME, Regli L, *et al.* New trends in the

medical management of glioblastoma multiforme: the role of temozolomide chemotherapy. Neurosurg Focus, 2006,20(4):E6
2 Schwartzbaum JA, Fisher JL, Aldape KD, *et al*. Epidemiology and molecular pathology of glioma. Nat Clin Pract Neurol, 2006,2(9):494-503
3 Fiscella M, Zhang H, Fan S, *et al*. Wip1, a novel human protein phosphatase that is induced in response to ionizing radiation in a p53-dependent manner. Proc Natl Acad Sci USA, 1997,94(12):6048-6053
4 Choi J, Appella E, Donehower LA. The structure and expression of the murine wildtype p53-induced phosphatase 1 (Wip1) gene. Genomics, 2000,64(3):298-306
5 Li J, Yang Y, Peng Y, *et al*. Oncogenic properties of PPM1D located within a breast cancer amplification epicenter at 17q23. Nat Genet, 2002,31(2):133-134
6 Gorgoulis VG, Vassiliou LV, Karakaidos P, *et al*. Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. Nature, 2005, 434 (7035):907-913
7 Bulavin DV, Demidov ON, Saito S, *et al*. Amplification of PPM1D in human tumors abrogates p53 tumor-suppressor activity. Nat Genet, 2002,31(2):210-215
8 Taylor WR, Stark GR. Regulation of the G2/M transition by p53. Oncogene, 2001,20(15):1803-1815
9 Saito-Ohara F, Imoto I, Inoue J, *et al*. PPM1D is a potential target for 17q gain in neuroblastoma. Cancer Res, 2003,63(8):1876-1883
10 Hirasawa A, Saito-Ohara F, Inoue J, *et al*. Association of 17q21-q24 gain in ovarian clear cell adenocarcinomas with poor prognosis and identification of PPM1D and APPBP2 as likely amplification targets. Clin Cancer Res, 2003,9(6):1995-2004
11 Castellino RC, De Bortoli M, Lu X, *et al*. Medulloblastomas overexpress the p53-inactivating oncogene WIP1/PPM1D. J Neurooncol, 2008,86(3):245-256
12 Lu X, Ma O, Nguyen TA, *et al*. The Wip1 Phosphatase acts as a gatekeeper in the p53-Mdm2 autoregulatory loop. Cancer Cell, 2007,12(4):342-354
13 Lu X, Nguyen TA, Zhang X, *et al*. The Wip1 phosphatase and Mdm2: cracking the "Wip" on p53 stability. Cell Cycle, 2008,7(2):164-168
14 Rodriguez M, Aladowicz E, Lanfrancone L, *et al*. Tbx3 represses E-cadherin expression and enhances melanoma invasiveness. Cancer Res, 2008,68(19):7872-7881
15 Lu X, Nguyen TA, Moon SH, *et al*. The type 2C phosphatase Wip1: an oncogenic regulator of tumor suppressor and DNA damage response pathways. Cancer Metastasis Rev, 2008,27(2):123-135
16 Choi J, Nannenga B, Demidov ON, *et al*. Mice deficient for the wild-type p53-induced phosphatase gene (Wip1) exhibit defects in reproductive organs, immune function, and cell cycle control. Mol Cell Biol, 2002,22(4):1094-1105
17 Gartel AL, Kandel ES. RNA interference in cancer. Biomol Eng, 2006,23(1):17-34
18 Wang X, Tang R, Xue Z, *et a*l. Lentivector-mediated RNAi efficiently downregulates expression of murine TNF-alpha gene *in vitro* and *in vivo*. J Huazhong Univ Sci Technolog [Med Sci], 2009,29(1):112-117
19 Cenciarelli C, Budoni M, Mercanti D, *et al*. *In vitro* analysis of mouse neural stem cells genetically modified to stably express human NGF by a novel multigenic viral expression system. Neurol Res, 2006,28(5):505-512
20 Meunier A, Latremoliere A, Dominguez E, *et a*l. Lentiviral-mediated targeted NF-kappaB blockade in dorsal spinal cord glia attenuates sciatic nerve injury-induced neuropathic pain in the rat. Mol Ther, 2007,15(4):687-697
21 Parssinen J, Alarmo EL, Khan S, *et al*. Identification of differentially expressed genes after PPM1D silencing in breast cancer. Cancer Lett, 2008,259(1):61-70
22 Macurek L, Lindqvist A, Voets O, *et al*. Wip1 phosphatase is associated with chromatin and dephosphorylates gammaH2AX to promote checkpoint inhibition. Oncogene, 2010,29(15):2281-2291
23 Zhang X, Lin L, Guo H, *et al*. Phosphorylation and degradation of MdmX is inhibited by Wip1 phosphatase in the DNA damage response. Cancer Res, 2009,69(20):7960-7968
24 Loukopoulos P, Shibata T, Katoh H, *et al*. Genome-wide array-based comparative genomic hybridization analysis of pancreatic adenocarcinoma: identification of genetic indicators that predict patient outcome. Cancer Sci, 2007,98(3):392-400
25 Demidov ON, Kek C, Shreeram S, *et al*. The role of the MKK6/p38 MAPK pathway in Wip1-dependent regulation of ErbB2-driven mammary gland tumorigenesis. Oncogene, 2007,26(17):2502-2506

(Received Mar. 30, 2010)