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Full Length Research Paper

Construction and identification of mouse RelB siRNA-expressing lentiviral vectors

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To determine the silencing efficiency of target genes by lentiviral vector-derived siRNA, this work constructed mouse RelB siRNA-expressing lentiviral vectors. Positive clones were screened by polymerase chain reaction (PCR) and identified by enzyme digestion as well as subsequent sequencing. RM-1 cells were transfected with liposome encapsulated siRNA, and Western blot and PCR were employed to detect the expressions of RelB in RM-1 cells followed by determination of the silencing efficiency. 293T cells were cotransfected with lentiviral vector and virus packaging plasmids mediated by calcium phosphate, and the virus titer was detected by measuring the GFP expression. PCR assay and sequencing demonstrated the RelB siRNA-expressing lentiviral vectors were successfully constructed. Real time- polymerase chain reaction (RT-PCR) and western blot showed the most effective target sequence of RelB was 5'-TGTCGTCAGGATCTGCTTC-3'. The lentivirus RNAi vector of relB which significantly inhibited relB expression was constructed successfully.

Key words: siRNA-expressing lentiviral vector, construction, identification.

INTRODUCTION

The introduction of endogenous or exogenous double-stranded RNA (dsRNA) into cells may specifically result in degradation of homologous mRNA and suppression of gene expression. This post transcriptional gene silencing (PTGS) is also known as RNA interference (RNAi). RNAi is a system within living cells that helps to control which genes are active and how active they are. PTGS is a tool to knock out specific gene in a variety of species through introducing dsRNA. *In vivo* and *in vitro* studies have confirmed that the short double-stranded RNA (21-23 bp, siRNA) can lead to gene block in mammal cells, which is more effective and complete than nucleosidase and antisense RNA are (Fire, 1999). The interference effects of siRNA are highly specific and any error in the siRNA may cause failure of RNAi (Brummelkamp, 2002).

However, not all siRNAs are effective. The silencing efficiency of different siRNAs varies. The most effective siRNA can generate the silencing efficiency of greater than 90% at RNA and protein levels (Holen et al., 2002). Well designing of siRNA is the key step in RNAi. Therefore, it is necessary to design several target sequences according to design principles and screen the most effective sequence.

The introduction of siRNA, siRNA expressing vectors or SECs into mammal cells plays a critical role in the RNAi. Currently, the methods with which the target genes are cloned into target cells or tissues include transfection through eukaryotic expression plasmid and viral vector-mediated gene transfer. The transfection through eukaryotic expression plasmid is frequently applied in *in vitro* cells with active proliferation achieving favorable transfection efficacy, but the target gene usually lose over time. However, viral vector-mediated gene transfer can integrate the target genes into host genome achieving favorable stability of expression.

MATERIALS AND METHODS

Materials

RPMI1640, fetal bovine serum (FBS), trypsin (Invotrogen, USA), plasmid DNA extraction kit, Gel extraction kit, Plasmid purification kit

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(Omiga, USA), lentiviral packaging system (College of Life Sciences at Wuhan University), mouse RM-1 prostate cancer cells (Cell Bank of Chinese Academy of Sciences), antibodies (cell signal, USA), and Lipofectamine^{TM}2000 (Invitrogen, USA) were used in the present study.

Methods

Designing and synthesis of siRNA

The design of siRNA and construction of recombinants were based on the full length mRNA sequence of mouse RelB in Genebank (ACCESSION: M83380). According to the principles of siRNA designing, the online design software (Ambion) was employed to design 5 target sequences with 21 bp. In addition, randomized negative sequences with disturbed order of some bases were applied as controls. BLAST software was used for homology analysis of selected target sequences and to exclude the possibility of non-specific inhibition of other genes. The hairpin siRNAs were as follows:

1:5′TGTCGTCAGGATCTGCTTCTTCAAGAGAGAAGCAGATCCTG ACGACATTTTTTC 3′
3′ACAGCAGTCCTAGACGAAGAAGTTCTCTCTTCGTCTAGGACT GCTGTAAAAAAGAGCT 5′
2:5′TTGGAAATCATCGACGAATTTCAAGAGAATTCGTCGATGATT TCCAATTTTTTC 3′
3′AACCTTTAGTAGCTGCTTAAAGTTCTCTTAAGCAGCTACTAAA GGTTAAAAAAGAGCT 5′
3:5′GAAGTCCACCAACACATCGTTCAAGAGACGATGTGTTGGTG GACTTCTTTTTTC 3′
3′CTTCAGGTGGTTGTGTAGCAAGTTCTCTGCTACACAACCACC TGAAGAAAAAAGAGCT 5′
4:5′AGAGGACATATCCGTGGTGTTCAAGAGACACCACGGATATG TCCTCTTTTTTTC 3′
3′TCTCCTGTATAGGCACCACAAGTTCTCTGTGGTGCCTATACAG GAGAAAAAAAGAGCT 5′
5:5′GCCAACCTTGATCAGTCTTTTCAAGAGAAAGACTGATCAAG GTTGGCTTTTTTC 3′
3′CGGTTGGAACTAGTCAGAAAAGTTCTCTTTCTGACTAGTTCCA ACCGAAAAAAGAGCT 5′

A phosphate group was connected to the 5' end of 5 siRNA, and 6 Ts and XhoI restriction site were added to 3' end of the antisense chain. Then, after confirming the correctness, the sequences were synthesized in Shanghai Sangong Co., Ltd.

Construction of viral vectors.recombinant plasmid

The siRNA blank vectors were digested with XhoI and KspAI in a mixture containing 1 µl of XhoI, 1 µl of KspAI, 2 µl of multi-core buffer, 1 µl of BSA, 1 µg of plasmid and 14 µl of H_2O at 37°C for 2 h. The mixture after digestion was subjected to agarose gel electrophoresis (7 g/L) and the linear vectors were retrieved followed by purification with a DNA gel purification kit. The siRNAs (20 ng) were incubated with 1 µl of linear vectors, 1 µl of 10 X T_4 DNA ligase buffer, 1 µl of T_4 DNA ligase, and 5 µl of H_2O at 16°C overnight.

Colony PCR

Under sterile condition, 20 µl of double distilled water were added to each well of the plate and 12 colonies from each dish were independently added to wells followed by incubation at 37°C for 1 h under continuous shaking at 220 rpm. The forward primer was 5' CAGCAGAGATCCAGTTTGGTTAGTA 3' and the reverse primer was 5' CTCGTGAAGCGAGCTTATCGATACC 3'. The mixture (10 µl) included 0.05 µl of each primer, 1 µl of $MgCl_2$, 0.3 µl of Taq DNA Polymerase, 1 µl of 10 X PCR buffer, 0.2 µl of dNTP Mix (10 mM), and 1.5 µl of colony solution. The PCR conditions included an initial denaturation at 94°C for 5 min, and 30 cycles of denaturation at 94°C for 30 s, annealing at 53°C for 30 s, extension at 72°C for 30 s and a final extension at 72°C for 10 min. In addition, blank plasmids were also used as template, PCR was performed under similar conditions and the products were regarded as negative controls. Finally, the PCR products were subjected to electrophoresis and positive colonies were selected. The positive colonies were confirmed by digestion with KspAI and HindIII followed by sequencing.

Detection of silencing efficiency

Cell culture: Cells were maintained at 37°C in a humidified 95% air and 5% CO_2 environment followed by digestion and passaging. Then, 2.5 ml of cell suspension were added to each well of a 6-well plate and cells were used for detection when 90% of confluence reached. The liposome-mediated delivery of siRNAs was performed 24 h later according to manufacturer's instruction (InVitrogen). The cells were divided into the following groups: (1) pll-sh-relB$_1$ group; (2) pll-sh-relB$_2$ group; (3) pll-sh-relB$_3$ group; (4) pll-sh-relB$_4$ group; (5) pll-sh-relB$_5$ group; (6) unrelated sequence group; and (7) negative control group. A total of 4 mg of DNA were added to each well and the content of endo-free plasmid was quantitated. The cells were harvested and lysed followed by extraction of proteins and analysis.

RT-PCR assay

Two-step RT-PCR was performed for reverse transcription according to manufacturer's onstructions (TaKaRa).

Total RNA extraction

Cell suspension was centrifuged at room temperature for 6 min at 1000 rmp/min. The supernatant was removed and Trizol (1 ml/5-10 X 10^6 cells) was added to the sediment followed by mixing. The mixture was then transferred to a 0.1 ml tube and kept at room temperature (15~30°C) for 5 min, and 0.2 ml of chloroform were added followed by violent oscillation for 15 s followed by being kept at room temperature 2~3 min. Then, the mixture was centrifuged at 2~8°C for 15 min at 10500 rpm/min. The supernatant was obtained and isopropanol of equal volume was added followed by being kept at room temperature for 10 min and subsequent centrifugation at 10500 rmp/min for 10 min at 2~8°C. The supernatant was removed and 1 ml of 75% ethanol was added to the sediment followed by centrifugation at 8500 rpm/min for 5min at 2~8°C. The supernatant was removed and the sediment was dissolved in DEPC treated water followed by aliquot. Then, 2 µl of RNA were subjected to electrophoresis and another 2 µl of RNA to colorimetric analysis.

The conditions for reverse transcription included 42°C for 20 min, 99°C for 5min and 5°C 5 min. The mixture (25 µl) for PCR of RelB cDNA included 1 µl of reverse transcription products, 1×PCR buffer, 1.5 mmol/L $MgCl_2$, 0.15 mmol/L dNTPs, 0.4 mol/L each primer, 1 U Taq DNA polymerase, and double distilled water. The PCR conditions included an initial denaturation at 94°C for 3 min and 32

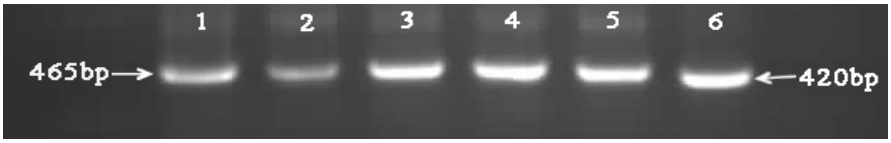

Figure 1. Confirmation of positive colonies by PCR: 1:pll-sh-relb$_1$; 2:pll-sh-relb$_2$; 3:pll-sh-relb$_3$; 4:pll-sh-relb$_4$; 5:pll-sh-relb$_5$;6: blank plasmid.

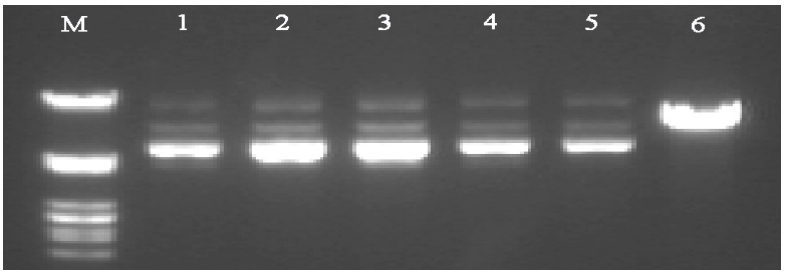

Figure 2. Confirmation by digestion with KspAI. After recombinant, the restriction site disappeared and linear DNA could not be obtained). M, lambda DNA;1, pll-sh-relb$_1$;2, pll-sh-relb$_2$;3,pll-sh-relb$_3$;4,pll-sh-relb$_4$;5,pll-sh-relb$_5$;6,pll3.7.

cycles of denaturation at 94°C for 30 s, at 55°C for 45 s, and 72°C for 1 min and a final extension at 72°C for 5 min. According to the mouse RelB cDNA sequence (ACCESSION: M83380), the primers were designed: forward: 5'CCGGCACAGCTTTAACAACCT 3', reverse: 5'CGTTTGCTCTCGATTCCATGTG 3'. Mouse β-actin was applied as internal reference and the primers were as follows: forward: 5-CTGTCCCTGTATGCCTCTG-3, reverse: 5-ATGTCACGCACGATTTCC-3'. The reagents and conditions for PCR of β-actin were similar to that of RelB. The PCR products were subjected to 1% agarose gel (containing EB) electrophoresis. Experiment was repeated three times.

Western blot

The cells were lysed with cell lysis solution followed by protein extraction. Briefly, the cell lysis solution (100 µl/5 X 10^6cells) was added to cells and then centrifugation was performed. The supernatant was obtained and aliquots were stored at - 70°C. The protein samples were mixed with 5 X SDS loading buffer at a ratio of 4:1 (v/v), which was then boiled at 100°C for 5 min. An appropriate amount of protein samples and 5 µl of protein marker were subjected to SDS PAGE (120 gel separating gel, 50 gel stacking gel). Then, the proteins were transferred onto NC membranes followed by blocking with 5% non-fat milk for 2 h at room temperature. These membranes were incubated with rabbit anti-mouse antibody (primary antibody; 1:1000) at 4°C overnight and then goat anti-rabbit antibody (secondary antibody; 1:500) at room temperature for 1 h. Chemiluminescence (ECL) was performed with films.

Virus packaging

293T cells were employed as packaging cells. When the cell confluence reached 30%, 1 µg of pll-sh-relb$_1$ and 1.5 µg of packaging plasmid were cotransfected into 293T cells and the medium was refreshed 7 h later. The supernatant was obtained 24 and 48 h later. The supernatant containing lentivirus was centrifuged at 2000 rpm for 7 min and filtered through a 0.45 µm filter followed by storage at -70°C for use. The 293T cells were seeded in a 24-well plate and the virus containing solution was added to cells at a ratio of 1:1 (v/v) when the cell confluence reached 90~100%. The transfection efficiency was detected under a fluorescence microscope. The virus containing samples and standard virus containing solution were diluted into a series of concentrations followed by transfection of 293T cells. The virus title was determined.

RESULTS

Construction of vectors

In the present study, 5 kinds of vectors carrying different specific DNAs were designed and were transcribed to siRNA to down-regulate mRNA expression of RelB. In addition, another vector carrying unrelated DNA was also designed as control. These vectors were then used to transfect RM-1 cells and positive colonies were confirmed by PCR. The length of PCR products in blank plasmid was 420 bp and that in positive vectors was 465 bp. The difference in the length of two products abovementioned was identical to that of target sequence, which suggested the target sequence has been cloned into vectors (Figure 1). Furthermore, sequencing showed the insertion sequence was the same as the designed sequence and missing and substitution of base were not noted. Moreover, digestion with KspAI and Hind III also confirmed the correctness (Figures 2 and 3).

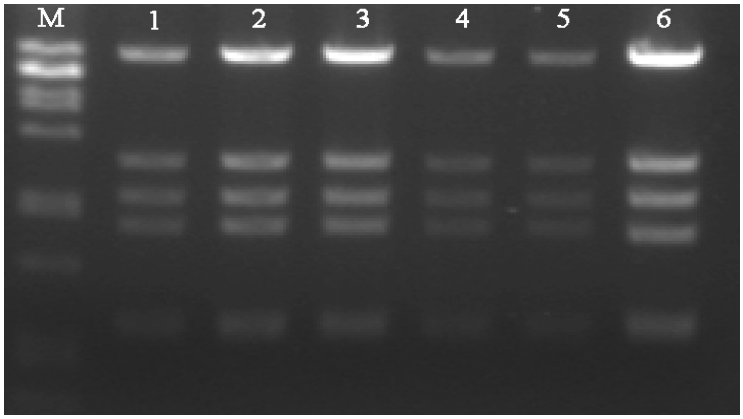

Figure 3. Confirmation by digestion withHindIII. After recombinant, the restriction site was not changed and the DNA bands after digestion were similar to those before digestion). M, lambda DNA; 1,pll-sh-relb$_1$; 2,pll-sh-relb$_2$; 3,pll-sh-relb$_3$; 4,pll-sh-relb$_4$; 5,pll-sh-relb$_5$; 6,pll3.7.

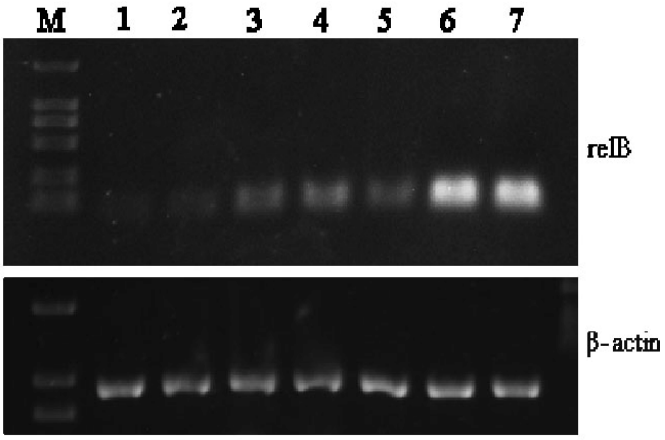

Figure 4. Detection of RelB mRNA expression by RT-PCR: 1.pll-sh-relB$_1$ group; 2.pll-sh-relB$_2$ group; 3.pll-sh-relB$_3$ group; 4.pll-sh-relB$_4$ group; 5.pll-sh-relB$_5$ group; 6. unrelated sequence group); 7.negative control group.

Transfection efficiency

A total of 7 groups were set: (1) pll-sh-relB$_1$ group; (2) pll-sh-relB$_2$ group; (3) pll-sh-relB$_3$ group; (4) pll-sh-relB$_4$ group; (5) pll-sh-relB$_5$ group; (6) unrelated sequence group; (7) negative control group. When compared with unrelated sequence group and negative control group, the mRNA expressions of RelB in the pll-sh-relB$_1$ group and pll-sh-relB$_2$ group were markedly decreased, and furthermore, the mRNA expression in the pll-sh-relB$_1$ group was lower than that in the pll-sh-relB$_2$ group. Compared with pll-sh-relB1 group, the mRNA and protein expression of relB in pll-sh-relB3 group, pll-sh-relB4 group and pll-sh-relB5 group significantly decreased. No significant difference in the mRNA expression was noted between unrelated sequence group and negative control group.

In addition, the expression levels RelB mRNA in the pll-sh-relB$_3$ group, pll-sh-relB$_4$ group and pll-sh-relB$_5$ group were remarkably lower than those in the unrelated sequence group and negative control group. Moreover, there was no marked difference in the mRNA expression of β-actin between seven groups. Our findings suggested the mRNA expression of RelB was dramatically decreased by pll-sh-relB$_1$ after transfection with recombinant vectors (Figure 4).

Western blot was employed to detect the protein expression of RelB, and absorbance was determined at 595 nm. The absorbance in 7 groups was 0.284, 0.198, 0.276, 0.295, 0.285, 0.307 and 0.296, respectively. The amount of samples in the pll-sh-relB$_2$ group was 20 μl.

Results showed all the 5 kinds of vectors could suppress the protein expression of RelB. When compared with unrelated sequence group and negative control group, the RelB protein levels in the PLL-sh-RelB$_1$ group and PLL-sh-RelB$_2$ group were markedly down-regulated. No significant difference in the RelB expression was found between unrelated sequence group and negative control group. Furthermore, there was no difference in the protein expression of β-actin between different groups. Therefore, our results revealed PLL-sh-RelB$_1$ and PLL-sh-RelB$_2$ could dramatically suppress the protein expression of RelB. The PLL-sh-RelB$_1$ had the highest suppression efficiency and was suitable for lentiviral packaging (Figure 5).

Viral packaging

Then, pll-sh-relb$_1$ and packaging plasmid were applied to co-transfect 293T cells. The fluorescence intensity was

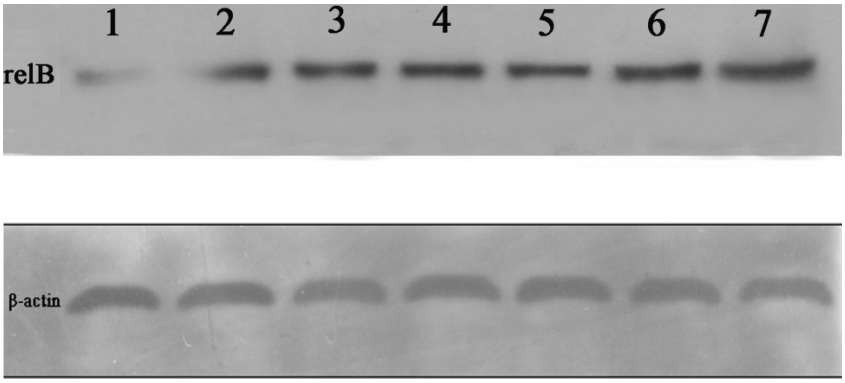

Figure 5. Detection of RelB protein expression by Western blot: 1.pll-sh-relB$_1$ group; 2.pll-sh-relB$_2$ group; 3. pll-sh-relB$_3$ group; 4. pll-sh-relB$_4$ group; 5.pll-sh-relB$_5$ group; 6. unrelated sequence group; 7. negative control group.

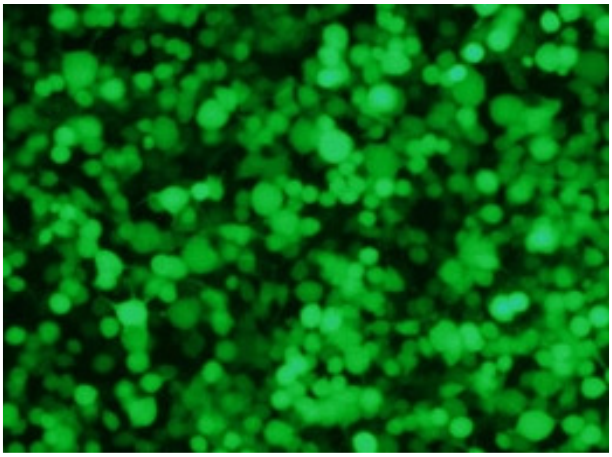

Figure 6. Representative photograph under a fluorescence microscope (×250). 293 T cells were observed after 24 h of transfection with lentivirus at a volume ratio of 1:1.

markedly increased after 24 h of transfection and favorable cell growth was observed, which implied successful packaging. The transfection efficiency was about 100% when the ratio of volume was 1:1 (Figure 6). In addition, the virus titer was 8 x 10^{10} pfu/L and qualified for further studies.

DISCUSSION

RNAi was first found in 1998 in Caenorhabditis elegans. RNAi has high sequence specificity and can efficiently and specifically silence genes. Therefore, RNAi has been a potent tool applied in the functional genomics researches. siRNA is the effector molecule and can bind to RNAi induced silencing complex (RISC) followed by melting which results in silencing of target gene (Elbashir et al., 2001). RNAi technique is a silencing technique with gene-specificity developed in the past few years. RNAi had the characteristics of high efficiency and high specificity. To date, RNAi has been applied in numerous fields: (1) Based on RNAi technique, gene pool scanning has been a tool applied in the identification of drug targets, and these targets play important roles in the occurrence and development of diseases (Martin and Caplen, 2007) ; (2) Application of siRNA and antisense oligonucleotide can inhibit one or more reductase subunits in a specific way, which provide a novel method for the pharma-cotherapy with selective or complementary molecules (Reid et al., 2009); (3) RNAi has the potential to treat diseases which are related to dominant gene mutation (Irene and Marta, 2009); (4) In the treatment of cancers, RNAi can induce gene rearrangement in undifferentiated somatic cells (Graeme, 2009).

It has been confirmed that in plants and mollusks the effects of RNAi can transmit between cells. But there remains still controversy in the transmission of the effects of RNAi in mammal cells. The transmission of the effects of RNAi in human cells was independent of cell-cell contact, and miR-122 could effectively expressed in human liver cancer cells (Thomas and Helen, 2010). In the present study, mouse prostate cancer cells were employed. In practice, although the RNAi technique still requires further development, the basic researches using RNAi are valuable (Baum et al., 2007). In the future, the RNAi technique as a therapeutic strategy depends on the development in the efficient transfection, which is still a challenge in RNAi field (Aigner, 2007).

To precisely silence target genes, it is critical to effectively transfect cells as more as possible with siRNA. *In vitro* studies on silencing efficiency should be based on the optimized transfection processes which can assure the effective silencing of target genes in cells (Reid et al., 2009). Recently, in the RNAi field, more attention has been paid to the viral vector mediated RNAi. Transfection with viral vectors can solve some problems such as low

transfection efficiency and instability in plasmid transfection and some cells can not be transfected, and also expand the fields in which transfection is applied. The common viral vectors include adenoviral vector, retroviral vector, lentiviral vector, etc. Adenovirus is linear double-stranded DNA virus without envelope and can be effectively transfected into different mammal cells. However, virus can not be integrated into the host genome. Retrovirus is plus-strand RNA virus and has broad host range.

In addition, transfection with retrovirus has high efficiency and retrovirus can be integrated into host genome. However, retrovirus can only be transfected into cells undergoing division. Lentiviruses can transfect into not only cells undergoing division but resting cells. As compared to two kinds of virus above, lentivirus is suitable to transfect embryonic stem cells or embryo to generate transgenic animals through RNAi (Rubinson et al., 2003). Lentivirus is replication-defective retrovirus and is developed on the basis of HIV-1. In addition, the genetic materials of lentivirus can be integrated into host genome exerting sustained effects (Wu et al., 1998). Therefore, lentivirus is a promising gene therapy vector. Several *in vivo* and *in vitro* studies have confirmed the significance of lentivirus mediated RNAi in the functional genomic study and gene therapy (Hui et al., 2004; Singer et al., 2005).

Nuclear factor κB (NF-kB)/ Rel family plays a crucial role in the inflammation and immune response (Hume et al., 2001). In mammalians, a total of 5 members have been identified (Jimi and Ghosh, 2005): RelA, c-RelB, RelB, NF-kB1 and NF-kB2. In the allograft rejection, the mature dendritic cells (DCs) of the donor can present MHC molecules or antigenic peptide-MHC molecules to T cells of the recipient and then activate T cells in the presence of costimulators (CD_{40}, CD_{80}, CD_{86},etc.) and corresponding ligands of T cells eliciting immune response. The immature DCs do not express costimulators and are not involved in the immune response. Furthermore, these DCs can selectively interact with antigen specific T cells of the recipient and then induce immune tolerance through inducing apoptosis, incompetence and increase of regulatory T cells.

The NF-κB family member RelB has many distinguishing features compared to the other NF-κB proteins, such as its unique amino-terminal leucine zipper motif. RelB also preferentially interacts with p100 rather than the usual NF-κB inhibitory molecules (e.g. IκBα). RelB has lower relative DNA binding activity at prototypical response elements, and can both activate and inhibit transcription of target genes. Specific nuclear DNA κB binding sites are efficiently recognized by RelB/p52 DNA heterodimers, allowing for transcription of certain κB-regulated target genes. RelB can modulate the maturation of DCs (Dong et al., 2005). The knockout of RelB may impair the maturation of DCs. In the present study, a total of 5 kinds of lentiviral vectors carrying siRNA of target sequence at different sites: PLL-sh-$RelB_1$, PLL-sh-$RelB_2$, PLL-sh-$RelB_3$, PLL-sh-$RelB_4$ and PLL-sh-$RelB_5$ which locate at 137, 757, 850, 955 and 1768, respectively. After transfection into RM-1 cells, the effective lentiviral vector was screened. Western blot showed the protein expression of RelB was down-regulated in all groups. Moreover, PLL-sh-RelB1 group had the highest suppression efficiency and therefore site 137 in RelB gene is an effective site for specific silencing.

Nuclear transcriptiton factor kappaB (NF-κB) is a multidirectional nuclear transcription factor and involved in numerous pathophysiological processes including inflammation, cell proliferation, transformation and apoptosis (Hume et al., 2001). In mammals, all proteins of the NF-κB family have five subunits including NF-κB1 (p50), NF-κB2 (p52), RelA (p65), RelB and c-Rel, and distinct subunits have varied functions (Jimi and Ghosh, 2005). Study showed the radiation resistance of prostate cancer was related to the activation of RelB subunit of NF-κB. Through immunohistochemistry, Josson et al.(2006) revealed the expressions of NF-κB family members in the normal prostate, prostatic intraepithelial neoplasia and prostate cancer (Dong et al., 2005). However, when the radiation resistance of prostate cancer cells occurred, the RelB expression was markedly increased and the expressions of other subunits were not dramatically changed. These findings suggest radiotherapy can increase the RelB expression in the prostate cancer cells and RelB enhances the radiation resistance of these cells. Xu et al. (2007) have demonstrated radiotherapy could increase the RelB expression in the cytoplasm and the nucleus, and the RelB DNA-binding activity was higher than RelA DNA-binding activity after radiotherapy. This result implies promotion of RelB nuclear translocation may be an important mechanism in the radiation resistance of prostate cancer cells. Therefore, to transfect prostate cancer cells with specific siRNA targeting RelB gene may inhibit the RelB expression and increase the sensitivity of these cells to radiotherapy.

In the present study, a total of 5 kinds of lentiviral vectors carrying siRNA of RelB at different sites were constructed: PLL-sh-RelB1, PLL-sh-RelB2, PLL-sh-RelB3, PLL-sh-RelB4 and PLL-sh-RelB5 which locate at sites 137, 757, 850, 955 and 1768, respectively. According to the principles for siRNA design (Ambion), five sequences with 2A in the initiating terminal were used as target sequences. The percentage of GC was 30-52% in the five sequences which not only ensures the stability of DNA but promotes the effective DNA unzipping. These principles increase the possibility in finding effective target sequences. NF-κB is widely expressed in a lot of tissues including mouse prostate cancer cells (RM-1 cells). In the present study, siRNAs of target sequences were transfected into RM-1 cells and the optimal vector was

screened. RT-PCR showed the RelB expression was down-regulated in the RM-1 cells after transfection. Additionally, vectors carrying one of 5 target sequences could decrease the mRNA expression of RelB, and the amplitude of this decrease was more obvious after transfection with PLL-sh-RelB1 and PLL-sh-RelB2.

In addition, western blot assay was employed to detect the protein expression of RelB. Results showed vectors carrying one of 5 target sequences could decrease the protein expression of RelB in the RM-1 cells and the amplitude of this decrease was the most obvious after transfection with PLL-sh-RelB1. These findings were consistent with RT-PCR and suggested the site 137 is a specific site of suppression of RelB expression. The lentiviral vector can effectively integrate the genome into the neurons playing an important role in the treatment of neurological diseases. Currently, lentivirus has been applied in the endogenous gene silencing through siRNA, and the efficiency of lentivirus mediated gene silencing is higher than that of plasmid vectors. In the present study, 5 plasmid vectors were used to transfect the RM-1 cells and the most effective plasmid vector was identified. Lentiviral packaging system was employed to generate a lentivirus carrying PLL-sh-RelB1 for future study.

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