EJGO European Journal of Gynaecological Oncology
Original Research

RNAi silenced NUP88 gene suppresses growth and invasiveness of human breast cancer cell line MCF-7

Y. Li[1], C. Li[1], N. Li[2], L. Jiang[3], W. Yang[4], Y. Wang[1], B. Wu[1], C. Shi[1], Z. Zhu[5]

[1]Department of Clinical Biological Sample Center, [2]Department of Intensive Medicine, [3]Department of General Surgery, [4]Department of Ultrasonography, [5]Department of Oncology, the First Affiliated Hospital of Jinzhou Medical University, Jinzhou (China)

Summary

Objective: To construct and recombine a lentivirus-mediated short hairpin RNA (shRNA) vector targeting *NUP88* gene and to observe the effect of silencing *NUP88* gene on the biological behavior of MCF-7 cells by RNA interference (RNAi) technology, so as to find a new target for the treatment of breast cancer. *Materials and Methods:* The recombinant lentiviral vector of *NUP88*-shRNA was constructed and then transfected into MCF-7 cells. Then, the changes of biological behavior of MCF-7 cells were detected by reverse transcription polymerase chain reaction (RT-PCR), Western-blot, methyl thiazolyl tetrazolium (MTT), flow cytometry, and Transwell assay. *Results:* The results of RT-PCR and Western-blot showed that the expression of *NUP88* mRNA and protein was significantly reduced in *NUP88*-shRNA group, as compared with that in the blank and negative groups ($p < 0.01$). The proliferation of MCF-7 cells in the *NUP88*-shRNA group was prominently reduced after transfected with lentivirus-*NUP88*-shRNA ($p < 0.05$). Flow cytometry results showed that the apoptosis rate increased significantly in the *NUP88*-shRNA group as compared with the other two groups ($p < 0.05$). After routine culture for 24 hours, the transmembrane cells in the NUP88-shRNA group were notably less than that in the blank or negative control group ($p < 0.05$). *Conclusion:* Recombinant *NUP88*-shRNA lentivirus can successfully inhibit the expression of *NUP88* gene in MCF-7 cells and consequently inhibit the proliferation and invasive ability of human breast cancer cells.

Key words: NUP88; Human breast cancer cell MCF-7; RNAi; Lentiviral vector.

Introduction

Breast cancer is one of the most common malignant tumors in women. It has been reported that its incidence rate occupies 7-10% of all kinds of malignant tumors and has an annually increasing trend [1]. In recent years, studies on the gene mutation and its functions in the occurrence, development, and metastasis of breast cancer have become a hotspot in this field [2-5].

The current treatments for breast cancer mainly include local treatment and systemic management. Tumor molecular targeted therapy is a new biotherapeutic model aiming to reverse the malignant biological behaviors at the molecular level of tumorigenesis, such as cell signaling pathways, proto-oncogene and tumor suppressor genes, cytokines and receptors, anti-tumor angiogenesis and suicide genes, and thereby inhibiting the growth of tumor cells, or even completely dissipating the tumor. Although targeted therapy cannot replace the traditional chemotherapy currently, it can be used as a new treatment choice for the patients with failed chemotherapy. Targeted therapy can also be used as the preferred treatment for the elderly and the patients with poor physical status. Thus, it has become a hot spot for researchers and clinicians at present.

Recent studies have shown that *NUP88* is overexpressed in many malignant tumors such as human ovarian cancer and breast cancer. It is overexpressed at the margins of colorectal cancer tumors, suggesting that it is closely related to tumor invasiveness [6-10]. Some studies have shown that *NUP88* protein is highly expressed in breast cancer tissues and related to the histological grade, TNM stage, with or without lymph node metastasis and other clinicopathological features of breast cancer. Thus, it has currently been proposed as a tumor marker.

RNA interference (RNAi) is a sequence-specific post-transcriptional gene silencing phenomenon mediated by double-stranded RNA, which is used to efficiently reduce or turn off the expression of specific genes. RNAi has the characteristics of high specificity, high stability, high efficiency, and concentration-dependence, which play an important biological role in maintaining genome stability and regulating gene expression. With the in-depth research on RNAi, as a tool for gene silencing, RNA interference technology has been widely used in gene function researches, disease targeted therapy, and screening new drug targets. Lentivirus vectors are gene therapy vectors based on human immunodeficiency virus [11-14]. For some difficult-to-transfect cells, such as primary cells, stem cells, undifferentiated cells, and so on, it can greatly improve the transduction efficiency of the target gene and greatly increases the probability of integrating the target gene into

Revised manuscript accepted for publication June 11, 2018

Table 1. — *Primers and shRNA sequences.*

Gene		Sequences (5'-3')
Nup88	Forward	TAAAGGAAGGGCGTATACCG
	Reverse	AAGCAGAGTACAGCACACGC
ß-actin	Forward	GGAAATCGTGCGTGACATTA
	Reverse	GGAGCAATGATCTTGATCTTC
**NUP88*-shRNA1	Forward	TGATCTCAGTTATTGTCGAGAACTCGAGTTCTCGACAATAACTGAGATCTTTTTTC
	Reverse	TCGAGAAAAAAGATCTCAGTTATTGTCGAGAACTCGAGTTCTCGACAATAACTGAGATCA
NUP88-shRNA2	Forward	TGATCTCAGTTATTGTCGAGAACTCGAGTTCTCGACAATAACTGAGATCTTTTTTC
	Reverse	TCGAGAAAAAAGATCTCAGTTATTGTCGAGAACTCGAGTTCTCGACAATAACTGAGATCA
NUP88-shRNA3	Forward	TCGGCTGAAGATAACTATGGTTCTCGAGAACCATAGTTATCTTCAGCCGTTTTTTC
	Reverse	TCGAGAAAAAAGCAAAGATGAAGTAGTGGCATCTCGAGATGCCACTACTTCATCTTTGCA
NUP88-shRNA4	Forward	TCACAACATCATGTAGCACTTACTCGAGTAAGTGCTACATGATGTTGTGTTTTTTC
	Reverse	TCGAGAAAAAACACAACATCATGTAGCACTTACTCGAGTAAGTGCTACATGATGTTGTGA
Negative	Forward	CCGGTTCTCCGAACGTGTCACGTTTCAAGAGAACGTGACACGTTCGGAGAATTTTTG
	Reverse	AATTCAAAAATTCTCCGAACGTGTCACGTTCTCTTGAAACGTGACACGTTCGGAGAA

**NUP88-shRNA1 are the optimal sequences.*

the genome of the host cell. This provides a favorable approach for the study on RNAi, cDNA cloning, and reporter gene. At present, in cell-related experimental operations, the transduction efficiency of the gene can be greatly improved by virus-mediated experiments, especially for some cells that are difficult to be transfected by the conventional method, so as to achieve efficient transient expression of the target gene.

In this experiment, the recombinant lentivirus-mediated *NUP88*-shRNA vector was constructed and transfected into human breast cancer cell line MCF-7. The *NUP88* gene in MCF-7 cell was silenced by RNAi technique. The proliferation, adhesion, invasion and metastasis of MCF-7 cell after *NUP88* silencing were observed, aiming to discover out a new target for the clinical gene therapy of breast cancer and explore an efficient and feasible method for the treatment of breast cancer.

Materials and Methods

Primers and shRNA sequences design and synthesis were performed. (Table 1). The sequences showed no homology with other genes as blasted on the National Center for Biotechnology Information (NCBI) database.

The primers were ligated to the linearized vector by T4 DNA ligase in appropriate buffer. Then the mixture was added into 800 µl SOC medium and incubated at a constant temperature in a water bath for 45 minutes. 150 µl transformed competent cells were transferred to LB agar medium with Amp resistance (100 µg/ml) containing 20 mmol/l MgSO4 and cultured at 37°C for 16 hours for the construction of lentiviral vectors.

At two hours before transfection, the culture medium of 293T cells at the logarithmic growth phase was changed to serum-free medium. The diluted DNA was mixed with the diluted Lipofectamine 2000 and incubated at room temperature for 20 minutes, and then the mixture was transferred to the 293T cells with serum-free medium and incubated in a 5% CO_2 incubator at 37°C for eight hours. After washed with PBS, the cell culture medium was changed with 25 ml complete medium and further cultured for 48 hours. Then the supernatant was collected.

At the day before the determination, 293T cells were collected and plated in 96-well plates at a density of 4×10^4 cells per well in a volume of 100 µl. Virus solutions were prepared by ten-fold serial dilution in sterile EP tubes and then added into the corresponding cell wells at 90 µl per well and incubated for 24 hours, followed by incubation with 100 µl complete medium for another four days. Then the fluorescence in cells was observed under a fluorescence inverted microscope.

MCF-7 cells were seeded in six-well plates at a density of 5×10^5/well and cultured for 24 hours. According to the formula: MOI = (TU value/mL × volume of virus liquid) / cell number to be transfected, MCF-7 cells were transfected with the lentiviral virus solution at the MOI of 10, 20, 50 and 100, leaving two wells as a negative control. After incubating at 37°C for one hour, the cells were replaced with fresh medium and cultured for 48-72 hours. The eGFP was detected under a fluorescence microscope. The maximum MOI which did not cause obvious CPE was taken as the optimal MOI of the lentivirus carrying the target gene.

MCF-7 cells were divided into three groups: silence group, negative control group, and blank group. The silence group were subdivided into *NUP88*-shRNA1, *NUP88*-shRNA2, *NUP88*-shRNA3, and *NUP88*-shRN4 groups. MCF-7 cells were cultured in six-well plates for 24 hours. The lentivirus at the best MOI was added into each group and incubated at 37°C for one hour. Then the cells were cultured with fresh 1640 medium for 48-72 hours. Cells in each group were collected for the total RNA extraction. The mRNA expression of *NUP88* was detected using reverse transcription polymerase chain reaction (RT-PCR) following the instruction supplied by the manufacturer. The PCR products were analyzed by 1% agarose gel electrophoresis and the optical density (OD) of the bands was analyzed. All experiments were repeated three times. The virus solution with the highest silencing efficiency was chosen for the following experiments. The relative expression of NUP88 mRNA = $OD_{NUP88}/OD_{ß\text{-actin}}$.

The third generation of MCF-7 cells were transfected with the aforementioned lentivirus and cultured for 72 hours. Then the total protein of each group were extracted and quantified and then separated by 8% sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE). After transferred onto the membrane, proteins were stained with ponceau solution. After elution for three time, the membrane was incubated with the primary rabbit anti-human monoclonal antibody (1:1000) overnight at 4°C, followed by the incubation with secondary goat anti-Rb antibody at room temperature for one hour. After incubated with BCIP/NBT staining working solution at room temperature for three hours, the

protein bands were analyzed using a gel imaging system. The OD values were measured for three times and the net OD values were needed.

The third generation of MCF-7 cells were seeded in 96-well plates at 10^4 cells per well. Each 96-well plate was divided into three groups of 9 wells each. The first group was *NUP88*-shRNA1 group, the shRNA with the best silencing efficacy, incubated with 200 µl *NUP88*-shRNA1 lentiviral vector mixed medium at the best MOI. The second group was negative control group, treated with 200 µl *NUP88*-control vector mixed medium. The third group was blank group, directly incubated with 200 µl culture medium. The culture medium was changed at 24, 48, 72, 96, and 120 hours after transfection, respectively. Twenty µl of methyl thiazolyl tetrazolium (MTT) solution was added into each well, and the cells were incubated for another four hours. After the supernatant was discarded, 150 µl dimethylsulphoxide (DMSO) was added into each well. Then the plates were shaken for ten minutes to fully dissolve the crystal and measured using a microplate reader at a wavelength of 490 nm. The results were zeroed with a blank cell-free well. All experiments were run in triplicate. The time was used as the horizontal axis and the OD value was used as the vertical axis to draw the curve.

The cells in each group were harvested after 24 hour culture. After washed twice with phosphate buffered saline (PBS), the cells were adjusted to 1×10^6/ml with binding buffer. 500 µl of cell suspension was incubated with 10 µl PE-labeled Annexin V in dark for 30 minutes at room temperature. Then 5 µl PI was added into each well and incubated in dark for another five minutes. Then the cells in each group were detected using a flow cytometer. The four quadrants of the FACS graph represented different meanings: the lower left contained normal cells, the lower right contained early apoptotic cells, the upper right contained late apoptotic cells, and the upper left contained necrotic cells. The results were analyzed according to the trend of cell distribution in each quadrant.

Precooled 1640 medium and Matrigel were mixed at a ratio of 1:1. 100 µl of the mixture was uniformly spreaded on the bottom of the upper chamber and incubated for four to five hours at 37°C. MCF-7 cells of each group at the logarithmic growth phase were harvested at 72 hours after transfection to produce the cell suspension. Then, 200 µl of 5×10^4 cell suspension was added into the upper chamber, six wells in each group. 500 µl of 1640 medium containing 20% FBS was added into the lower chamber. After cultured in an incubator at 37°C for 48 hours, the chambers were taken out and fixed with 4% paraformaldehyde. After HE staining, the chambers were observed under an inverted microscope. Five fields were randomly selected for counting and the average was obtained.

All data were analyzed using SPSS18.0 software. The measurement data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to compare the differences among groups. $P < 0.05$ was considered statistically significant.

Results

By sequencing, the authors found that the four lentiviral *NUP88*-shRNAs and negative control vectors were successfully constructed without base mutation. The four pairs of sequences were all consistent with the expected DNA sequences, indicating that the synthetic *NUP88*-shRNA sequence was correctly inserted. DNA recombination was successfully prepared for the following experiments. After transfection with the lentiviral vectors for 48 hours, HEK293T cells showed strong green fluorescence under a fluorescence microscope. Meanwhile, cytopathic effect (CPE) was observed, presenting as retracted cell antennae, swelling, and distension in cells, and some cells were shed and suspended (Figures 1A, B).

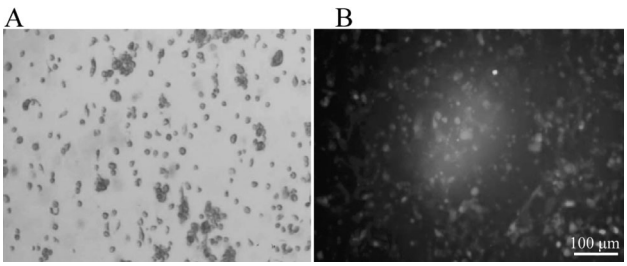

Figure 1. — HEK293T cells transfected with lentiviral vector for 48 hours. A) Virus packaging, under a light inverted microscope (×100). B) Fluorescence distribution, under a fluorescence inverted microscope (×100).

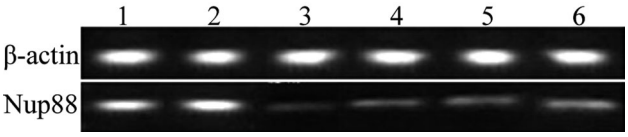

Figure 2. — NUP88 mRNA expression in each group detected by RT-PCR. Lane 1: Negative control group. Lane 2: Blank control group: Lane 3: *NUP88*-shRNA1 group. Lane 4: *NUP88*-shRNA2 group. Lane 5: *NUP88*-shRNA3 group. Lane 6: *NUP88*-shRNA4 group.

The titers of LV-*NUP88*-RNAi-1, LV-*NUP88*-RNAi-2, LV-*NUP88*-RNAi-3, LV-*NUP88*-RNAi-4, and LV-NUP88-negative control were all 4E+8 TU/mL, meeting the requirements of lentiviral interference for the following experiments. The best MOI was 20 and the optimal transfection volume for each lentiviral vector was 20 µl.

The expression of NUP88 mRNA in MCF-7 cells was detected by RT-PCR. The authors found that the expression of *NUP88* mRNA in MCF-7 cells transfected with *NUP88*-shRNAs was significantly reduced as compared with that in the blank control group or negative control group ($p < 0.01$), while it was not different between the negative control group and the blank control group ($p > 0.05$). NUP88 mRNA expression in *NUP88*-shRNA1 group was prominently lower than that in the *NUP88*-shRNA2, *NUP88*-shRNA3, and *NUP88*-shRNA4 groups ($p < 0.05$, Figure 2).

The WB results showed that the Nup88 protein expression in MCF-7 cells was significantly decreased by transfection with *NUP88*-shRNA ($p < 0.01$). No difference in *Nup88* protein expression was found between negative control group and blank group ($p > 0.05$). The *NUP88* protein expression in *NUP88*-shRNA1 group was lower than that in the other three groups ($p < 0.05$), indicating that the in-

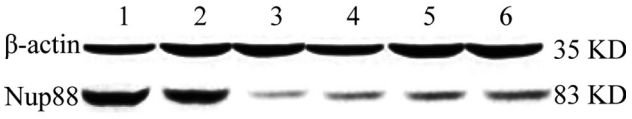

Figure 3. — *NUP88* protein expression detected by Western-blot. Lane 1: Negative control group. Lane 2: Blank control group. Lane 3: *NUP88*-shRNA1 group. Lane 4: *NUP88*-shRNA2 group. Lane 5: *NUP88*-shRNA3 group. Lane 6: *NUP88*-shRNA4 group.

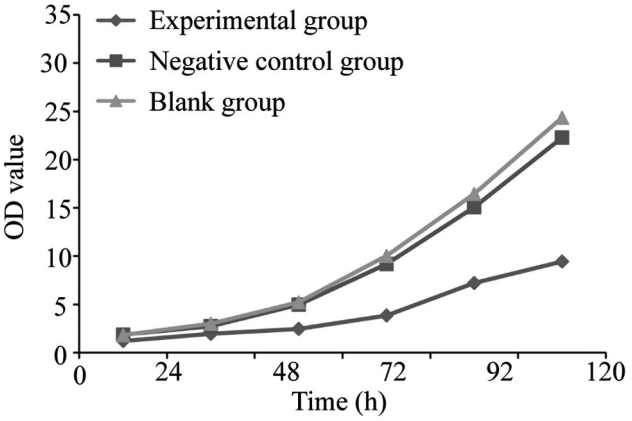

Figure 4. — Growth curve of MCF-7 cells determined by MTT.

hibitory rate of *NUP88*-shRNA1 on *NUP88* protein in MCF-7 cells was higher than that in the other three groups (Figure 3).

Cell growth curve was established following the proliferation of MCF-7 cells transfected with *NUP88*-shRNA1 or negative-shRNA, or treated with blank medium for 24-120 hours. The results showed that cells in each group grew gradually over time, while cells in the blank group and the negative group proliferated notably faster than those in the *NUP88*-shRNA group ($p < 0.05$). However, there was no significant difference between the blank group and the negative group ($p > 0.05$). The results indicated that the recombinant lentivirus *NUP88*-shRNA can inhibit the proliferation of breast cancer cell MCF-7 via inhibiting the expression of *NUP88* gene (Figure 4).

Table 2. — *The apoptosis rate of the three groups cultured for 24 hours by flow cytometry.*

Group	Apoptosis rate	*p* value
Blank	3.56 ± 0.35	> 0.05
Negative control	3.75 ± 0.29	> 0.05
*NUP*88-shRNA	28.56 ± 0.25	< 0.01*

*$p < 0.01$: statistical significance.

The apoptosis of MCF-7 cells detected by flow cytometry showed that there was no significant difference in the apoptosis rate between blank control group (3.56 ± 0.35) and negative control group (3.75 ± 0.29, $p > 0.05$). However, transfection with lentivirus *NUP*88-shRNA caused the apoptosis rate of MCF-7 remarkably increased (28.56 ± 0.25, P<0.05; Table 2). The results showed that the recombinant lentiviral vector *NUP88*-shRNA can promote the apoptosis of MCF-7 through inhibiting the expression of *NUP88* gene (Figure 5).

Transwell assay was used to investigate the invasive ability of MCF-7 cells transfected with recombinant lentivirus. After cultured for 24 hours, the numbers of transmembrane cells in the blank group, negative control group and *NUP88*-shRNA group were 253.4 ± 13.4, 259.2 ± 14.6, and 102.5 ± 7.9, respectively. Compared with the blank group and the control group, the number of transmembrane cells in the *NUP88*-shRNA group was significantly decreased ($p < 0.05$), while there was no significant difference between the blank group and the control group ($p > 0.05$). Inhibitory rate = (blank group - *NUP88*-shRNA group) / blank group. The results suggested that the recombinant lentiviral vector *NUP88*-shRNA can significantly inhibit the invasiveness of breast cancer cells MCF-7.

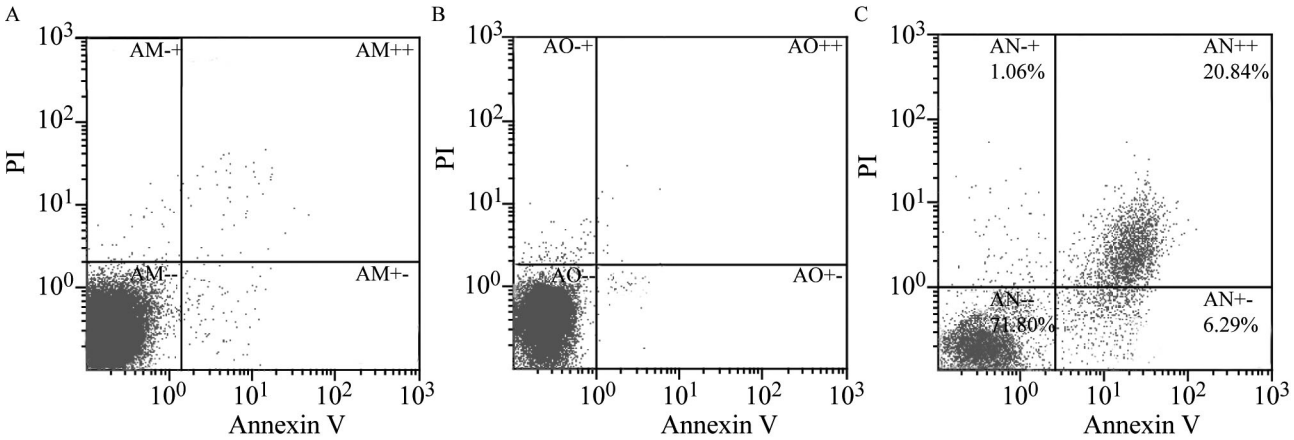

Figure 5. — The apoptosis of MCF-7 detected by flow cytometry. A) Blank group. B) Negative control group. C) *NUP88*-shRNA group.

Discussion

With the change of people's lifestyle and the influence of social, environmental and other factors, the incidence of breast cancer shows an increasing trend year by year, which seriously threatens the general health of women. Surgery is the most commonly used traditional treatment for breast cancer, followed by radiotherapy, chemotherapy, and endocrine therapy. However, no matter what method is used, there is still relatively large damage to the body. Therefore, to discover a method with less side effects and high efficacy is very urgent. From the phenomenon of "co-suppression" proposed by Rich Jorgensen *et al.* [15] at Arizona University in 1996 to the RNA interference (RNAi) first proposed by Fire *et al.* at Carnegie Institute in 1998 [16], RNAi is rapidly developed into a powerful tool for analyzing gene function, opening up a whole new path for the treatment of breast cancer [17-19].

The nuclearpore complex (NPC) is the only channel found in recent years that penetrates the nuclear membrane for nuclear-cytoplasmic substance exchange. It is a large protein complex structure across the lipid bilayer. Structurally, NPC consists mainly of cytoplasmic rings, nuclear rings, and spokes. *NUP88* is an antigen in tumor cells recognized by the monoclonal antibody (Mab) C6 and is highly expressed in many tumors. Agudo *et al.* [20] found that high expression of *NUP88* gene in breast cancer is associated with its high aggressiveness. Studies by Schneider *et al*. [21] have shown that the *NUP88* gene may serve as a more specific phenotypic marker of some specific tumors to guide the clinical practice Generally, in clinical and experimental studies, RNA interference is to construct a lentivirus or adenovirus for silencing *NUP88* gene, and then investigate whether the invasion and metastasis of breast cancer cells will be changed [22-25], in order to further provide better clinical targets or to provide a scientific theoretical basis.

The advantage of lentivirus is that it can infect a variety of cells difficult to be infected, such as primary, stem, and neuronal cells. Moreover, lentivirus can insert foreign genes into the host genome to achieve stable transfection. Although the packaging of adenovirus is relatively large (up to 8K), its cycle is longer than the lentivirus, and the exogenous genes cannot be inserted into the host genome. Thus, in this study, lentivirus was used.

The efficiency of RNAi silencing depends on whether the targets screening of the target gene is reasonable and accurate. In this study, the authors designed the shRNAs on the functional region of *NUP88* gene in GenBank (NM_002532) database according to the shRNA design principle. Four *NUP88*-shRNAs and a negative control were confirmed to have no homology with other genes. After sequencing, the results showed that the synthetic sequences were completely consistent with the designed ones.

RT-PCR and Western-blot results showed that the mRNA and protein expression of *NUP88* in MCF-7 cells was significantly decreased after *NUP88*-shRNA was transfected into MCF-7, indicating that breast cancer cells were successfully transfected with NUP88-shRNA, and NUP88 mRNA and protein expression was successfully inhibited in MCF-7 cells. On the contrary, the negative control group and the blank group showed no significant reduction in NUP88 mRNA expression, and the difference between them was not statistically significant.

The results of MTT assay showed that the cells in the blank group and the control group grew rapidly with no significant difference between the two groups ($p > 0.05$), while the proliferation of the *NUP88*-shRNA group was significantly reduced after lentivirus transfection ($p < 0.05$), indicating that the recombinant lentiviral vector *NUP88*-shRNA can inhibit the proliferation of breast cancer cells via inhibiting the expression of *NUP88* gene.

Transwell chamber test showed that the number of transmembrane cells in the blank group, the negative control group, and the *NUP88*-shRNA group were 253.4 ± 13.4, 259.2 ± 14.6, and 102.5 ± 7.9, respectively, after the chemotaxis was performed for 24 hours by conventional culture of tumor cells. Compared with the control groups, the number of transmembrane cells was reduced significantly ($p < 0.05$), but there was no significant difference between the blank and the negative control groups ($p > 0.05$), indicating that the recombinant lentiviral vector *NUP88*-shRNA can significantly inhibit the invasiveness of breast cancer cells.

In summary, the study successfully constructed a RNAi lentiviral vector targeting *NUP88* gene, and identified the interference efficiency. After successfully transfected into MCF-7 cells, the optimal *NUP88*-shRNA1 with the highest interference efficiency was screened and used in the following experiments. Further function tests were carried out to prove that *NUP88* gene silencing could significantly inhibit the proliferation of MCF-7 cells and promote their apoptosis as well as inhibit the invasiveness of MCF-7 cells. The present results laid the foundation for the *in vivo* experiments aiming to further study the gene therapy for breast cancer.

References

[1] Denic S., Agarwal M.M.: "Breast cancer protection by genomic imprinting in close kin families". *BMC Med. Genet.*, 2017, *18*, 136.
[2] Ye X., He Q., Zhou X.: "Study on the adverse effects following chemotherapy for breast cancer diagnosis during pregnancy: The first case report in China". *Medicine (Baltimore)*, 2017, *96*, e8582.
[3] Wang Y., Zhu J., Gou J., Xiong J., Yang X.: "Phyllodes tumors of the breast in 2 sisters: Case report and review of literature". *Medicine (Baltimore)*, 2017, *96*, e8552.
[4] Jiang Y., He Q., Li S., Shi C., Yang X.: "Reversible severe fatty liver induced by capecitabine: A case report". *Medicine (Baltimore)*, 2017, *96*, e8547.
[5] Jung J.H., Son S.H., Kim D.H., Lee J., Jeong S.Y., Lee S.W., et al.: "CONSORT-Independent prognostic value of asphericity of pretherapeutic F-18 FDG uptake by primary tumors in patients with breast cancer". *Medicine (Baltimore)*, 2017, *96*, e8438.

[6] Genenncher B., Lipka V., Petutschnig E.K., Wiermer M.: "Nucleoporin NUP88/MOS7 is required for manifestation of phenotypes associated with the Arabidopsis CHITIN ELICITOR RECEPTOR KINASE1 mutant cerk1-4". *Plant Signal. Behav.*, 2017, *12*, e1313378.

[7] Li J., Zhao J., Li Y.: "Multiple biological processes may be associated with tumorigenesis under NUP88-overexpressed condition". *Genes Chromosomes Cancer*, 2017, *56*, 117.

[8] Li Y., Zhang X., Ge J., Liu X., Xu S., Zhu Z., et al.: "Can Nup88 expression be associated with atypical endometrial hyperplasia and endometrial cancer? A preliminary study". *Pathol. Res. Pract.*, 2016, *212*, 274.

[9] Naylor R.M., Jeganathan K.B., Cao X., van Deursen J.M.: "Nuclear pore protein NUP88 activates anaphase-promoting complex to promote aneuploidy". *J. Clin. Invest.*, 2016, *126*, 543.

[10] Park G.T., Frost J.M., Park J.S., Kim T.H., Lee J.S., Oh S.A., et al.: "Nucleoporin MOS7/Nup88 is required for mitosis in gametogenesis and seed development in Arabidopsis". *Proc. Natl. Acad. Sci. U. S. A.*, 2014, *111*, 18393.

[11] Rodrigues T.B., Dhandapani R.K., Duan J.J., Palli S.R.: "RNA interference in the Asian Longhorned Beetle:Identification of Key RNAi Genes and Reference Genes for RT-qPCR". *Sci. Rep.*, 2017, *7*, 8913.

[12] Majumdar R., Rajasekaran K., Cary J.W.: "RNA Interference (RNAi) as a Potential Tool for Control of Mycotoxin Contamination in Crop Plants: Concepts and Considerations". *Front. Plant. Sci.*, 2017, *8*, 200.

[13] Mathur K., Anand A., Dubey S.K., Sanan-Mishra N., Bhatnagar R.K., Sunil S.: "Analysis of chikungunya virus proteins reveals that non-structural proteins nsP2 and nsP3 exhibit RNA interference (RNAi) suppressor activity". *Sci. Rep.*, 2016, *6*, 38065.

[14] Unniyampurath U., Pilankatta R., Krishnan M.N.: "RNA Interference in the Age of CRISPR: Will CRISPR Interfere with RNAi". *Int. J. Mol. Sci.*, 2016, *17*, 291.

[15] Jorgensen R.A., Cluster P.D., English J., Que Q., Napoli C.A.: "Chalcone synthase co-suppression phenotypes in petunia flowers: comparison of sense vs. antisense constructs and single-copy vs. complex T -DNA sequences". *Plant Mol. Biol.*, 1996, *31*, 957.

[16] Fire A., Xu S., Montgomery M.K., Kostas S.A., Driver S.E., Mello C.C.: "Potent and specific genetic interference by double -stranded RNA in Caenorhabditis elegans". *Nature*, 1998, *391*, 666.

[17] Bobbin M.L., Rossi J.J.: "RNA Interference (RNAi)-Based Therapeutics: Delivering on the Promise". *Annu. Rev. Pharmacol. Toxicol.*, 2016, *56*, 103.

[18] Moon S.L., Dodd B.J., Brackney D.E., Wilusz C.J., Ebel G.D., Wilusz J.: "Flavivirus sfRNA suppresses antiviral RNA interference in cultured cells and mosquitoes and directly interacts with the RNAi machinery". *Virology*, 2015, *485*, 322.

[19] Stebbing J., Zhang H., Xu Y., Grothey A., Ajuh P., Angelopoulos N., Giamas G.: "Characterization of the Tyrosine Kinase-Regulated Proteome in Breast Cancer by Combined use of RNA interference (RNAi) and Stable Isotope Labeling with Amino Acids in Cell Culture (SILAC) Quantitative Proteomics". *Mol. Cell. Proteomics*, 2015, *14*, 2479.

[20] Agudo D., Gómez-Esquer F., Martínez-Arribas F., Núñez-Villar M.J., Pollán M., Schneider J.: "Nup88 mRNA overexpression is associated with high aggressiveness of breast cancer". *Int. J. Cancer*, 2004, *10*, 717.

[21] Schneider J., Martnez-Arribas F., Torrejon R.: "Nup88 Expression is Associated With Myometrial Invasion in Endometrial Carcinoma". *Int. J. Gynecol. Cancer*, 2010, *20*, 804.

[22] Zhao Z.R., Zhang Z.Y., He X.Q., Hu Y.M., Tian Y.F., Zhang L.J., Sun X.F.: "Nup88 mRNA overexpression in colorectal cancers and relationship with p53". *Cancer Biomark.*, 2010, *8*, 73.

[23] Cheng Y.T., Germain H., Wiermer M., Bi D., Xu F., García A.V.: "Nuclear pore complex component MOS7/Nup88 is required for innate immunity and nuclear accumulation of defense regulators in Arabidopsis". *Plant Cell*, 2009, *21*, 2503.

[24] Shu W.X., Chen Y., He J.: "Effects of gambogic acid on the regulation of nucleoporin Nup88 in HL-60 cells". *Zhonghua Zhong Liu Za Zhi*, 2008, *30*, 484.

[25] Andres-Hernando A., Lanaspa M.A., Rivard C.J., Berl T.: "Nucleoporin 88 (Nup88) is regulated by hypertonic stress in kidney cells to retain the transcription factor tonicity enhancer-binding protein (TonEBP) in the nucleus". *J. Biol. Chem.*, 2008, *283*, 25082.

Corresponding Author:
ZHITU ZHU, M.D.
Department of Oncology
The First Affiliated Hospital of
Jinzhou Medical University
No. 2, Section 5, Renmin Street, Guta District
Jinzhou City, Liaoning Province 121000 (China)
e-mail: cnzhituzhu@163.com