Chem. Res. Chin. Univ., 2017, 33(3), 388—391 doi: 10.1007/s40242-017-7090-1

In vivo Inhibitory Effect of Lentivirus-mediated RNA Interference Targeting RhoC on Growth of SKOV3 Cells

PAN Ying, WANG Ke, LIU Yichen, QIN Rui, CAO Lu, WANG Jia,
ZHOU Guanghong and ZHANG Aichen*
China-Japan Union Hospital of Jilin University, Changchun 130031, P. R. China

Abstract To investigate the inhibitory effect of lentivirus-mendiated RNA interference targeting RhoC on the growth of SKOV3 cells(ovarian cancer SKOV3 cells) *in vivo*, the vector expressing RNA interference targeting RhoC gene(LV-shRhoC) was constructed and the virus particles were packaged. The infection effiency of SKOV3 cells by the virus was estimated by green fluorescent protein expression on a fluorescence microscope and the expression of RhoC gene in the SKOV3 cells was detected by reverse transcription real time polymerase chain reaction(PCR). Furthermore, human ovarian cancer SKOV3 cells, empty vector infected SKOV3 cells and interfered-vector infected SKOV3 cells were respectively seeded into nude mice, and the shape, mass, volume and histophathological changes of the transplanted tumors were observed 20 d later the mice were sacrified. The results show that lentivirus packaging particles can effectively infect SKOV3 cells and the lentivirus-mediated RNA interference can significantly inhibit the expression of RhoC gene in SKOV3 cells, the mass and volume of the transplanted tumor in the mice of the specific-control group(Lv-shRhoC) are all lower than the corresponding ones in the mice of negative- and blank-control groups(Lv-NC and SKOV3). Moreover, the histopathlosical secion investigation shows that the nuclear Karyotype and histopathologic mitotic figure of SKOV3 cells in mice of the specific-control group are clearly lower than those in the mice of the negative-control group.Thus it is concluded that silencing RhoC gene by means of lentivirus-mediated RNA interference targeting RhoC can obviously inhibit the growth of ovarian cancer cells(SKOV3) *in vivo*, which is a new strategy for the gene therapy of ovarian cancers.

Keywords Ovarian; RhoC gene; Lentivirus vector; RNA interference

1 Introduction

Ovarian carcinoma is a common cancer of the female reproductive systems, which is seriouly harmful to the health of the majority of female compatriots. Clinically, surgery was adopted to cure oophoroma followed by radiotheraphy and chemotherapy at present. Since a wealth of clinical experience has been accumulated, a portion of ovarian cancer patients have been relieved from pain. However, the early diagnosis of the malignant tumor of ovarian cancer patients, the effects of treatment and the relapse rate after treatment as well as 5-year survival rate are not satisfactory[1,2]. With the studies of the oncogenesis of ovarian carcinoma and the ovarian cancer, gene therapy of cancer has become a new domain of exploring cancer treatment[3—5]. RhoC is a member of Rho(Ras-homologous) subfamily proteins and shows a high expression in ovarian cancer, which has been recognized by the majority of experts and scholars[6]. A lot of researches have confirmed that the high expression of RhoC gene in ovarian cancer is positively related to the invasive ability of malignant tumor[7,8]. Our previous studies also demonstrated that RhoC gene plays an extremely important role in the development of ovarian cancer cells. Furthermore, the silent eukaryotic expression vector of RhoC-miRNA was constructed and used to successively make the transfection of SKOV3 cells, and it was found that the cells transfected showed an obviously slow growth rate and an increase in cell apoptosis, which further indicated that RhoC gene possessed the characteristics of cancer gene at the cell level *in vivo*[9,10].

In this study, the authors have inhibited the expression of RhoC gene by means of lentivirus-mediated RNA interference targeting RhoC *in vivo* so as to lay a theoretical foundation for the gene therapy of ovarian cacinoma.

2 Experimental

2.1 Reagents

Fetal bovine serum(FBS) and high glucose Dulbecco's modified Eagle medium(DMEM) were purchased from Hyclone Company, TRIzol from Invitrogene Company and SYBR Prime Script Kit from TaKaRa Company.

2.2 Construction of Lentivirus Vector and Encapsulation of Virus

According to the sequence of DNA of *RHOC*(Genbank

*Corresponding author. E-mail: zhangaichen1213@126.com
Received March 9, 2017; accepted May 2, 2017.
Supported by the Project of the Department of Science and Technology of Jilin Province, China(No.20090747).

NO:NM_001042678.1), we designed the sequence of RNA interference target, on the basis of which the lentivirus-mediated vector was constructed. We investigated the lentivirus vector interfering the expression of RhoC gene and the encapsulation of virus(Guangzhou Funuo Biotechnology Co., China) and named the encapsulated virus vector as Lenti-shRhoC 1(RhoC silence vector 1), Lenti-shRhoC 2(RhoC silence vector 2) and Lenti-NC(contrast vector), respectively.

2.3 Cells Culture

The human SKOV3 cells purchased from Shanghai Cell Band of Chinese Academy of Sciences were incubated in DMEM supplemented with 10% fetal bovine serum at 37 °C in a humidified atmosphere with 5% CO_2 in an incubator.

2.4 Lentivirus Lenti-shRhoC of SKOV3 Cells and Infection of Lenti-NC

SKOV3 cells were seeded at a density of 1×10^5 cell/well in a 6-well plate and incubated for 48 h, then the medium was discarded and 1 mL of fresh medium and 20 μL of the encapsulated virus(1×180 pfu/mL) were added to the plate in turn. After 48 h, fluorescence microscope observation was carried out on an inverted microscope for the 3 groups, *i.e.*, transfected Lenti-shRhoC cells named interfered group, transfected Lenti-NC named negative control group and untransfected cells named blank control group.

2.5 Expression of RhoC Gene

Total RNAs were extracted from three groups of cell samples(specific-control, negative-control and blank-control groups), respectively, with TRIZOL agent and reversely transfected into cDNA then to detect RhoC gene expression *via* real-time PCR using SYBR Prime Script RT-PCR Kit. The primer for the PCR amplification of RhoC gene expression was upstream primer 5′-ACCTGCCTCCTCATCGTCTTC-3′ and downstream primer 5′-CACCTGCTTGCCGTCCACC-3′ with a length of 105 bp of PCR product, and the primer for the PCR amplification of inner reference gene GAPDH was upstream primer 5′-TGCACCACCAACTGCTTAGC-3′ downstream primer 5′-GGCATGGACTGTGGTCATGAG-3′ with a lenth of 87 bp of PCR product. The gene amplification was performed by ABI7500fast. The conditions for PCR were (1) 95 °C 30 s; (2) 40 cycles of reaction at 95 °C 5 s and 60 °C 30 s; (3) 95 °C 15 s, 60 °C 60 s, 95 °C 15 s. The relative quantity was calculated by virtue of $-2^{\Delta\Delta T}$ method and the data analysis was automatically completed with the software SPSv1.40.

2.6 Construction and Tumorigenicity of Subcutaneous Transplanted Oncoma of Human Ovarian Cancer in Nude Mice

2.6.1 Preparation of Tumor-bearing Mice

At 12 h prior to seeding, the culturing media of SKOV3 cells grown in the logarithmic phase, transfected Lenti-NC cells and Lenti-shRhoC cells were replaced with the fresh culturing medium and then they were digested with a pancreatin into monoplast suspensions, respectively. Then the cells of the 3 groups were separately collected by means of centrifugation at 1000 r/min and washed twice with phosphate buffer solution (PBS) and resuspended to adjust to a density of 5×10^6 cell/mL, which were respectively injected into 10 mice in each group subcutaneously at a level of 10^6 cells(about 0.2 mL) with a 1 mL-injector.

2.6.2 Measurement of Oncoma Volume

Having been seeded for 20 d, the tumor-bearing mice were sacrificed and the whole transplant oncoma tissue was ablated as quickly as possible. After the length and width of the maximum area of the above mentioned tumors were measured with a vernier, the tumor volume was calculated with the aid of equation $V=ab^2/2$, where a is the length of the tumor and b is the width of it. The ablated oncoma was arrested in a 10% formalin solution for hematoxylin and eosin(HE) staining.

2.6.3 Histopathological Observation of the Transplanted Oncoma

The oncoma tissues arrested in 10% formalin were conventionally embedded with paraffin and then cut into sections with a thickness of 5 μm that were respectively soaked in dimethylamine twice for 10 min and then dewaxed so as to make them transparent, followed by dewatering in gradient alcohol for 3—5 min and thorough washcouting with distilled water for 10 min and blotting. Then the sections were stained in a hematoxylin solution for 3 min and blotted with blotting paper. After that, the sections were soaked again for 3—5 s and washed with water and put into water at 60 °C, at which they were kept for 10 min to lead to the recovery of the colour and blotted to dry, followed by staining in an eosin solution for 25 s and drying. Finally, the dried sections were submitted to neutral gum mounting and histopathological observation under a microscope.

2.7 Statistical Analysis

The data were statistically analyzed with the software SPSS19.0, the oncoma volume was expressed as averaged value±standard error($\overline{X}\pm S$), the comparison between the groups was submitted to t test and the enumeration data were subject to Chi-square test, with $P<0.05$ being statistical significant.

3 Results

3.1 Construction of Lentivirus-mediated RNA Interference Targeting RhoC Vector

Based on the cDNA sequence of gene *RHOC* and design software of RNA interference, the interfering target sequence of gene *RHOC* was designed to 2 strands, the first strand with the sequence of gacctgcctcctcatcgtc, the limen of which is located at the 387th ribonucleotide of the cDNA ribonucleotide sequence, and the second strand with the sequence of cgaaccggatcagtgcctt, the limen of which is located at the 776th ribonucleotide of the cDNA ribonucleotide sequence. The above-mentioned 2 strands were respectively cloned into Lentivirus psiHIV-H1 vector(Fig.1) and named as psiHIV-RhoC1

and psiHIV-RhoC2, separately.

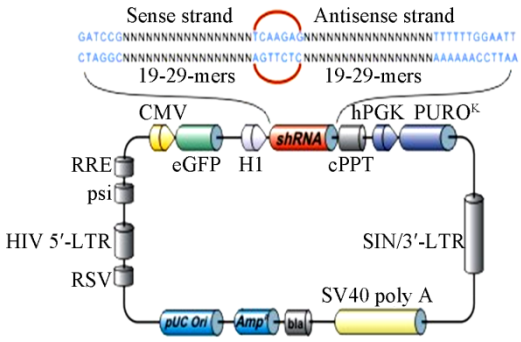

Fig.1 Construction of psiHIV-H1 vector

3.2 Vector with Lentivirus Embedded to Infect SKOV3 Cells

Having been infected with the vector embedding Lentivirus for 48 h, SKOV3 cells were observed under a fluorescence microscope, indicating that SKOV3 cells affected by Lentivirus all show fluorescence due to the expression of protein, while SKOV3 cells uncommunicated by Lentivirus do not show fluorescence(Fig.2), proving the successful embedding of Lentivirus Lenti-shRhoC and its acting as an interfering vector, which can be integrated into SKOV3 genome of targeted cells so as to realize the stable expression of interfere sequence.

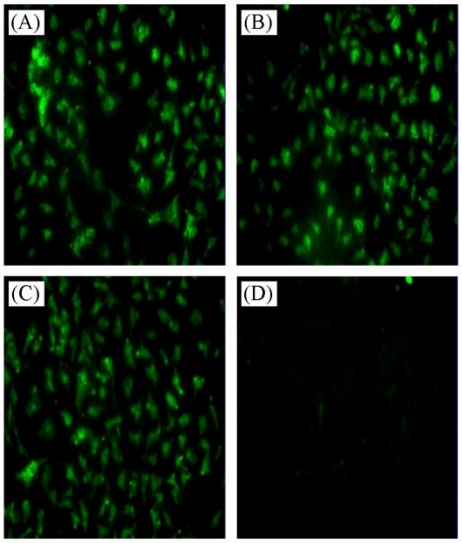

Fig.2 Fluorescence microscope photographs of SKOV3 cells infected by the packed lentiviral vectors(40×)

(A) psiHIV-RhoC1; (B) psiHIV-RhoC2; (C) negative control; (D) blank control.

3.3 Fluorescence Quantified Expression of RhoC Gene by RT-PCR

The amplification results of RhoC gene expression by means of RT-PCR show that the expression of RhoC gene in psiHIV-RhoC1 group is down regulated by a factor of more than 100 compared with that in the blank-control group, and the expression of RhoC gene in psiHIV-RhoC2 group is down regulated by a factor of more than 100 compared with that in the blank-control group, indicating that the expression efficiency of RhoC mRNA in SKOV3 cells of RhoC silent group is obviously decreased compared with that of the blank-control group. The expression efficiency of RhoC mRNA in SKOV3 cells in the negative-control group does not change compared with that in the blank-control group and there is no statistical difference between the two groups[Fig.S1(see the Electronic Supplementary Material of this paper and Fig.3)]. These results show that Lentivirus-mediated RNA interference targeting RhoC significantly reduces the expression of RhoC mRNA in SKOV3 cells.

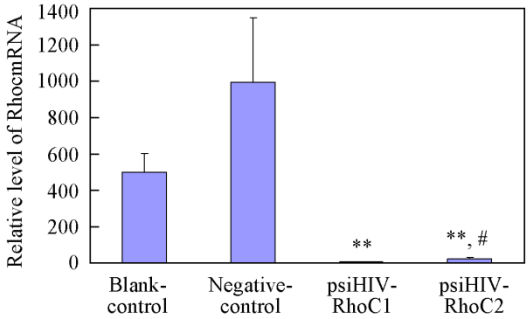

Fig.3 RhoC mRNA expression by siRNA in SKOV3 cells

n=3. ** Compared with blank control group, P<0.01; # compared with psiHIV-RhoC1 group, P<0.05.

3.4 Mass and Volume of the Hypodermic Transplanted Oncoma from Sacrificed Mice

The mass and volume of the tumor tissues were all obviously lower in the specific-control group than the corresponding ones in the negative- and blank-control groups (Lenti-NC and SKOV3), with P<0.01, being statistically significant, while the comparison of the above-mentioned parameters between the latter two groups shows no statistical difference, with P>0.05(Fig.4).

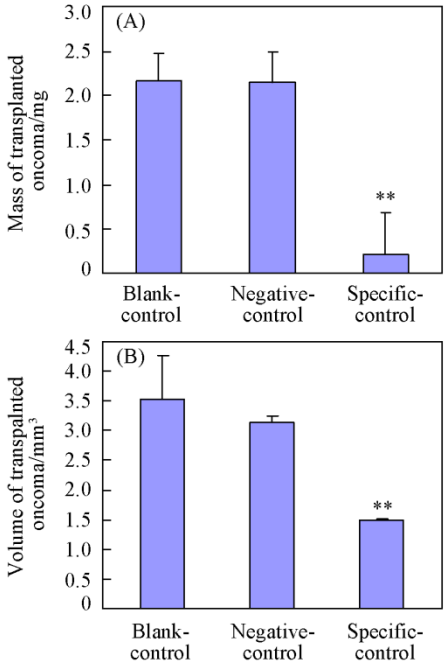

Fig.4 Mass(A) and volume(B) of transplant oncoma in each group of mice

** Compared with blank control group, P<0.01.

3.5 Histopathological Observation of Hypodermic Transplanted Tumors in Nude Mice

After the ablated hypodermic transplanted tumor tissues were dissected along the diameters of them, it was visible to the naked eye that the transplanted tumors in the blank- and negative-control groups were all growing in the manner of hump or leaf shapes, with texture somewhat hard and dissection plane grey.

Having been stained in HE solution, the sections of hypodermic transplanted tumors were observed under a microscope, showing that the shapes of the cells of the transplanted oncoma are different in size, with the nuclei large and deeply-stained, atypia being obvious and histopathological figures. Thus they are defined as malignant tumors(Fig.5).

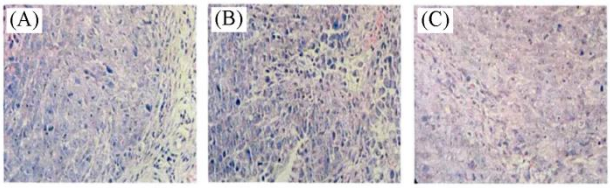

Fig.5 Pathological observation of transplanted tumor of the three groups of nude mice by HE staining(200×)

(A) Specific-control group(Lenti-shRhoC group); (B) negative-control group(Lenti-NC group); (C) blank-control group(SKOV3 group).

4 Discussion

With the help of multiple downstream molecules to regulate the activity of cytoskeleton, RhoC participates in cell migration so as to change the reconstruction of extracellular matrix and basement membrane and thus to further adjust the invasiveness and metabasis of tumors[11,12]. RhoC has become a new target for the treatment of ovarian cancers[13]. Lentivirus-mediated RNA interference targeting RhoC to inhibit the expression of RhoC gene has been considered as an effective measure for the gene therapy of tumors[14,15]. We adopted Lentivirus-mediated RNA interference targeting RhoC gene to construct the affecting vector to encapsulate Lentivirus that infected ovarian cancer SKOV3 cells, followed by the observation of SKOV3 cells infected by the packed Lentivirus vector with an inverted fluorescence microscope. The results proved that in the SKOV3 cells infected by the packed Lentivirus, the interfering RNA was efficiaciouslly espressed and the fluorescence quantification of RhoC gene expression by virtue of RT-PCR confirmed that specific Lentivirus-mediated RNA interference effectively inhibited the RhoC mRNA expression in SKOV3 cells. Thus it was clear that Lentivirus-mediated RNA interference targeting RhoC could significantly suppress the expression of RhoC gene in ovarian cancer SKOV3 cells.

On the basis of the experiments, we prepared the explanted tumors in nude mice with the help of Lentivirus-mediated RNA interference targeting RhoC and investigated the inhibition effect of silenced RhoC gene on the growth of the transplanted tumors of SKOV3 cells. The experimental results showed that the growth of the reimplantation tumor in the mice of specific-control group was obviously inhibited, with the tumorigenic rate, mass and volume of the transplanted tumor all lower than the corresponding ones in the mice of negative- and blank-control groups. The histopathologic observation of the sections of the transplanted tumors of the nude mice showed that in the negative- and blank-control groups, the cells of the transplanted tumors of ovarian cancer cells(SKOV3) were different in shape and size, the nuclei were large and deeply-stained, and the cells showed pathological mitotic figures, while in the specific-control group, there were no above-mentioned phenomena, which might be explained by the assumption that in the specific-control group, the RhoC gene was so considerably inhibted that the capabilities of the metastasis and invasion of the cancer cells were reduced. Thus it was further confirmed that Lentivirus-mediated RNA interference targeting RhoC to silence RhoC gene could significantly inhibit the growth of ovarian cancer cells, which can be a novel protocol for the gene treatment of cancers.

Electronic Supplementary Material

Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s40242-017-7090-1.

References

[1] Sapiezynski J., Taratula O., Rodriguez L., Minko T., *J. Control Release*, **2016**, *243*, 250

[2] Ren F., Shen J., Shi H., Hornicek F. J., Kan Q., Duan Z., *Biochim. Biophys. Acta*, **2016**, *1866*(2), 266

[3] Tait D. L., Obermiller P. S., Jensen R. A., Holt J. T., *Hematol Oncol. Clin. North Am.*, **1998**, *12*(3), 539

[4] Casado E., Nettelbeck D. M., Gomez-Navarro J., Hemminki A., Gonzalez B. M., Siegal G. P., Barnes M. N., Alvarez R. D., Curiel D. T., *Gynecol. Oncol.*, **2001**, *82*(2), 229

[5] Park J. W., Kim M., *Eur. J. Gynaecol Oncol.*, **2016**, *37*(3), 295

[6] Ridley A. J., *J. Microsc.*, **2013**, *251*(3), 242

[7] Zhao Y., Zong Z. H., Xu H. M., *Gynecol. Oncol.*, **2010**, *116*(3), 563

[8] Han Z. Q., Zhang A. L., Wu M. F., Liu Y. L., Chen G., Li F. J., Gao Q. L., Liao G. N., Lu Y. P., Wang S. X., Ma D., *Chinese Journal of Oncology*, **2004**, *26*(7), 385

[9] Pan Y., Du Z. W., Leng W. C., Zhou J. W., Wang Y. J., Sheng M. J., Wang J. R., Zhang G. Z., *Chem. Res. Chinese Universities*, **2011**, *27*(1), 70

[10] Pan Y., Zhang W. Y., Zou J. Y., Sheng M. J., Xuan L. L., Hai D. Y., *Chinese Medical Journal*, **2009**, *89*(21), 1498

[11] Gou W. F., Zhao Y., Lu H., Yang X. F., Xiu Y. L., Zhao S., Liu J. M., Zhu Z. T., Sun H. Z., Liu Y. P., Xu F., Takano Y., Zheng H. C., *BMC Cancer*, **2014**, *14*, 477

[12] Chen X., Chen S., Xiu Y. L., Sun K. X., Zong Z. H., Zhao Y., *Mol. Cancer*, **2015**, *14*, 31

[13] Sang X. B., Sun K. X., Wang L. L., Chen S., Wu D. D., Zong Z. H., Zhao Y., *Oncol. Rep.*, **2016**, *36*(6), 3267

[14] Wang H., Zhao G., Liu X., Sui A., Yang K., Yao R., Wang Z., Shi Q., *Exp. Clin. Cancer Res.*, **2010**, *29*, 123

[15] Kaushal N., Durmaz Y. Y., Bao L., Merajver S. D., Elsayed M. E., *Mol. Pharm.*, **2015**, *12*(7), 2406