Lentivirus-mediated shRNA interference targeting vascular endothelial growth factor inhibits angiogenesis and progression of human pancreatic carcinoma

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Abstract. Angiogenesis is known to be essential to the survival, growth, invasion and metastasis of cancer cells. Vascular endothelial growth factor (VEGF) is an important factor regulating tumor angiogenesis. In the present study, we analyzed the effect of lentivirus-mediated shRNA interference targeting vascular endothelial growth factor (VEGF) on angiogenesis and progression in the pancreatic cancer cell line Patu8988 *in vitro* and *in vivo*. The study aimed to construct a recombinant lentivirus carrying targeted VEGF shRNA (LV-RNAi) to be used to transfect Patu8988 cells, and we investigated its anti-angiogenic and growth inhibitory effects on pancreatic cancer. VEGF expression was measured by RQ-PCR, western blotting and enzyme-linked immunosorbent assay (ELISA). In subcutaneous transplantation models, tumor volumes were determined, and the expression levels of VEGF and CD34 were assessed by immunohistochemistry. Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) was used to determine apoptosis. In the orthotopic transplantation models, tumor volume and liver metastasis were determined. We successfully constructed LV-RNAi and confirmed that it knocked down the VEGF gene at the mRNA and protein levels in Patu8988 cells. In the subcutaneous transplantation models, tumors with low levels of VEGF expression exhibited reduced pancreatic carcinoma angiogenesis and growth, and the apoptotic index was significantly higher. In the orthotopic transplantation models, tumors with low levels of VEGF expression exhibited significantly reduced pancreatic carcinoma growth, but no significant difference was observed between the three mouse groups, LV-RNAi, LV-NC and the control, in regards to liver metastasis. In summary, lentivirus-mediated RNAi silencing of VEGF inhibited tumor angiogenesis and growth, and increased apoptosis of the pancreatic cancer cell line Patu8988. VEGF targeted gene silencing approach has the potential to serve as a novel treatment for pancreatic cancer.

Introduction

Pancreatic cancer is a highly lethal disease that is usually diagnosed at an advanced stage for which there is little or no effective therapy. It remains the fourth most common cause of cancer-related death in the Western world (1). Due to the aggressive natural history of this disease, most patients with pancreatic cancer present with local invasion or distant metastasis at the time of diagnosis, and less than 20% of patients are candidates for surgery with curative intent (2). The overall 5-year survival rates are reported to be below 5% (3). No adjuvant treatments have shown efficacy in improving survival to date. Thus, new approaches including gene therapy are definitely required to improve treatment results (4,5).

Angiogenesis is necessary for successful tumor growth (6,7), and inhibition of VEGF represents the most validated anti-angiogenic approach described thus far (8,9). VEGF is a key stimulating factor for angiogenesis of cancer, and it contributes to the malignant development and metastasis of tumors through many processes. In addition, VEGF is highly expressed in most human tumors (10-14).

RNA interference (RNAi) has emerged as a powerful tool to induce lose-of-function phenotypes by post-transcriptional silencing of gene expression (15,16). Lentiviral vectors have provided a huge advance in technology and offer the means to achieve significant levels of gene transfer *in vitro* and *in vivo* (17,18).

In this study, we used the lentiviral vector mediating RNAi to deliver a specially designed small hairpin RNA for the human VEGF gene (LV-RNAi) into pancreatic carcinoma cell line Patu8988 to observe the gene therapeutic effects on angiogenesis and progression.

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Materials and methods

Animals and cell lines. BALB/c nude mice were obtained from the Shanghai Experimental Animal Center (Shanghai, China) and maintained according to guidelines of the Animal Research Committee of Soochow University (Suzhou, China).

Human pancreatic cancer cell line Patu8988 was provided by Professor Chang-Geng Ruan, Jiangsu Institute of Hematology, and maintained in RPMI (Roswell Park Memorial Institute)-1640 medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) at 37˚C in a humidified atmosphere containing 5% CO_2. The cultures were passaged 2 or 3 times weekly to maintain log-phase growth.

Lentiviral vectors for VEGF shRNA. Small hairpin RNA (shRNA) targeting human VEGF (GenBank, NM 001025366) was designed as follows. The underlined part in the sense strand is the target sequence of the VEGF gene which is 19-bp long, the italicized characters in the sense strand is the loop sequence of the hairpin. According to the sense strand, the antisense was also synthetized: sense, 5'-GATCCC(G)CCATGAACTTTCTGCTGTC***TTGATATCCG***GACAGCAGAAAGTTCATGGTTTTTTCCAAC-3'; antisense, 3'-GG(C)GGTACTTGAAAGACGACAGAACTATAGGCCTGTCGTCTTTCAAGTACCAAAAAAGGTTGAGCT-5'.

The recombinant lentivirus gene transfer vector targeting VEGF pGCSIL-GFP-VEGF (LV-RNAi) encoding the green fluorescent protein (GFP) sequence was constructed and gifted by Dr L. Li followed by chemically synthesized shRNAs and lentiviral vector constructed as previously described (19,20). The targeting sequence of the shRNA was confirmed by sequencing. The lentiviral vector pGCSIL-GFP-Negative (LV-NC) containing an invalid RNAi sequence (GeneChem, Shanghai) was used to monitor non-specific responses caused by heterologous siRNA. The LV-RNAi and the LV-NC were prepared to $5x10^9$ Tu/ml (transfection units/ml).

Lentiviral vector transfection. Cells were subcultured at $5x10^4$ cells/well into 6-well tissue culture plates overnight. The viral supernatant was then added into cells at a multiplicity of infection (MOI) of 10 with ENi.S and 5 μg/ml Polybrene. The infected cells were considered to be the LV-RNAi and the LV-NC group, respectively, and the Patu8988 cells without infection were considered as the control group. Flow cytometry was used to detect the transfection efficiency, and fluorescence microscopy was used to observe the cells which released fluorescence. The three groups mentioned above were used in the experiments below.

Real-time quantitative RT-PCR. Total RNA was collected using TRIzol reagent following the manufacturer's instructions. The concentration and purity of the total RNA were detected with an ultraviolet spectrophotometer and then reversely transcribed into cDNA with MMLV. Total RNA (2 μg) was converted to cDNA in 40 μl and stored at -20˚C until use. The transcriptional level of VEGF was analyzed using the MJ Research DNA Engine Opticon 2 System with SYBR-Green fluorochrome. The GAPDH gene was used as an internal control. PCR was carried out with cDNA derived from 50 ng of RNA, 1 unit Taq polymerase and reaction kits in a final volume of 25 μl. Each cycle of PCR included 15 sec of denaturation at 95˚C, 20 sec of primer annealing at 58˚C and 20 sec of extension/synthesis at 72˚C. The primer sequences were as follows: 5'-GCTTTACTGCTGTACCTCCAC-3' (sense) 5'-TCCAGGGCTTCATCGTTA-3' (antisense) for VEGF (239 bp); 5'-GCAAGTTCAACGGCACAG-3' (sense) 5'-GCCAGTAGACTCCACGACAT-3' (antisense) for GAPDH (140 bp).

Western blotting. Cells were washed twice and lysed on ice. After centrifugation, the supernatants were collected. Protein concentrations were determined using the Bio-Rad DC Protein Assay system. The β-actin gene was used as an internal control. The following steps were as previously described (20).

Detecting VEGF levels in culture supernatants by ELISA. Cells were seeded in new cell culture bottles, and after a 72-h culture supernatants were collected and cell counting was performed. The expression of VEGF in the collected supernatants was tested by a human VEGF ELISA kit (R&D Co.) according to the handbook. VEGF concentration/cell count was considered as the VEGF expression level. VEGF expression level in samples of the control group was considered as 1, and the expression of VEGF in the other groups was calculated by comparison to the control. Duplicate wells were set, and the entire experiment was repeated twice.

Subcutaneous transplantation model. A total of 18 BALB/c-nu mice, 5-weeks old and 20-24 g in weight, were bred in a specific pathogen-free (SPF) condition and maintained at a constant humidity and temperature (25-28˚C). All mice underwent subcutaneous injection of a 200-μl cell suspension of Patu8988 cells ($1.0x10^7$) in the infra-axillary region, respectively. Two weeks later, the animals were randomly divided into three groups and intratumorally injected only once with 400 μl normal saline, 400 μl LV-NC or 400 μl LV-RNAi, respectively. The size of the tumors was measured in a blinded manner once a week with calipers, and the volume was determined using the simplified formula of a rotational ellipsoid ($L \times W^2 \times 0.5$).

Immunohistochemical staining. Tumors were harvested from mice 5 weeks after treatment, and VEGF expression and microvessel density (MVD) of the tumor specimens were determined by immunohistochemistry. The tissue specimens fixed with formalin solution were embedded in paraffin wax, serially sectioned at 4 μm and immunohistochemically stained using the SP method according to the manufacturer's instructions for the SP kit. The primary antibodies were diluted to 1:50 for VEGF (as mentioned above) and 1:100 for CD34 (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Rectal cancer slides served as the positive control, and PBS was used to replace the primary antibodies to serve as the negative control. The expression of VEGF protein was scored semi-quantitatively. Sections were then evaluated for the presence of brown diaminobenzidine precipitates indicative of positive reactivity by microscopy. Ten visual fields (magnification, x200) were counted for each section. The brown staining in or around the nucleus was considered as positive reactivity for VEGF. CD34 is used as a biomarker in endothelial cells for the identification of new blood microvessels. One lumen of blood

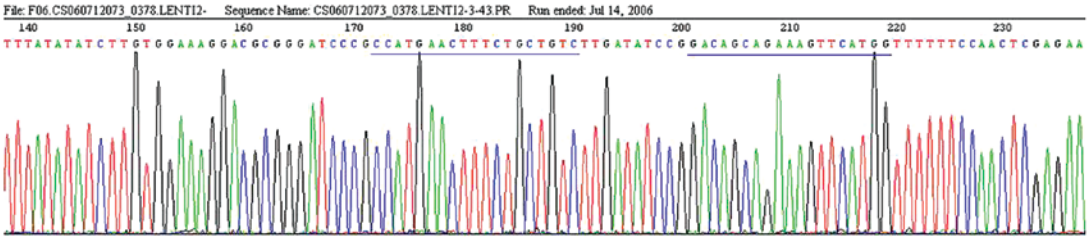

Figure 1. Part of the sequencing result for the recombinant vector LV-RNAi. The underlined part shows that the target sequences were constructed to the virus successively.

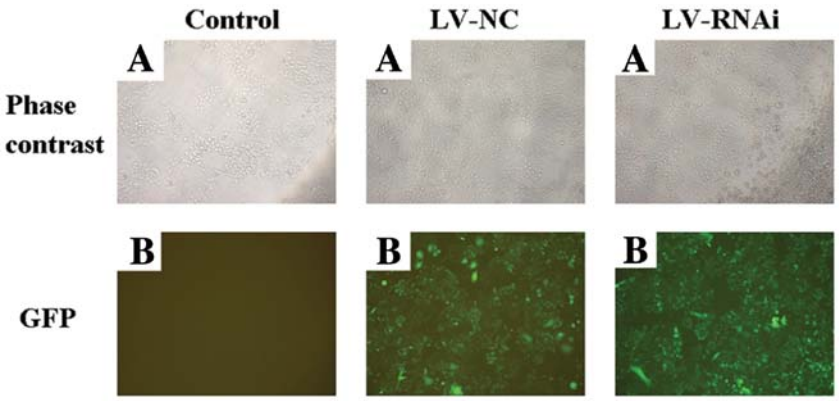

Figure 2. Transfection efficiency as assessed by fluorescence microscopy. Patu8988 cells were transfected with the lentivirus at MOI of 10 for 120 h. (A) Phase contrast and (B) GFP expression of the control, LV-NC and LV-RNAi groups were assessed under a fluorescence microscope (original magnification, x100).

vessels was assessed as one new blood capillary. The MVD value was determined based on Weidner's method (21).

Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay. Apoptotic tumor cells were detected with the TUNEL method, using an *in situ* cell death detection kit (Roche Diagnostics, Mannheim, Germany). The assay was performed according to the manufacturer's instructions. Briefly, after routine deparaffinization and treatment with H_2O_2 (3%), sections were digested with proteinase K (20 μg/ml, pH 7.4, 12 min) at 25˚C and incubated with the reaction mixture (1:40, 60 min) at 37˚C. Incorporated fluorescein was detected with horseradish peroxidase after a 30-min incubation at 37˚C and subsequently dyed with DAB. Cell with brown-colored nuclei were assessed as positive apoptotic cells, and the number of apoptotic cells counted for 1,000 tumor cells in one section for at least 5 high power fields, was scored as the apoptotic index (AI).

Orthotopic transplantation pancreatic cancer model. The establishment of the infra-axillary subcutaneous transplantation tumor model was performed as described in the 'Subcutaneous transplantation model' section. After reaching a specific volume, the tumors were resected under aseptic environment and washed twice in antibiotic-containing RPMI-1640 to prevent possible infection. Necrotic tissues were removed, and the remaining viable tumor tissues were cut into small pieces of 1 mm^3. Five-week-old BALB/c-nu mice, weighing 20-24 g, were anesthetized with urethane (4 ml/kg) by intramuscular injection. After the abdominal skin was sterilized, an incision was made in the upper left abdomen and the pancreas was exposed. Tumor pieces were attached to the pancreas using absorbable sutures. The pancreas was then returned to the peritoneum, and the abdominal wall and the skin were closed with silk sutures, respectively. The animals were allowed to recover for 24 h. Eighteen surviving mice were randomly divided into three groups (n=6) and intraperitoneally injected only once with 400 μl normal saline, 400 μl LV-NC or 400 μl LV-RNAi, respectively. All of the mice were sacrificed 6 weeks after treatment. Tumors were harvested from mice, and the volume was determined as previously described. Liver metastasis was also observed.

Statistical analysis. Statistical analysis was carried out using SAS 9.0 statistical software. Data are presented as the means ± standard deviation (SD). The Student's t-test or ANOVA was used to compare the means of different groups. Chi-square test was used to compare categorical variables and clinical pathological correlation. The relationships among VEGF and MVD were investigated by Spearman-rank correlation. A $P<0.05$ was considered to indicate a statistically significant difference.

Results

Sequencing result and transfection efficiency of the lentiviral vector. The result of sequencing for the recombinant vector confirmed that the target sequences were constructed to the lentivirus system pGCSIL-GFP successively (Fig. 1). We used a lentiviral vector system to express shRNAs directed against

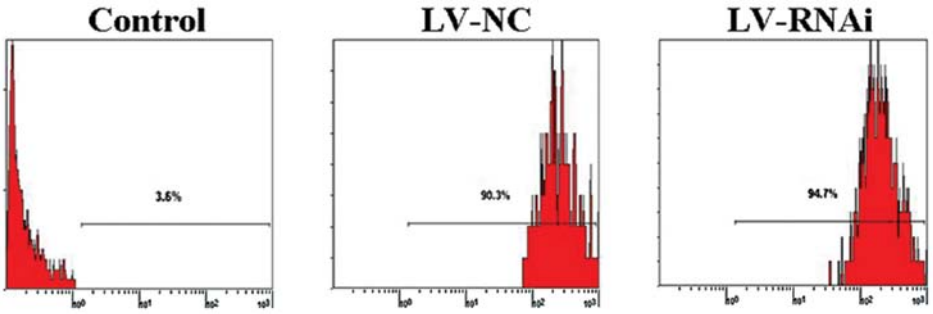

Figure 3. Transfection efficiency as assessed by flow cytometry. Patu8988 cells were transfected with the lentivirus. GFP expression was assessed using flow cytometry. The high and stable transfection of lentiviral vector was confirmed.

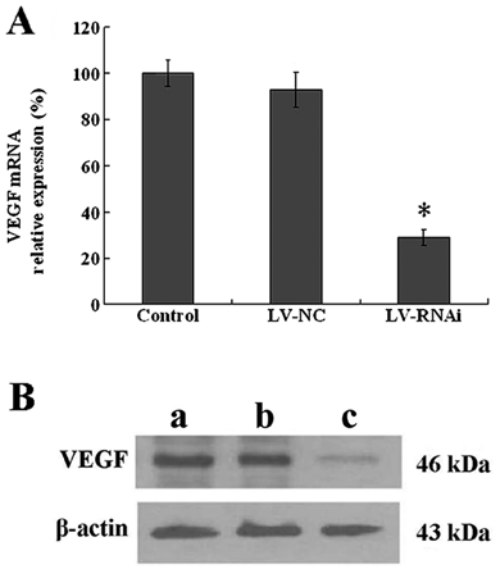

Figure 4. Knockdown effect of VEGF mRNA and protein expression by real-time PCR and western blot analysis. (A) Relative level of VEGF mRNA expression was analyzed using the the $2^{-\Delta\Delta Ct}$ method. *P<0.05 vs. the control group. (B) Antibody specific for VEGF (molecular weight, 46 kDa) was used for the protein level change, while β-actin (molecular weight, 43 kDa) was used as an internal control (a, control; b, LV-NC; c, LV-RNAi).

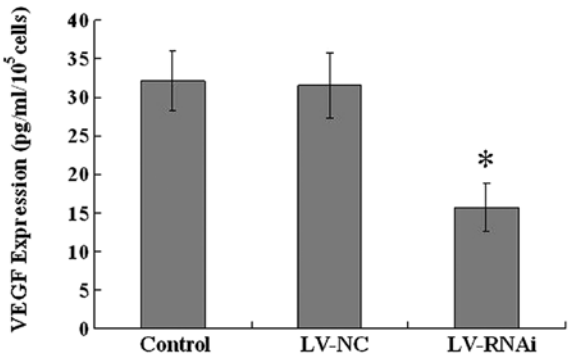

Figure 5. VEGF expression levels *in vitro* by ELISA assay. VEGF concentration/cell counting was considered as the VEGF expression level. The VEGF expression in culture supernatants of the LV-RNAi group was obviously downregulated vs. the control group (*P<0.05).

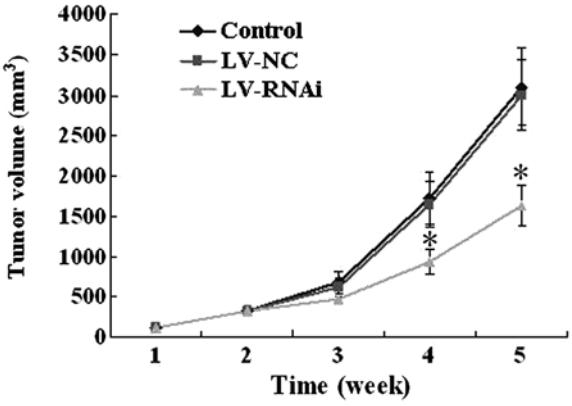

Figure 6. Inhibition of tumor growth in the subcutaneous transplantation model. The tumor volume was detected weekly following treatment. The tumor volume of the LV-NC group was obviously smaller (*P<0.05) than the tumor volume of the other two groups from week 4.

VEGF. In addition, GFP was as used as a reporter gene. After a single exposure of Patu8988 cells to the encoding lentivirus at MOI of 10 for 120 h, the cells were examined by fluorescence microscopy (Fig. 2) and flow cytometry (Fig. 3). A high percentage (>90%) of transfectants expressed GFP, indicating high and stable transfection of the lentiviral vector system.

Assessment of the VEGF silencing effect by RQ-PCR and western blotting. To detect the effect of VEGF silencing, real-time RQ-PCR and western blot analysis were performed to determine the mRNA and protein levels of VEGF following transfection. As shown in Fig. 4A, the relative VEGF mRNA expression (0.29±0.04, P<0.05) was significantly decreased in the LV-RNAi group compared with the LV-NC (0.93±0.07) and the control group (1.00±0.06), while no significant differences were noted between the LV-NC and control group (P>0.05). As shown in Fig. 4B, a 46-kDa protein band, VEGF protein, was detected in the control and the LV-NC group, but was weakly expressed in the LV-RNAi group.

VEGF expression levels in vitro. To detect the downregulation effect on VEGF expression, ELISA assay was performed. VEGF concentration/cell counting was considered as the VEGF expression level (Fig. 5). The VEGF expression level in the culture supernatants of the LV-RNAi group was 15.7±3.06 pg/ml/10^5cells, and in comparison to the control group (32.16±3.90 pg/ml/10^5cells), it was obviously inhibited (P<0.05), with a high inhibition efficiency (51.18%). There

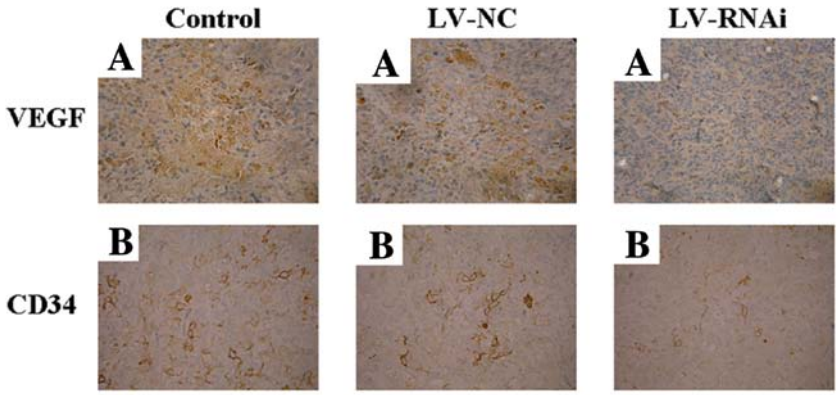

Figure 7. Expression of VEGF and CD34 in the subcutaneous transplantation model following treatment. The subcutaneously transplanted tumors of the control, LV-NC and LV-RNAi group were assessed by immunohistochemistry. (A) VEGF expression and (B) CD34 expression in tumor tissues (original magnification, x400). Expression of VEGF and CD34 in tumors of the LV-RNAi group was obviously downregulated when compared to the other two groups.

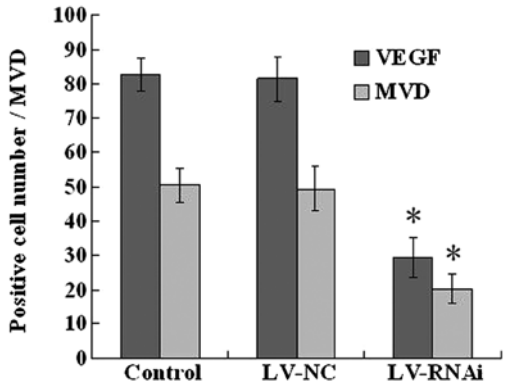

Figure 8. The positive cell number of VEGF and the MVD in the subcutaneous transplantation model after treatment. The expression of VEGF and the MVD value in tumors of the LV-RNAi group were obviously reduced (*P<0.05 vs. the control group).

were no significant differences between the LV-NC and the control group.

Tumor growth in the subcutaneous transplantation model. All of the 18 mice developed detectable tumors at the beginning of this experiment. Inhibition of growth was observed more significantly in mice after treatment with LV-RNAi for 5 weeks, when compared to the LV-NC (3,000±430 mm^3) or control group (3,100±480 mm^3). The average tumor volume (1,630±250 mm^3) in the LV-RNAi group was significantly lower than the other two groups (P<0.05) (Fig. 6). No significant differences were noted between the LV-NC and the control group.

Evaluation of expression of VEGF and CD34 by immunohistochemistry. In order to demonstrate the mechanism of the anti-angiogenic effect of LV-RNAi, the expression of VEGF and CD34 was assessed by immunohistochemistry in the nude mouse transplanted tumors after treatments. The LV-RNAi group exhibited downregulation of VEGF expression and a decrease in the MVD when compared to the LV-NC and control groups (P<0.05) (Figs. 7 and 8). There were no significant differences between the LV-NC and control group (P>0.05).

Apoptosis assay by TUNEL staining. The number of positive apoptotic tumor cells exhibiting brown nuclei in the TUNEL assay was determined (Fig. 9). Based on the TUNEL assay, we found that increased numbers of apoptotic pancreatic carcinoma cells were present in the subcutaneously transplanted tumors treated with LV-RNAi. When compared to the LV-NC (0.047±0.020) or control group (0.044±0.014), the AI (0.254±0.029) in the LV-RNAi group was significantly higher than that in the former two groups (P<0.05) (Fig. 10). These results indicate that inhibition of VEGF gene expression caused apoptotic cell death in pancreatic carcinoma cells *in vivo*.

Tumor growth and liver metastasis in the orthotopic transplantation pancreatic cancer model. All of the 18 mice developed orthotopic transplantation pancreatic carcinoma tumors in this experiment (Fig. 11A). Liver metastasis was detected (Fig. 11B) in the orthotopic transplantation pancreatic cancer model. Inhibition of growth was significantly observed in mice following treatment with LV-RNAi for 5 weeks, when compared to the mice treated with LV-NC (197±49 mm^3) or normal saline (213±52 mm^3). The average tumor volume (47±22 mm^3, P<0.05) in the former group was significantly lower than that in the latter two groups (Fig. 11C). The number of cases of liver metastasis in the LV-RNAi group (1/6, 16.67%) was less than that in the LV-NC (3/6, 50.00%) or the control group (4/6, 66.67%), while there was no significant differences between the LV-NC and the control group (P=0.58).

Discussion

Pancreatic adenocarcinoma is one of the deadliest human malignancies, accounting for more than 20% of gastrointestinal cancer-related deaths (22,23). At the time of diagnosis, the disease has often progressed to an advanced stage at which surgical resection is often not a viable option and at which time tumors are highly resistant to conventional chemotherapy

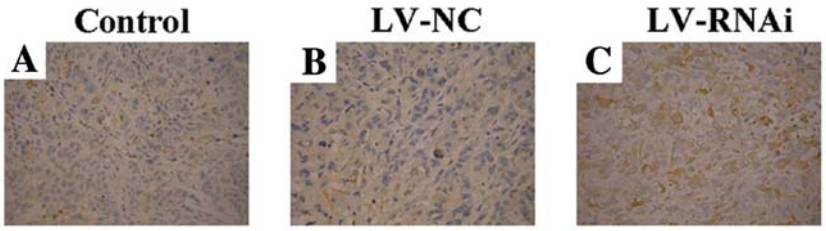

Figure 9. Apoptosis assay of the subcutaneously transplanted tumors by TUNEL staining. The number of positive apoptotic tumor cells with brown nuclei in the LV-RNAi group (C) was significantly increased when compared to the numbers in the (A) control and (B) LV-NC groups.

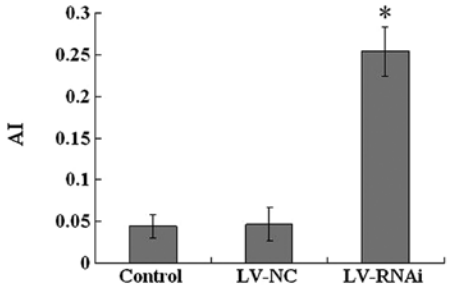

Figure 10. Apoptotic index (AI) of the subcutaneously transplanted tumors. AI in the LV-RNAi group was significantly higher than that in the other two groups (*P<0.05).

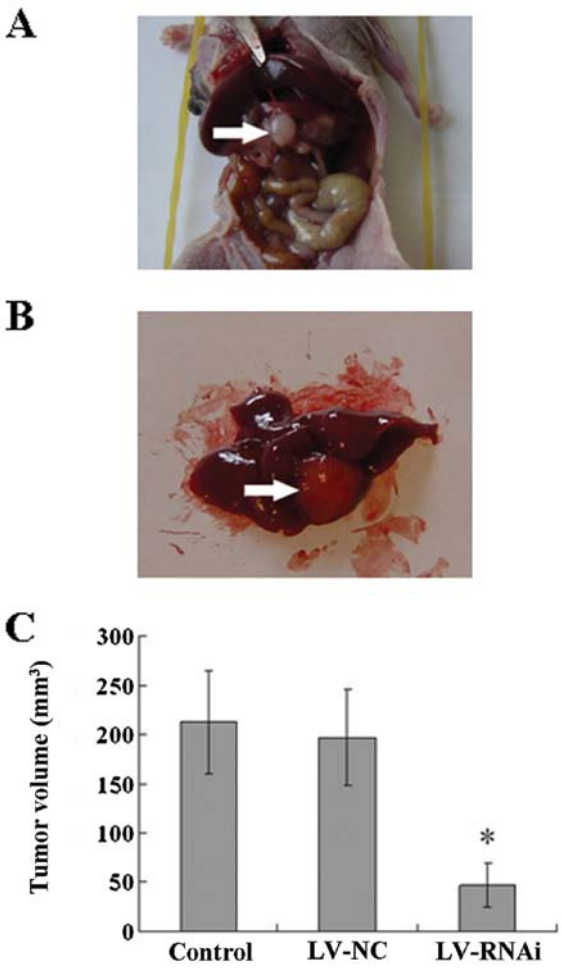

Figure 11. Inhibition of tumor growth in the orthotopic transplantation pancreatic cancer model. (A) The orthotopic transplantation pancreatic cancer model was established successfully (indicated by the arrow). (B) Liver metastasis in the orthotopic transplantation pancreatic cancer model (indicated by the arrow). (C) The tumor volume was detected after the tumors were harvested from mice. The tumor volume of the LV-NC group was obviously smaller (*P<0.05) than that of the other two groups.

and radiation treatments (24,25). The resistance of pancreatic adenocarcinoma to conventional treatment strategies has led to a search for novel targeted therapies that may be useful in eradicating this disease. Gene therapy has been recently emphasized for its contribution to a more favorable patient prognosis (26). In this study, we chose a lentiviral vector since it displays high efficiency in gene delivery and is expressed long-term. In addition, the vector can integrate genes into non-dividing cells with little immunologic reaction (17,18). Our results showed that a high gene transduction efficiency (>90%) was achieved at day 5 after exposure to lentiviral vectors, suggesting gene integration.

Small hairpin RNA (shRNA) mimic natural RNAi in ways that synthetic siRNA oligonucleotides do not (27). shRNA expression vector systems have been established to induce RNA interference (RNAi) in mammalian cells (28). Although these vectors provide certain advantages over chemically synthesized siRNAs, some disadvantages remain, including transient shRNA expression and low transfection efficiency, especially in non-dividing primary cells. To overcome these limitations, shRNA delivery systems using retroviral vectors (29), adenoviral vectors (30) and, more recently, lentiviral vectors (31) have been reported and proven safe for humans. Lentiviral vectors encoding antisense targeting sequences have been used in clinical trials with no obvious side effects (32,33). Lentivirus-delivered shRNAs are capable of specific, highly stable and functional silencing of gene expression in a variety of human cells including primary non-dividing cells and also in transgenic mice (34,35).

Tumor growth relies on angiogenesis, the formation of new blood vessels, to receive an adequate supply of oxygen and nutrients (6,7). In the absence of a blood vascular network, tumors are restrained in size due to limits in the diffusion of oxygen. Therefore, angiogenesis is an important process for growth, progression and metastasis of solid tumors (36). VEGF plays a central role in tumor angiogenesis; it is expressed in most tumors, often at substantially increased levels (37), which makes it a critical target for cancer therapy (38-42). Experiments with neutralizing antibodies and other inhibi-

tors have demonstrated that blockade of the VEGF pathway is sufficient to significantly suppress angiogenesis associated with solid tumor growth in many models. Subcutaneous and orthotopic models have been used to test the effects of inhibitors of the VEGF/VEGFR pathway on the growth of a variety of tumor cell lines (43). Angiogenesis in pancreatic carcinoma is based on the same fundamental principles of activation, proliferation and migration of endothelial cells. The expression of VEGF and CD34 in pancreatic carcinomas was demonstrated to be significantly higher than that in normal pancreatic tissue samples, respectively (44,45).

In the present study, we used lentivirus-mediated shRNA expression system targeting the VEGF gene to downregulate gene expression. After successful lentivirus-mediated VEGF RNA interference, the mRNA and protein expression of the VEGF gene was virtually knocked down *in vitro* as detected by real-time RT-PCR, western blot analysis and ELISA, respectively. Decreased expression of VEGF in the human pancreatic carcinoma cell line and in the xenografted tumors contributed to decreased angiogenesis, growth and metastasis. CD34 is a cell surface marker of progenitor cells and is frequently used as a new vessel marker and an indicator of microvessel density in tissues (46,47). Immunostaining assays revealed VEGF and CD34 in tumors were significantly decreased after LV-RNAi transfection. As shown in the subcutaneous and orthotopic xenografted pancreatic cancer in mice, downregulation of VEGF was found to lead to the suppression of cancer growth, resulting in reduced tumor size.

Additionally, overexpression of VEGF has been shown to be associated with enhanced tumorigenicity and tumor metastatic potential (8,48). Evidence now indicates that the direct receptor-mediated effects of VEGF on tumors such as VEGF induction of phosphatidylinositol-3'-kinase (PI3K) and Akt and enhancement of cell survival (49,50), affect clinical outcomes more decisively than do changes in blood flow and/or oxygenation (51). As is now recognized for co-expression of the kinase-impaired HER3 with HER2, (52) co-expression of kinase-defective VEGFR-1 with active VEGFR-2 increases Akt signaling (53) thus enhancing cell survival and tumor invasion (48). The pro-apoptotic effect of VEGF signaling inhibition is also apparent *in vitro* (54), confirming that the pro-apoptotic action is at least in part independent of the blood supply. In the TUNEL assay of the subcutaneous xenografted tumors, we found that decreased VEGF expression increased cell apoptosis of pancreatic carcinoma cells. Moreover, VEGF-dependent signaling cascades were found to increase cell motility via Src or Fak inducible tyrosine phosphorylation of adhesion substrates (55) and such motility is directly inducible *in vitro* by VEGF (56). In the orthotopic transplantation pancreatic cancer model, we found that decreased VEGF expression reduced the liver metastasis rate, although there was no significant difference detected by statistics which may have been due to the small sample size. Thus, more animal experiments nust be performed to further confirm the inhibitory effects of metastasis mediated by lentivirus-mediated shRNA interference targeting VEGF in pancreatic cancer *in vivo*. Therefore, the anticancer effects induced by lentivirus-mediated shRNA interference targeting VEGF require further investigation, and this will be the focus of our interest in future research.

In conclusion, our findings indicate that lentivirus-mediated shRNA interference targeting VEGF potently suppressed angiogenesis, growth and increased cell apoptosis in the Patu8988 pancreatic cancer cell line *in vivo*. Our findings support the theory that lentivirus-mediated shRNA interference targeting VEGF may be a promising mothod for the treatment of pancreatic carcinoma.

Acknowledgements

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