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Original Article

Effect of Ras homolog C gene silencing on the expression of oesophageal cancer-associated Ras homolog C and vascular endothelial growth factor

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Abstract: Objective: This study investigated the effects of Ras homolog (Rho) C gene silencing on RhoC and vascular endothelial growth factor (VEGF) expression in oesophageal cancer. Methods: Polymerase chain reaction (PCR), Western blot, in situ hybridization, and immunocytochemistry were employed. Results: pRNAT-U6.1-siRhoC1 expression can effectively interfere with RhoC mRNA expression. VEGF protein expression levels in the siRNA intervention group were significantly lower than that in the control group, and the knockdown of the RhoC gene could inhibit the expression of the VEGF protein. The invasion ability of EC9706 cells transfected by pRNAT-U6.1-siRhoC1 was significantly low. pRNAT-U6.1-siRhoC1 can down-regulate the expression of the RhoC gene in nude mice transplantation tumour cells. Conclusion: The in vivo experiments on EC9706 human oesophageal cancer cell lines proved that RhoC gene silencing can effectively decrease the expression of RhoC and VEGF gene in tumour tissues in nude mice transplantation.

Keywords: RhoC, gene silencing, protein, VEGF, oesophageal cancer

Introduction

RhoC is one of the Rho subfamily members. As a signal converter or molecular switch, RhoC functions in the cytoskeleton or the target protein and produces a variety of biological effects [1, 2]. The main function of RhoC involves regulation of cell motility and migration by pathways, such as regulating actin cytoskeleton, inducing actin, integrins and the aggregation of the associated protein, such as fibronectin (FN) [3, 4]. Clark [5] found that RhoC gene overexpression is closely related with tumour metastasis, and may function similarly to a 'switch' in the process of tumour metastasis. Van Golen [6, 7] also believed that RhoC performs a crucial function in promoting angiogenesis in breast cancer. However, foreign and domestic studies on the close correlation between RhoC overexpression and differentiation degree, invasion, metastasis and angiogenesis in oesophageal squamous cell carcinoma remain nonexistent.

The metastasis of original tumours to distant tumours include various steps, such as cell proliferation changes, intercellular adhesion changes, extracellular matrix (ECM) degradation, actin-mediated cell migration and angiogenesis [8-11]. RhoC could be involved in the key steps of tumour invasion and metastasis, such as regulation of cell morphology, cell proliferation, migration, transcription and angiogenesis. Domestic and foreign studies have shown that the RhoC gene and its expression products perform an important function in the occurrence and development processes of numerous malignancies [1-3, 12, 13].

For investigating inhibition in the process of invasion and metastasis in oesophageal squamous cell carcinoma by inhibiting RhoC activity or down-regulating RhoC expression and providing new ideas for targeted treatment of oesophageal cancer, pRNAT-U6.1-siRhoC interfere expression vector was transfected into EC9706 oesophageal cancer cells. Then, we observed

Table 1. Primer sequences

PCR verification primer	F: 5'-TGGACTATCATATGCTTACCGT-3', R: 5'-TAGAAGGCACAGTCGAGG-3'
RhoC mRNA detection primer (410 bp)	RhoC F: 5'-GGTGGACGGCAAGCAGGTGG-3', RhoC R: 5'-TCTTGCGGACCTGGAGGCCA-3'
Internal reference β-actin primer (126 bp)	β-actin F: 5'-CTCACCGAGCGCGGCTACAG-3', β-actin R: 5'-GGAGCTGGAAGCAGCCGTGG-3'
Hairpin siRNA oligodeoxynucleotide	Rho11: 5'-GATCCAGAAGCUGGUGAUCGUUGGTTCAAGAGACCAACGAUCACCAGCUUCUTTTTTTC-3', Rho12: 5'-TCGAGAAAAAAAGAAGCUGGUGAUCGUUGGTCTCTTGAACCAACGAUCACCAGCUUCUG-3'
	Rho21: 5'-GATCCGCUGGUGAUCGUUGGGGAUTTCAAGAGAAUCCCCAACGAUCACCAGCTTTTTTC-3', Rho22: 5'-TCGAGAAAAAAGCUGGUGAUCGUUGGGGAUTCTCTTGAAAUCCCCAACGAUCACCAGCG-3'
	Rho31: 5'-GATCCCAUUCCUGAGAAGUGGACCTTCAAGAGAGGUCCACUUCUCAGGAAUGTTTTTTC-3', Rho32: 5'-TCGAGAAAAAACAUUCCUGAGAAGUGGACCTCTCTTGAAGGUCCACUUCUCAGGAAUGG-3'
	C-1: 5'-GATCCTGATTGGCTGTTACTCACTTTCAAGAGAAGTCAGCGTAGAACAATCATTTTTTC-3', C-2: 5'-TCGAGAAAAAATGATTGTTCTACGCTGACTTCTCTTGAAAGTCAGCGTAGAACAATCAG-3'

cell invasiveness changes before and after transfection, as well as the expression levels of RhoC and VEGF in transfected cells. The effect of down-regulation of RhoC gene on biological behaviour of oesophageal squamous cell carcinoma was investigated, and the impact of RhoC gene expression on nude mice transplantation tumour and VEGF expression was viewed from the perspective in vitro to provide a new theoretical basis for oesophageal cancer gene therapy.

Materials and methods

General materials

Human oesophageal cancer cell line EC9706 cells were donated by the Molecular Tumour State Key Laboratory of Chinese Medical Academy Oncology Hospital. Fifteen male nude mice (BALB/c) with age of 4 weeks and weight of 16-18 g were provided by the Shanghai Hayes Experimental Animal Centre, raised in Henan Tumour Pathology Key Laboratory in the semi-barrier system with constant humidity and a thermostat. Goat anti-human RhoC antibody and mouse anti-human VEGF antibody were provided by Santa Cruz, USA. A SP immunohistochemistry kit was purchased from Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd. pRNAT-U6.1 interference expression vectors were purchased from American GenScript companies. Primer sequences are shown in **Table 1**.

Construction of pRNAT-U6.1-siRhoC interference expression vector

Human RhoC cDNA coding sequence (GenBank Accession No.: NM_001042679) was scanned using the Ambion designed siRNA target sequence analysis system. The final target sequences are as follows: 317-335 (AGAAGCTGGTGATCGTTGG), 321-339 (GCTGGTGATCGTTGGGGAT) and 582-600 bits (CATTCCTGAGAAGTGGACC) were designed according to the principles of siRNA target sequence via homology search in BLAST analysis. After transformation by *Escherichia coli* plasmids, screening and identification of the positive cloning vector, the transfected EC9706 cells were divided into five experimental groups: (1) experimental group one, transfected by siRNA oligonucleotides pRNAT-U6.1-siRhoC1; (2) experimental group two, transfected by siRNA oligonucleotides pRNAT-U6.1-siRhoC2; (3) experimental group three, transfected by siRNA oligonucleotides pRNAT-U6.1-siRhoC3; (4) unrelated siRNA control group, transfected by unrelated sequence siRNA interference expression vectors; (5) empty vector control group, transfected by interference expression vector with empty vector; and (6) blank control groups, untreated group. The interference expression vector with optimal inhibition was screened.

Detection of RhoC mRNA, protein and VEGF protein expression

Reverse transcription-polymerase chain reaction (RT-PCR): specific steps were performed in accordance with the kit instructions.

Western blot: cell protein samples were prepared. SDS-PAGE electrophoresis was carried out and transferred to films for immunoassays. Finally, DAB was added, and the samples were exposed in a darkroom. Results were obtained by a digital camera.

In situ hybridization: hybridization specific steps in situ hybridization were carried out according to the literature.

Detection of immunochemical cells: slides were immersed in sulphuric acid solution for full cleaning for 48 h, fully rinsed, dried and immersed in 5% ethanol for 24 h. APES was evenly smeared onto each slice and fixed by 80% ice-cold acetone for 30 min. Coverslips with cells were placed on a glass slide and washed for three times with phosphate-buffered saline (PBS) solution for 5 min. Then, 50 μL of 3% H_2O_2 solution was added, and the slips were placed at room temperature for 10 min and washed for three times with PBS for 5 min. Animal serum was dropped on the slides, which are placed at room temperature for 10 min. The first, second and third antibodies were dropped and incubated at 37°C for 1-2 h, 30 min and 40 min, respectively, followed by PBS washing for three times and 5 min. One drop of the reagents in groups A, B and C were respectively added to 850 μL distilled water and dropped onto a glass slide after full mixing. Slides were fully coloured in a cartridge at room temperature, and colouring was subsequently terminated by water. Slides were restained in haematoxylin for 1.5 min, washed with running water for 5 min, treated with 1% HCl alcohol for 10 s and washed with running water for 5 min. Then, samples were dehydrated using gradient alcohol (80%, 95%, 95%, 100% and 100%), transparently treated by xylene and mounted with neutral gum. Photos were obtained and analysed using digital cameras.

Invasiveness experiments in vitro by Boyden chamber

The Matrigel matrix film was placed at 37°C for 3 h and then at room temperature overnight. Cells in the three groups were prepared into a single-cell suspension, and a haemocytometer was used for counting, the number of cells in each group was adjusted to approximately 5×10^5/mL. The attractant was added to the lower chamber, and then Matrigel matrix membrane was tiled at the lower room. Three replicates were set in each group, and 25 μL cell suspension was added in the pores of the upper chamber. Then, the membranes were incubated in a CO_2 incubator for 6 h. The Matrigel matrix film was removed and fixed by 70% methanol for 45 min, stained with non-bound haematoxylin for 5 min and rinsed with water for termination. The number of cell migration in the membrane was counted under an ordinary optical microscope. Tumour cell migration ability was expressed using the number of transmembrane cells. The total number of the five fields in each pore under the high magnification microscope was summed up, and the average was obtained.

Animal experiments

Fifteen mice were randomly divided into three groups, with five members for each group. The three animal groups were the siRNA interference cell group (EC9706 cells vaccinated and transfected by pRNAT-U6.1-siRhoC1 interference expression vector), the unrelated siRNA cell control group (EC9706 cells seeded and transfected with siRNA oligonucleotides pRNAT-U6.1-siC) and the non-transfected cell group (EC9706 cells without being inoculated and transfected). Cells in six groups in the logarithmic phase were collected, prepared in PBS solution free of Ca^{2+}, Mg^{2+} (0.01 M, pH 8.0) and washed for two times to prepare 1 mL cell suspension. The scapular bone area on the back of nude mice in each group was taken as the injection point, and each group were injected with 0.2 mL subcutaneously with density of 1×10^7/mL. At five days after injection, the newborn tumour goitre was visible in the inoculated nude mice with naked eyes, diameter (a) and short diameter (b) of the tumour were measured every three days, tumour volume was calculated according to the formula: [V (cm^3) = $a\times b^2\times0.5$]. The tumour growth curve was plotted, and the rate of tumour formation was calculated. Mice were killed at four weeks after inoculation to observe tumour metastasis in nude mice. Tumour volume was measured on ice; one part was rapidly placed into a cryogenic refrigerator for RT-PCR experiments, and another part of the tumour tissues were fixed with paraformaldehyde to prepare paraffin sections. RT-PCR and in situ hybridization were used to detect the RhoC mRNA expression of nude mice transplantation tumour tissues, and immunohistochemistry was used to determine RhoC and VEGF protein expression in nude mice transplantation tumour tissues.

Statistical analysis

Statistical analysis was performed using SPSS13.0 software. All values were expressed as means ± standard deviation (SD). The signifi-

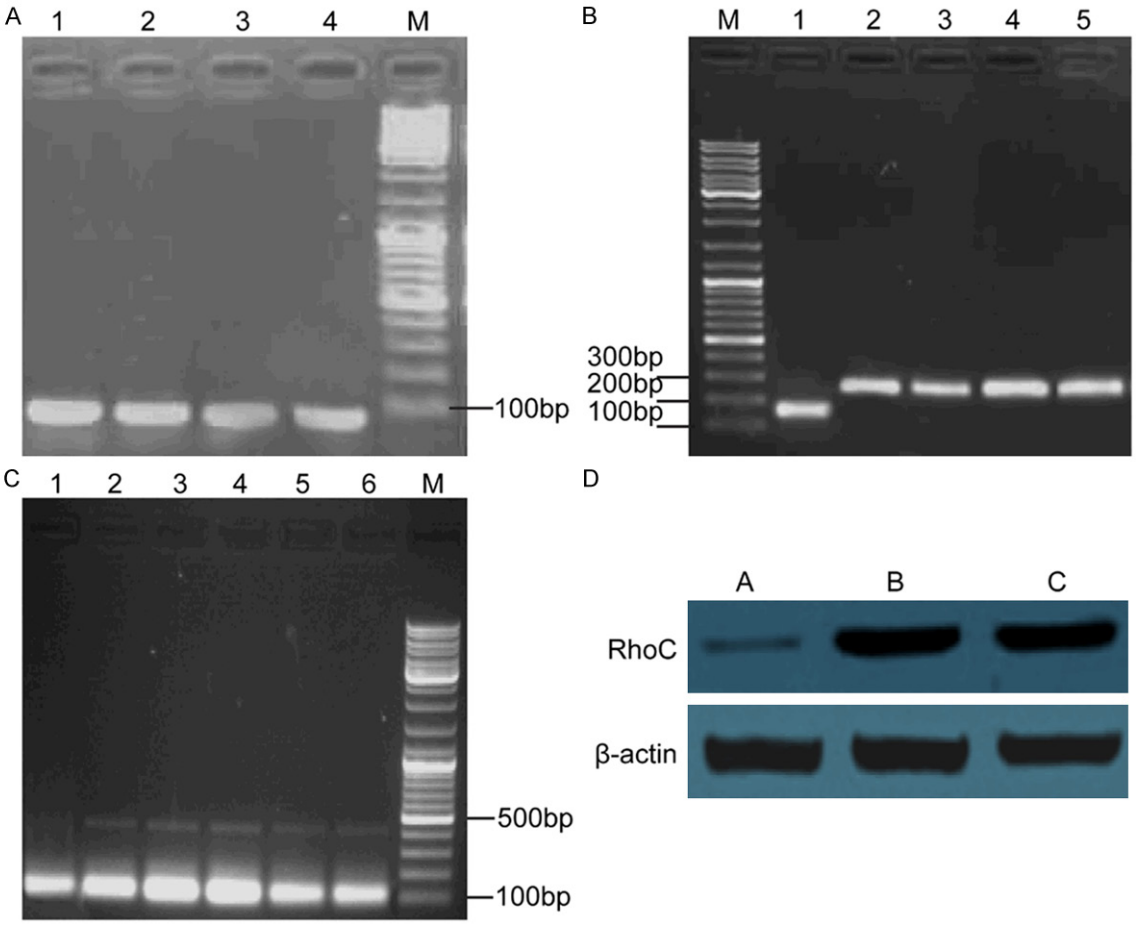

Figure 1. PCR visualized by gel electrophoresis and Western blot maps. A. siRNA hairpin DNA annealing result by eletrophoersis; B. Identification of recombinant plasmid pRNAT by PCR; C. Expression of RhoC mRNA in each transfected group; D. Expressionof RhoC protein in each group cells by Western blot: A-tranfecting pRNAT-U6.1-siRhoC1, B-tranfecting pRNAT-U6.1-siC, C-no tranfecting pRNAT-U6.1

cance of differences among the groups was determined by Student's t-test and one-way analysis of variance. A *P*-value of < 0.05 was considered as statistically significant.

Results

Successful construction of pRNAT-U6.1-siRhoC interference expression vector

The amplification product of empty vectors was approximately 150 bp, whereas the amplification results of various other positive clones were 200 bp, showing that annealed products (50-60 bp) were successfully connected to the interference expression vector without carriers (**Figure 1A**). Colonies with appropriate molecular size were verified by PCR, and DNA sequence analysis was performed. The sequencing results of the insert fragments in the positive recombinant, pRNAT-U6.1-siRhoC1/C2/C3, and the control interference expression vector pRNAT-U 6.1-siC was completely identical with the expectedly designed sequences (**Figure 1B**).

Experiment in vitro results of RNA interfering RhoC gene

Inhibitory effect of RhoC mRNA expression: cells that were transfected by only pRNAT-U6.1-siRhoC1 could interfere RhoC mRNA expression, and the other two unrelated control sequences failed to significantly interfere with the expression of RhoC mRNA (**Figure 1C**).

In situ hybridization results of RhoC mRNA expression: RhoC mRNA expressions in the untreated group and nonsense siRNA control group were significantly higher than that of RhoC siRNA the group, and the difference was statistically significant ($P < 0.05$). RhoC mRNA

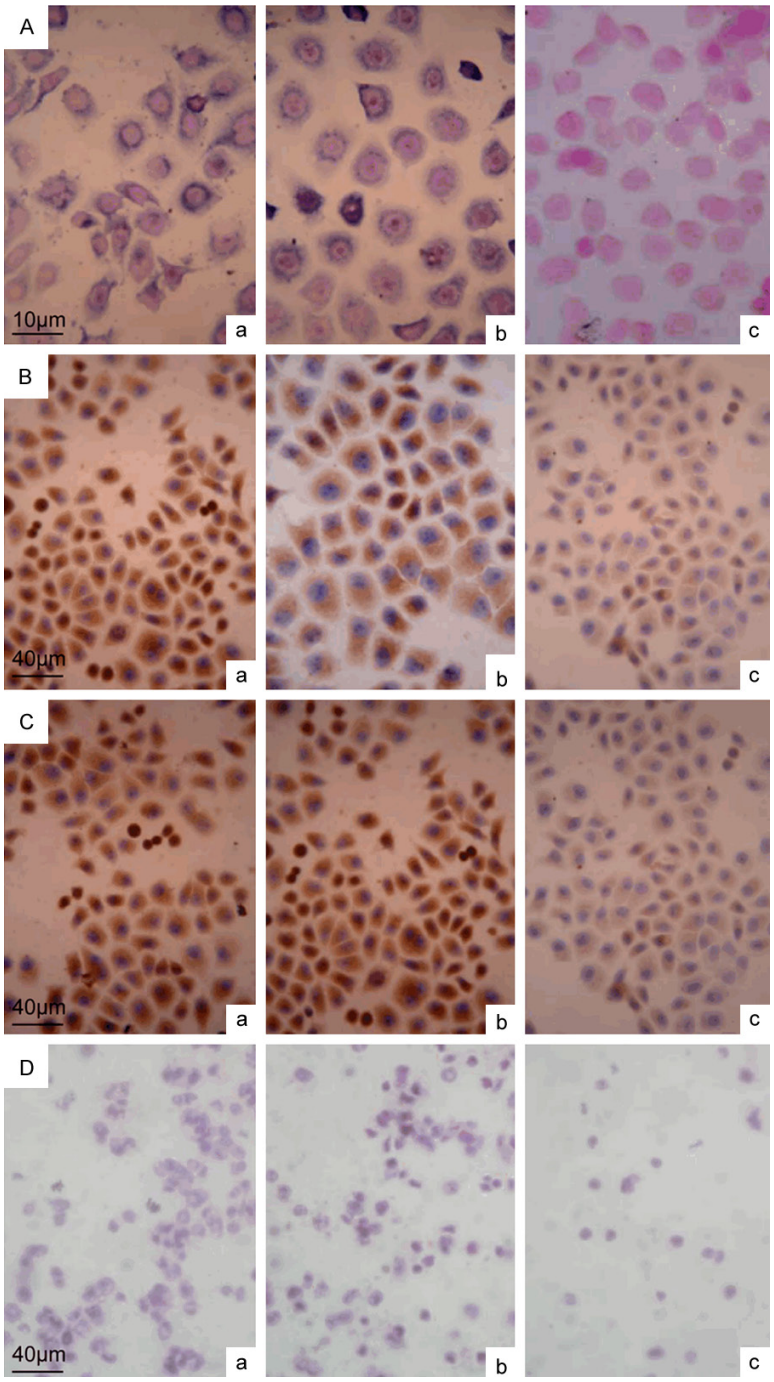

Figure 2. IHC and HE experimental results of RhoC mRNA and protein expression, VEGF protein expression and Boyden chamber invasion experiment in vitro. A-IHC for RhoC mRNA in EC9706, B-IHC for RhoC protein in EC9706, C-IHC for VEGF protein in EC9706, D-HE for boyden chamber assay in vitro cells. a indicates the untransfected group, b denotes the unrelated siRNA control group and c represents siRNA intervention group.

positive signals mainly showed purple and blue colours, which are located in the cytoplasm of oesophageal squamous cell carcinoma EC9706 cells (**Figure 2A**).

RhoC protein expression results: Western blot analysis results showed that apparent RhoC protein expression appeared in the unrelated siRNA control group and non-transfected cell group, and RhoC protein expression of the siRNA intervention group significantly decreased (**Figure 1D**).

The immunocytochemistry results of RhoC protein expression showed that the positive signals of RhoC protein could be evidently seen in the immune cell group, untransfected nonsense siRNA group and the control group. The protein expression is mainly localized in oesophageal squamous cell carcinoma EC9706 cell cytoplasm with light yellow to brown granules. Meanwhile, no evident RhoC protein expression was seen in the RhoC siRNA group. The differences among the three groups were statistically significant ($P < 0.05$) (**Table 2**; **Figure 2B**).

Immunocytochemistry results of VEGF protein expression showed that VEGF protein expression positive signals could significantly be seen in the untransfected nonsense siRNA group and the control group. Protein expression was mainly localized in the cytoplasm of oesophageal squamous cell carcinoma EC9706 cells with pale yellow to brown granules. Whereas no apparent VEGF protein expression appeared in the RhoC siRNA group, the differences among the three groups showed statistical significance ($P < 0.05$) (**Table 2**; **Figure 2C**).

The analysis results of cell invasive ability in the in vitro experiment in the three groups by Boyden chamber showed that, in the comparisons among the non-transfected group, trans-

Table 2. RhoC mRNA positive expression (RhoC mRNA +), RhoC protein positive expression (RhoC protein +), VEGF protein positive expression (VEGF protein +) numbers and transmembrane cells in the blank control group, the unrelated siRNA control group and the siRNA intervention group.

Index	n	Blank control group	Unrelated siRNA control group	siRNA intervention group	*F*	*P*
RhoC mRNA +	5×200	124.00±19.25	125.00±17.87	22.40±6.23	71.52	0.000
RhoC protein +	5×200	149.00±20.70	149.20±19.15	46.00±9.06	60.59	0.000
VEGF protein +	5×200	128.00±16.84	126.00±19.49	60.00±8.51	30.52	0.000
Transmembrane cells	5×200	121.40±6.80	125.40±19.25	59.80±8.23	110.66	0.000

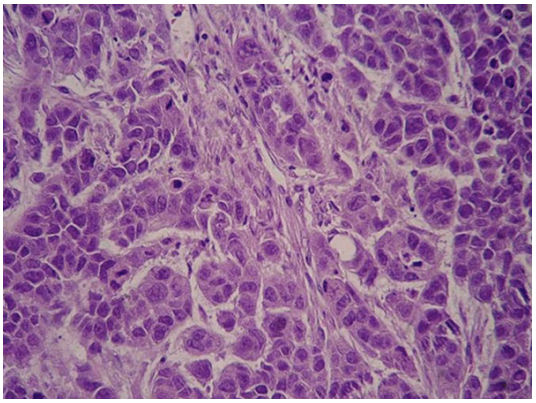

Figure 3. Histological morphology of nude mice transplantation tumour (HE×200).

fected group, unrelated siRNA control group and siRNA intervention group, the Matrigel transmemberane cell number in the siRNA intervention group significantly decreased (43.80 ± 7.01), with statistically significant difference ($P < 0.05$) (**Table 2**; **Figure 2D**).

Experiment in vitro results of RNA interference RhoC gene

Successful construction of EC9706 cell nude mice transplantation tumour model: subcutaneous tumours with different sizes in the three groups were formed, the tumour formation rate of the nude mice model was 100% (**Figure 4A**).

Histological morphology: we see tumour cell clusters of varying sizes, scattered spotty and small pieces of necrosis. Mesenchymes were formed by a small amount of fibrous connective tissues among tumour cell masses. Tumour cells were round with different sizes, the nucleoli were visible to be large with deeply stained and significant atypia and pathological nuclear division image was visible (**Figure 3**).

Effect of RhoC expression on tumour volume and inhibition: tumour volume sizes in the EC9706 cell group, the control group and SiRNA unrelated siRNA interference group were 2519.60±189.28, 2361.80±136.75 and 858.20±54.01, respectively. The differences among the three groups were statistically significant by rank-sum test ($P < 0.05$) (**Table 3**; **Figure 4B**).

Growth curve plotting of nude mice transplantation tumour: tumour volume in the SiRNA interference group slowly increased and was significantly smaller than that of the EC9706 cell group and the unrelated siRNA cell group. Differences among the two groups were statistically significant ($P < 0.001$) (**Figure 4C**).

RhoC protein expression: results detected by immunohistochemical method displayed that RhoC protein positive signals are mainly localized in the cytoplasm of the transplantation tumour tissue cells with brownish-yellow granules. RhoC protein positive staining in nude mice tumour of the SiRNA intervention cell group was weak. RhoC protein positive signals could be seen in the EC9706 cell group, unrelated siRNA control cell group and the RhoC protein expression group. According to rank-sum test, the difference was statistically significant ($P < 0.05$) (**Table 3**; **Figure 5A**).

RhoC mRNA expression: the results of RhoC mRNA expression in the tumour tissues detected by in situ hybridization showed that positive expression in the SiRNA interference cell group was weak, whereas RhoC mRNA positive signals (1/4) were seen in the cytoplasmic purple blue granules in the EC9706 cell group and unrelated siRNA control cell group. The expression differences among the three groups were statistically significant ($P < 0.05$). The detection results of RhoC mRNA in the nude mice tissues of the three groups by RT-PCR was processed by gel grayscale scanning and quantified as 0.33±0.06, 0.91±0.07 and 0.92±0.06, respectively. RhoC mRNA content was significantly

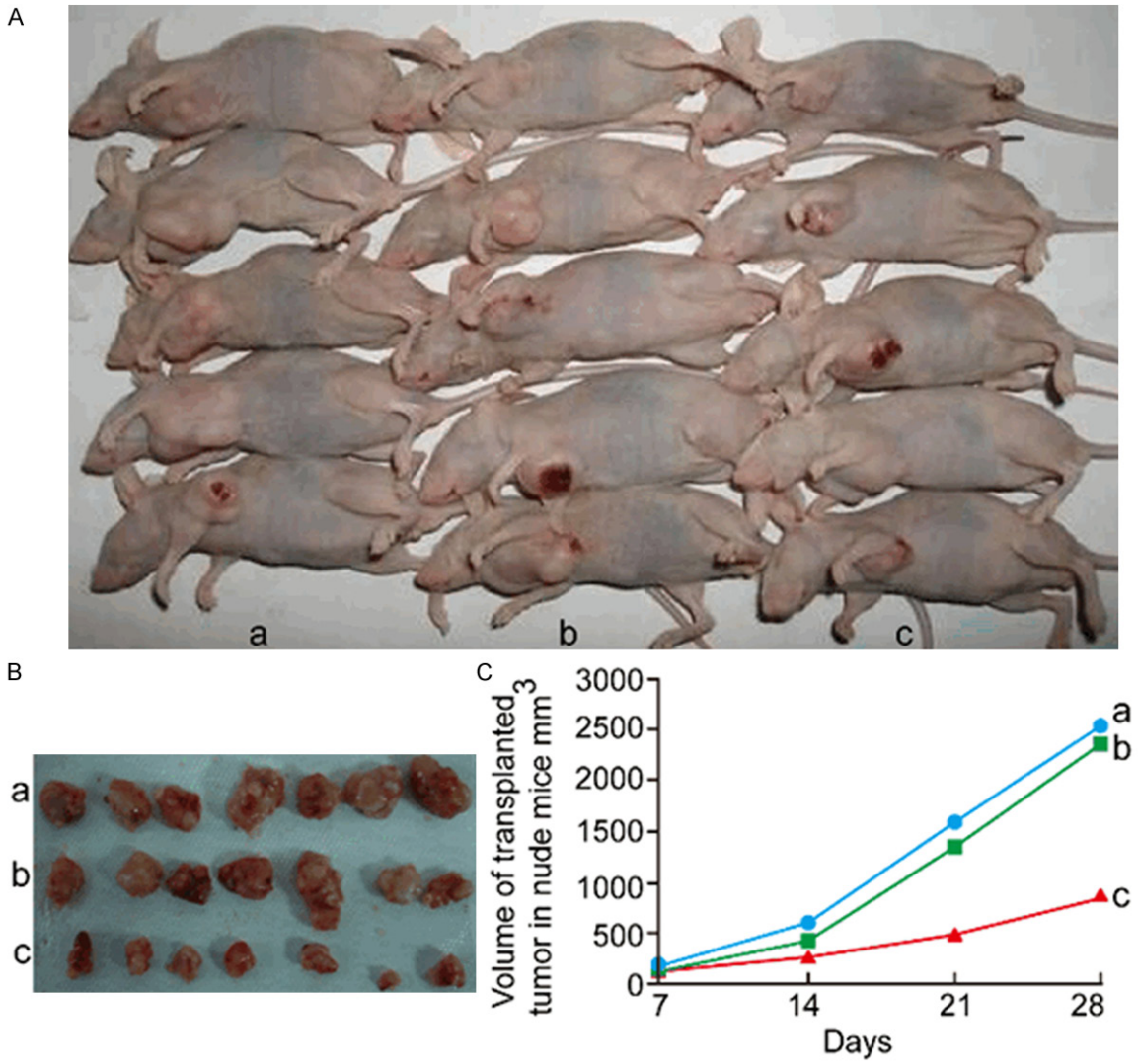

Figure 4. General morphology, volume and growth curve of nude mice in each group. Shape (A), Size (B) and Volume growth curve (C) of nude mice transplanted tumor in each group. a, EC9706 contorl group; b, pRNAT-U6.1-siC group; c, pRNAT-U.61-siRhoC1 group.

Table 3. Tumour volume (mm^3), RhoC mRNA, RhoC protein, RhoC mRNA relative content and VEGF protein expression in the siRNA intervention group, the unrelated siRNA control group and the EC9706 cell group

Index	siRNA intervention group	Unrelated siRNA control group	EC9706 cell group	χ^2/F	*P*
Tumor volume (mm^3)	864.20±22.22	2353.36±72.73	2530.41±43.82	219.593	0.000
RhoC mRNA	10.54±3.37	20.79±0.39	20.58±0.45	9.98	0.007
RhoC protein (n=5×200)	7.20±0.44	18.96±0.59	18.96±0.62	748.30	0.000
RhoC mRNA relative content (n=5)	0.33±0.06	0.91±0.07	0.92±0.06	143.71	0.000
VEGF protein (n=5×200)	76.19±2.07	117.96±1.97	118.23±2.15	684.65	0.000

lower in the siRNA interference cell group. The difference among groups was statistically significant ($F = 53.720$, $P < 0.05$) (**Table 3**; **Figure 5B**).

VEGF expression: immunohistochemistry results showed that VEGF protein positive signals are mainly located in the cytoplasm with brownish-yellow granules. VEGF protein positive

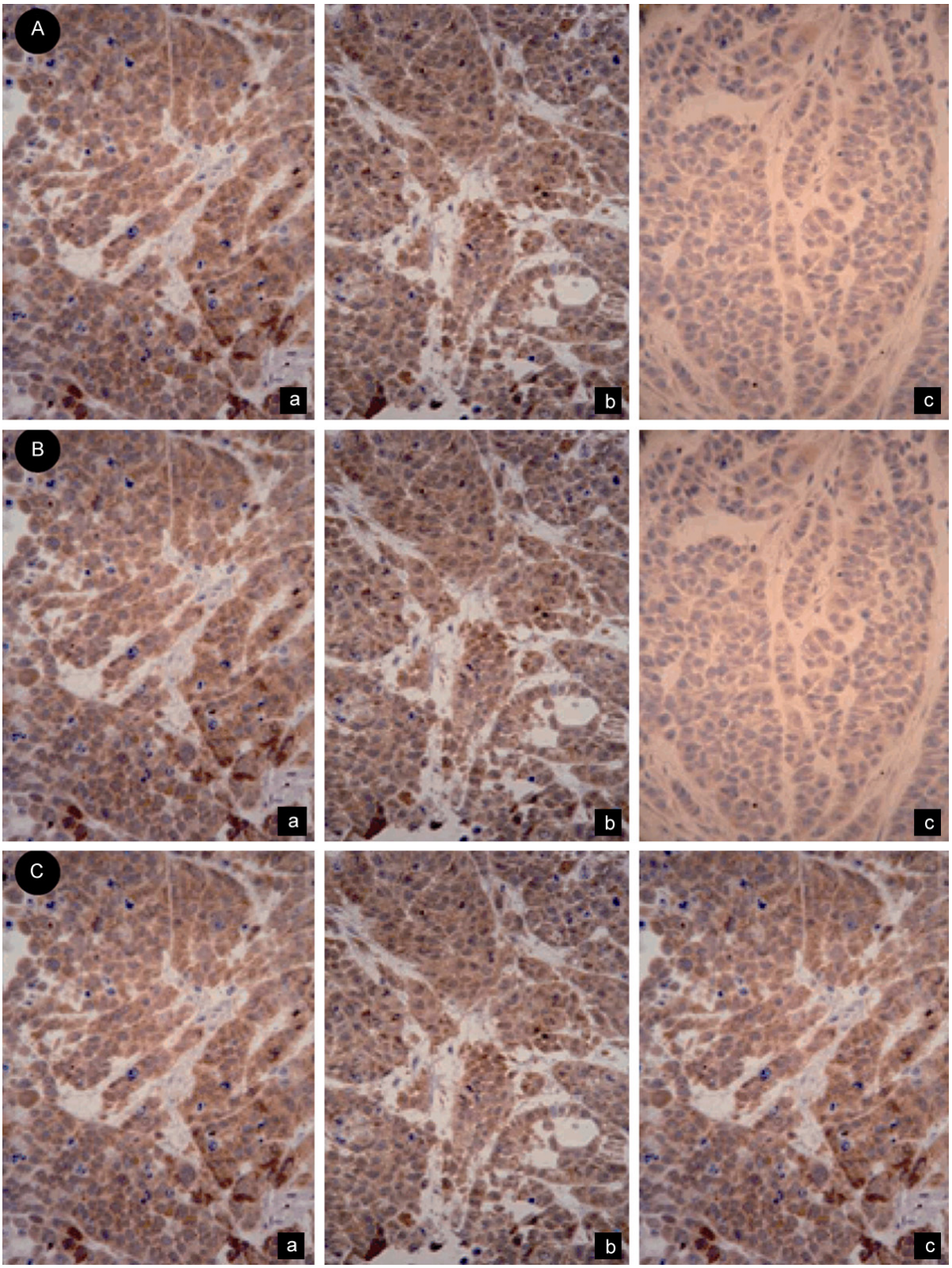

Figure 5. IHC and HE results of RhoC mRNA, protein and VEGF protein expression. A-IHC for RhoC protein in nude mice transplanted tumor, B-IHC for RhoC mRNA in nude mice transplanted tumor, C-IHC for VEGF protein in nude mice transplanted tumor. a represents the EC9706 cell group, b indicates the unrelated siRNA control cell group and c denotes the siRNA intervention group.

staining in the tumour tissues of the siRNA intervention cell group was rarely seen, whereas positive staining was observed in both the unrelated siRNA control cell group and EC9706 cell group. The differences in RhoC expression among the three groups were statistically significant ($P < 0.05$) (**Table 3**; **Figure 5C**).

Discussion

RNA interfering technology is the cell reaction process of introducing a double-stranded RNA to cell to cause the corresponding degradation of homologous mRNA by genetic engineering. Then, the double-stranded RNA could be specifically cut into multiple small interfering RNA (siRNA) with size of 21-25 nt by Dicer enzyme. siRNA bound with ribozyme to form 'RNA-induced silencing complex (RISC)' to cut homologous target mRNA molecules mediated by RISC, which belonged to post-transcriptional gene silencing [14-17]. RNAi technology is a highly effective gene silencing or gene knockout approach, which has become a powerful tool that is widely used to study a variety of human tumours [18-23]. In this study, the RhoC gene was considered as a target gene, and human RhoC gene siRNA oligonucleotide duplexes were designed according to the general principles of siRNA via the website (http://www.ambion.com/techlib/misc/siRNA_finder.html). Genetic engineering was further used to successfully construct three RhoC siRNA expression vectors (pRNAT-U6.1-siRhoC1, pRNAT-U6.1-siRhoC2 and pRNAT-U6.1-siRhoC3), as identified by PCR. Results showed that the gene was inserted correctly.

In this study, the measurement results of the RhoC mRNA expression levels of cells in each group by RT-PCR method indicate that the constructed pRNAT-U6.1-siRhoC1 expression vector can effectively interfere with the RhoC mRNA expression in cells, suggesting that RhoC gene silencing strains were successfully established by transfecting pRNAT-U6.1-siRhoC1 EC9706 to cells to further explore the cell biological behaviour of oesophageal cancer cells after RhoC gene silencing. The detection results of RhoC mRNA and protein expression by collective use of RT-PCR, Western blot, in situ hybridization and immunohistochemical methods showed that the RhoC mRNA and protein expression levels in the siRNA intervention group were significantly lower. Besides, immunohistochemistry was used to detect the VEGF protein expression in cells of each group, and the results showed that the expression levels of VEGF protein in siRNA treated group was significantly lower than that in the control group. These observations suggest that transfection of pRNAT-U6.1-siRhoC1 EC9706 cells can effectively close the RhoC mRNA and protein expression. RhoC gene knockdown could inhibit VEGF expression. Thus, RhoC gene silencing is presumed to inhibit the biological behaviours of oesophageal cancer, including proliferation and metastasis by inhibiting tumour angiogenesis.

To detect the changes of infiltration ability mediated by down-regulating RhoC gene expression in EC9706 oesophageal squamous cell carcinoma, we designed a Boyden chamber to simulate the in vivo process of partial tumour cells decomposing the extracellular matrix and passing through the basement membrane and provide a strong technical approach [24] for the detection of enhanced tumour cell invasion and metastasis ability. The results in this study showed that the infiltration capacity of EC9706 cells transfected by pRNAT-U6.1-siRhoC1 was significantly lower than that in the other two groups. These findings indicating that RhoC gene overexpression can promote the intracorporal infiltration capacity of EC9706 cells, further confirming the close relationship between RhoC gene with the invasion and metastasis of oesophageal squamous cell carcinoma.

However, the in vitro environment was considerably more complex compared with the environment in vivo. Thus, subcutaneous inoculation and transfection in nude mice were performed on EC9706 cells of each group to establish a human EC9706 cell line oesophageal carcinoma xenograft animal model. The tumour duration time of nude mice in EC9706/pRNAT-U6.1-siRhoC1 group was approximately 2-4 days longer than that in the other two groups, indicating that the tumour formation capacity of nude mice in the EC9706/pRNAT-U6.1-siRhoC tumour cell group decreased. After being cultured and collected, the EC9706 tumour cells of the three groups were inoculated subcutaneously in nude mice. Results showed that, at four weeks after vaccination, the differences of the transplanted volume among the three groups were statistically significant ($P = 0.000$). Nude mice transplantation tumour volume in the EC9706/pRNAT-U6.1-siRhoC1 cell group was significantly lower than that of the EC9706

cell group and the unrelated siRNA control cell group, suggesting that EC9706/pRNAT-U6.1-siRhoC1 exerted significant inhibition on tumour growth in nude mice.

In this study, RT-PCR, in situ hybridization and immunohistochemistry techniques were first combined to apply in the detection of RhoC mRNA and protein expression in the nude mice of each group. Results showed that RhoC expression could be reduced in the RhoC-siRNA interference group by constructing vectors in nude mice transplantation tumour tissues. These results suggest that EC9706/pRNAT-U6.1-siRhoC1 can inhibit malignancy growth by regulating intracorporal oesophageal cancer-related genes. Immunohistochemical method was further used to detect the VEGF protein in transplanted tumour tissues of the EC9706/pRNAT-U6.1-siRhoC1 cell group, the EC9706 cell group and the unrelated siRNA control cell group. The results showed VEGF expression in the transplanted tumour of EC9706/pRNAT-U6.1-siRhoC1 cell group was significantly decreased in comparison with this of the other two groups. RhoC in vivo experiments further confirmed the theory that RhoC overexpression could promote tumour angiogenesis by up-regulating the expression of VEGF expression. This study of oesophageal cancer genes was expected to provide new ideas for targeted therapy.

Conclusion

In vivo experiments showed that pRNAT-U6.1-siRhoC1 can decrease the expression of RhoC gene in nude mice transplantation tumour tissues, inhibit the growth of tumours in nude mice and reduce the VEGF protein expression of transplanted tumour tissues. Thus, pRNAT-U6.1-siRhoC1 plays important roles in the inhibition of tumour angiogenesis process. A recombinant plasmid of pRNAT-U6.1-siRhoC1 was successfully constructed and transfected into the EC9706 cell line in vitro with stable RhoC silencing. Both invasion and metastasis abilities are reduced, and the RhoC mRNA and protein expression in EC9706 cells can be effectively closed. Knockdown of the RhoC gene could inhibit the expression of VEGF protein. Human oesophageal cancer EC9706 cell lines in vivo experiments proved that RhoC gene silencing can effectively decrease the expression of RhoC and VEGF genes in tumour tissues in nude mice transplantation. These results provide foundation for the in-depth study of the relationship between RhoC and oesophageal cancer.

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Disclosure of conflict of interest

None.

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