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Regulating Oncogenic lncRNA VPS9D1-AS1 with Cholesterol-Modified siRNA for Esophageal Squamous Cell Carcinoma Therapy

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Abstract: Esophageal carcinoma is the tenth most common cancer in the world. long non-coding RNA (lncRNA) is a type of RNA molecule that has no or limited coding ability. Many studies have found that it plays an important role in the occurrence and development of tumors. This article aims to investigate the expression and clinical significance of lncRNA VPS9D1-AS1 in patients with esophageal squamous cell carcinoma (ESCC). Additionally, it aims to examine the effects of targeted silencing of VPS9D1-AS1 on the growth and migration of ESCC cells *in vivo* and *in vitro*, and to explore the mechanism of action. This study compared the expression difference of lncRNA VPS9D1-AS1 in ESCC tissues and normal tissues through TCGA and UCSC databases, and analyzed the correlation between VPS9D1-AS1 expression and the prognosis of ESCC patients; clinical samples from ESCC patients were collected and qRT-PCR (Fluorescent quantitative polymerase chain reaction) was used to detect the relative expression of VPS9D1-AS1 and analyze the correlation between the expression of VPS9D1-AS1 and the onset and development of ESCC. Specific small interfering RNA was used to knock down the expression of VPS9D1-AS1 in ESCC cells, and the effect of VPS9D1-AS1 on the proliferation and migration of esophageal squamous cell carcinoma cells was detected by CCK-8 and Transwell assays. A subcutaneous transplantation tumor model of ESCC cells was established, and cholesterol-modified si-VPS9D1-AS1 was injected around the tumor to detect the impact of VPS9D1-AS1 on ESCC tumor growth *in vivo*. Western blot was used to detect the expression of apoptosis-related proteins in the tumor tissues of mice in each group. Finally, it was found that lncRNA VPS9D1-AS1 was highly expressed in ESCC tissues and significantly correlated with the prognosis of ESCC patients. Knocking down VPS9D1-AS1 regulates the proliferation, migration, and apoptosis of ESCC cells, and inhibits the growth of transplanted tumors *in vivo*, indicating it is a potential diagnostic and therapeutic target for esophageal squamous cell carcinoma.

Keywords: Esophageal Cancer, lncRNA VPS9D1-AS1, siRNA, Cell Proliferation, Cell Migration

1. Introduction

Esophageal cancer (esophageal carcinoma) is the tenth most common cancer worldwide and the sixth leading cause of cancer-related deaths [1]. However, most esophageal cancers are detected at an advanced stage, when treatment options are limited and prognosis is poor, and conventional

synchronized chemoradiotherapy or induction chemotherapy in combination with it does not improve the response rate or survival of patients [2]. Studies have shown that the number of esophageal cancer cases is expected to increase to 957,000 by 2040 if current trends continue [3]. Therefore, the discovery of early diagnostic and prognostic biomarkers associated with esophageal cancer, or the development of new targets for drug action against characteristic molecules, is of great importance for the prevention, treatment, and prognosis of esophageal cancer.

Long Non-Coding RNAs (lncRNAs) are a class of RNA molecules with no or limited coding ability that are more than 200 nucleotides in length. It has been found that, in addition to its involvement in genomic imprinting and X-chromosome inactivation, it can also participate in tumorigenesis, progression, invasion, and metastasis by regulating various physiological activities of cells [4-6], and can even participate in metabolic reprogramming of cancer cells [7, 8], and thus it has also become a hot spot of research in the field of cancer in recent years. Currently, many databases around the world collate information about lncRNAs, which have been widely characterized in several species [9], and upregulation of LINC01296, and LOC100133669 have both been shown to be associated with poor prognosis of ESCC patients. LncRNA CASC9 has also been emphasized to play a role in novel diagnostic and prognostic biomarkers and potential therapeutic ESCC key roles in novel diagnostic, prognostic biomarkers, and potential therapeutic targets for ESCC [10]. However, the role of lncRNAs in ESCC prognosis is still poorly understood. In this study, we screened lncRNA VPS9D1-AS1 by bioinformatics high-throughput analysis, and then verified the differential expression of lncRNA VPS9D1-AS1 in normal tissues and esophageal cancer tissues, and explored its specific effect on the development of ESCC.

RNA interference (RNAi) is a biological process in which exogenous or endogenous double-stranded RNA (dsRNA) is introduced into cells and causes degradation of mRNAs homologous to dsRNA, which in turn inhibits the expression of the corresponding genes [11]. As an important technology in RNAi, small interfering RNA (siRNA) can selectively silence the target genes and thus down-regulate the expression of target genes. In recent years, siRNA drugs have been approved and marketed, and although none of the approved siRNA drugs are currently used for the treatment of cancer [12], selective gene silencing therapy with siRNAs has been proved to be revolutionary in cancer treatment. Therefore, we also synthesized cholesterol-modified si-VPS9D1-AS1 to verify its inhibition on ESCC tumor growth *in vivo*.

2. Materials and Methods

2.1. Database Information

The harmonized and standardized pan-cancer dataset: TCGA TARGET GTEx (PANCAN, N=19131, G=60499) was downloaded from the UCSC database, from which the expression data of the ENSG00000261373 (VPS9D1-AS1) gene was further extracted from the individual samples, and the esophageal squamous carcinomas with a significantly high expression of VPS9D1-AS1 were selected for the study after the comparison.

2.2. Main Reagents

RPMI 1640 medium was purchased from Gibco, fetal bovine serum was purchased from BI, reverse transcription kit (GoTaq 2-Step qRT-PCR System) and qPCR kit (GoTaq qPCR Master Mix) were purchased from Applied Biological Materials, Trizol was purchased from Tiangen Biochemistry Co. Ltd., Pierce® BCA Protein Assay Kit was purchased from Shanghai Biyuntian Biotechnology Co., Cell Counting Kit-8 (CCK-8) was purchased from KGI Biologicals, and Transwell for cell migration assay was purchased from Millipore. All the siRNAs were obtained from GenePharma and the sequences are listed in Table 1.

Table 1. *siRNA interference sequences.*

Name	Sequences
si-VPS9D1-AS1-1	Forward chain: 5'-GGCCUCUCAAACUUAAUGUTT-3' Reverse chain: 5'-ACAUUAAGUUUGAGAGGCCTT-3'
si-VPS9D1-AS1-2	Forward chain: 5'-GCUCUACCACUGUUACUUATT-3' Reverse chain: 5'-UAAGUAACAGUGGUAGAGCTT-3'
si-NC	Forward chain: 5'-ACGUGACACGUUCGGAGAATT-3' Reverse chain: 5'-TTCTCCGAACGTGTCACGTTT-3'

*In the table, si-VPS9-1 refers to si-VPS9D1-AS1-Homo-1717, and si-VPS9-2 refers to si-VPS9D1-AS1-Homo-883.

2.3. Clinical Sample Tissue and Laboratory Animal Sources

Esophageal squamous carcinoma tissues were collected from 20 patients with esophageal squamous carcinoma who attended Nanjing General Hospital of Nanjing Military District, Nanjing, Jiangsu Province, China, from December 2014 to November 2015, and pathogen-free grade BALB/c nude mice were purchased from Nanjing Collective Pharmacology and Wellness Biotechnology Co.

2.4. Bioinformatics Analysis

LncRNA VPS9D1-AS1 expression in ESCC patients was analyzed with the help of TCGA and GTEx databases. Kaplan Meier survival curves and staging comparison box plots of VPS9D1-AS1 were obtained with the help of the UCSC Xena database platform.

2.5. qRT-RCR

Total RNA was extracted from frozen tissues and cell cultures using Trizol reagent, and the total RNA was reverse transcribed into cDNA according to the instructions of the Reverse Transcription Kit, and then the PCR reaction was performed with GAPDH as the internal reference gene. The RT-qPCR reaction conditions were as follows: 95°C for 3 min, 95°C for 15 s, and 60°C for 1 min for 40 cycles. The relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Each sample was repeated 3 times. The primer sequences of VPS9D1-AS1 and GAPDH are shown in Table 2.

Table 2. *The sequences of qPCR primers.*

Primer Name	Forward (5'-3')	Reverse (5'-3')
VPS9D1-AS1	ATGGGGCCTCTGGGGAAGT	TACGGGAAGGCAGGACT
GAPDH	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGGTCATGAG

2.6. Cell Lines and Culture

Human ESCC cell lines (TE13, Eca109) were cultured using 1640 medium containing 0.1% Pen/Strep and 10% fetal bovine serum in a 37°C and 5% CO_2 incubator. The cells were digested and passaged when the confluence of adherent growth was about 80%. Cells in the logarithmic proliferation phase were taken for subsequent transfection experiments.

2.7. In Vitro Activity Assay

TE13 and Eca109 cells were inoculated in 12-well plates at a density of 8×10^4 cells/ml. si-VPS9D1-AS1 (group transfected with si-VPS9-1) and negative control si-NC group (negative control siRNA, si-NC group) were set up when the cell fusion reached 60%.

Cell Proliferation Assay: TE13 and Eca109 cells after transfection with siRNA were digested and treated with trypsin to make a homogeneous cell suspension, which was inoculated in 96-well plates at 2×10^4 cells/ml per well, liquid-sealed, and set up with 5 replicate wells, and incubated for 48 h to allow the cells to proliferate and adhere to the wall. 10 μl CCK8 was added to each well protected from light and the absorbance at 450 nm was detected by a Waltham enzyme labeler (USA) after 4 h in a water bath at 37°C protected from light.

Cell Migration Assay: TE13 and Eca109 cells after transfection with siRNA were reconditioned with empty medium to a cell density of 1.5×10^5 cells/ml. 100 μl of empty medium and 100 μl of cell suspension were added to each well in a Transwell, and 600 μl of complete medium was added to the lower chamber, with 3 replicate wells for each group; the plates were spread out and placed in an incubator for 48 h. After the liquid in the wells in the chamber and 24 plates were discarded, they were washed with PBS, fixed in methanol for 30 min, inverted in the chamber and dried for 10-15 min, and stained with 0.25% crystal violet solution for 15 min. The lower layer of the migration cells was observed in an inverted fluorescence microscope, and pictures were taken in three fields of view, and cell counting was performed and recorded.

2.8. Mouse Subcutaneous Transplantation Tumor Formation Experiment

Male BALB/c-nude mice (6-8 weeks old) were maintained under pathogen-free conditions in the Laboratory Animal Center of China Pharmaceutical University. Subsequently, procedures were performed in accordance with the institutional ethical guidelines for animal experiments. Briefly, 2×10^6 TE13 and Eca109 cells were subcutaneously injected into the mice, respectively.

Two weeks after the inoculation, when the tumor growth of all 8 nude mice reached about 100 mm^3, the 4 mice inoculated with the same cell line were divided into two groups, and the tumors were injected with equal amounts of 10 μg of si-NC or si-VPS9D1-AS1 with 1.5 μl of JetPEI *in vivo*, and the diameter and weight of the transplanted tumors of the nude mice were measured every three days, respectively. Mice were executed after 14 days, and tumor blocks and liver and lung tissues were stripped and fixed with formaldehyde. Tumor volume was calculated according to the formula: tumor volume (mm^3) $=0.5\times length\times width^2$ and tumor growth curves were plotted.

2.9. Western Blotting

The exfoliated tumor mass was lysed with RIPA lysis solution, and the protein was extracted and quantified by BCA kit; the sample was loaded at 60 μg per well, subjected to SDS-PAGE electrophoresis, and transferred to PVDF membrane, keeping the gel and membrane moist throughout the whole process; the membrane was closed in 5% skimmed milk powder solution for 3 h; the antibody against Bcl-2 and BAX (1:500) was added, and the bed was shaken at 4°C overnight; the antibody against rabbit (1: 5000) was added and incubated at room temperature for 1 h. Using GAPDH as an internal reference protein, and the results were observed using a gel imaging system.

2.10. Statistical Methods

GraphPad Prism software was used for statistical analysis. Measurements that were normally distributed were expressed as $\bar{x}\pm s$. Comparisons of measures between two groups were performed by t-test, and comparisons of differences between multiple groups were performed by one-way ANOVA or nonparametric tests. Comparisons of normally distributed measures were performed by t-test. The test level was $\alpha = 0.05$.

3. Results

3.1. Database Validation of VPS9D1-AS1 Expression in ESCC Tissues Was Significantly Higher Than Controls

Expression of lncRNA VPS9D1-AS1 in tissues: as shown in the TCGA and GTEx databases, the expression of VPS9D1-AS1 was significantly higher in ESCC tissues when compared with normal samples ($P<0.0001$, Figure 1A, C). Kaplan Meier survival curve analysis showed that the overall survival rate of patients with VPS9D1-AS1 high-expression group was significantly lower than that of patients with low

VPS9D1-AS1 expression (P<0.01, Figure 1B), the median survival of patients with low expression of VPS9D1-AS1 (45.37 months) was 23 months longer than the patients with high expression of VPS9D1-AS1 (22.7 months). Therefore, it is proposed that VPS9D1-AS1 may play an important role in the development of esophageal squamous carcinoma.

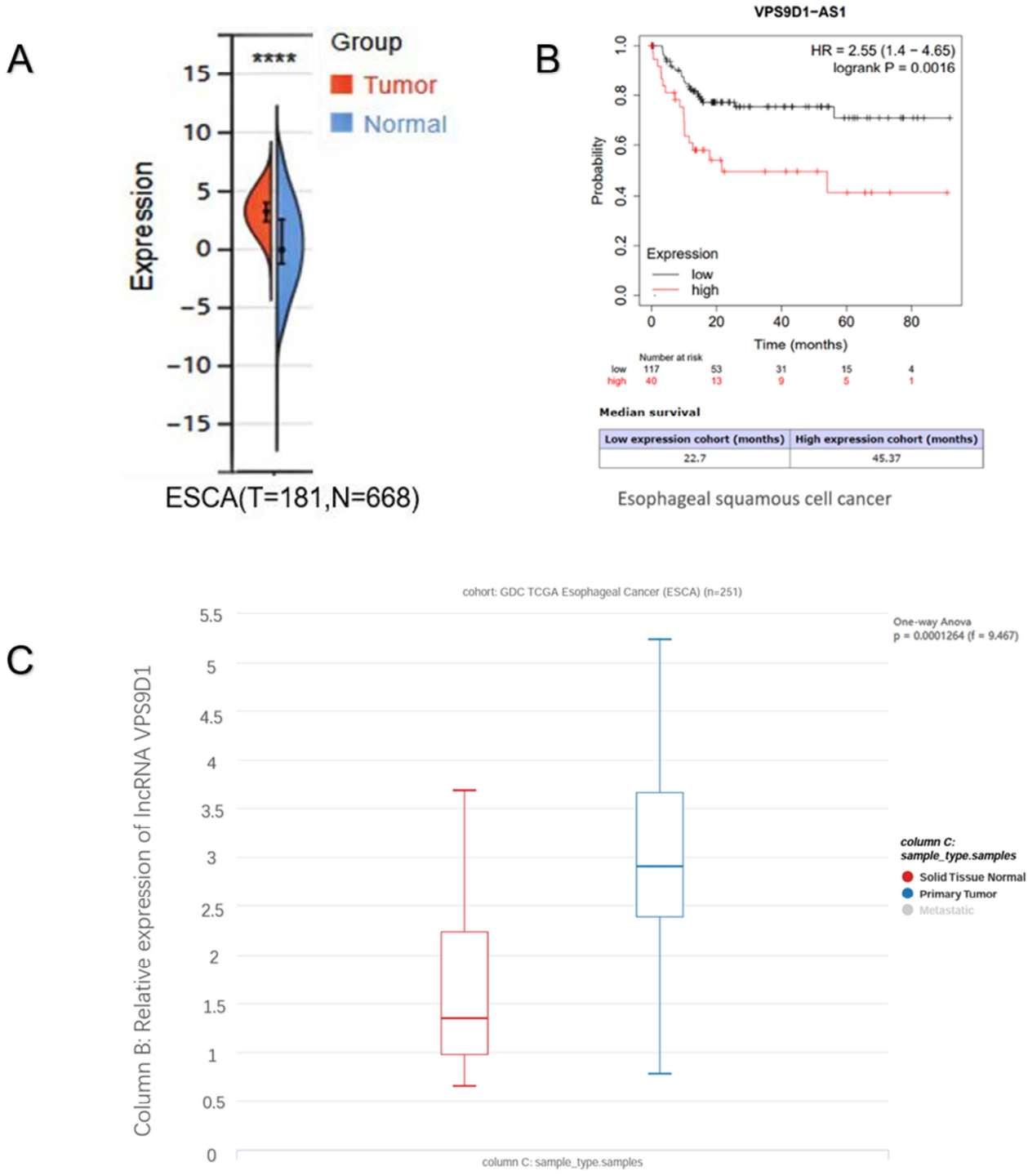

Figure 1. *Results of bioinformatics analysis. (A): Different expression multiples of VPS9D1-AS1 in ESCC and normal tissues;(B): Kaplan Meier survival curve between different VPS9D1-AS1 expression levels and overall survival rate of patients with ESCC;(C): Comparison of VPS9D1-AS1 expression stages in esophageal squamous cell carcinoma.*

3.2. High Expression of VPS9D1-AS1 in Clinical Sample Tissues

To further determine the clinical significance of VPS9D1-AS1 in patients with esophageal squamous carcinoma, we examined the clinical tissues and paracancerous tissues from 20 ESCC patients, and the results showed that VPS9D1-AS1 expression was higher than that of paracancerous tissues in 14 patients (Figure 2).

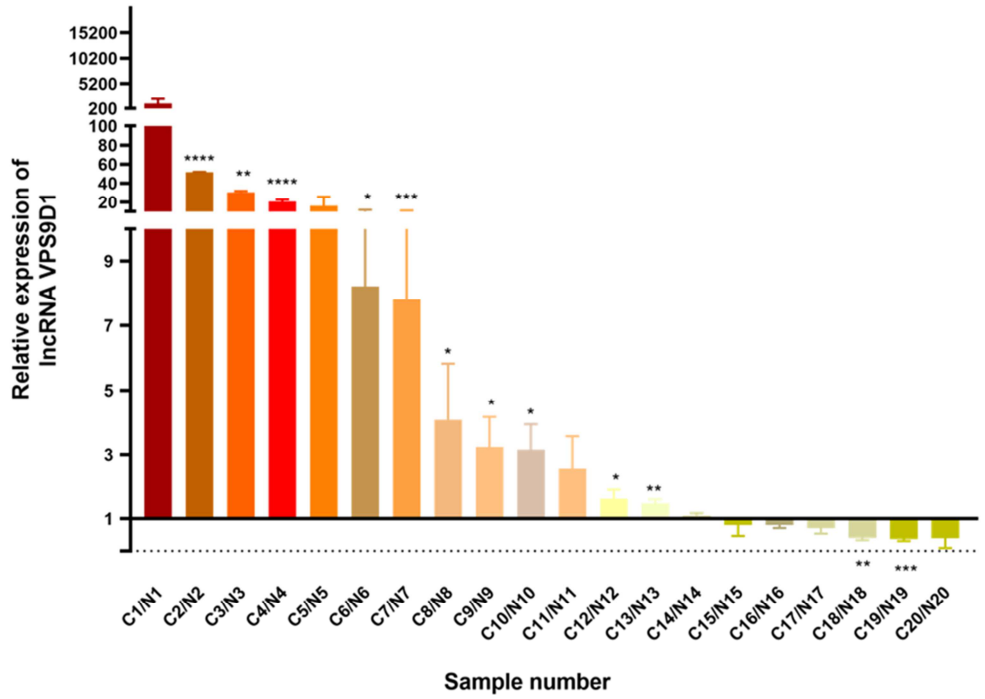

Figure 2. *Relative expression of VPS9D1-AS1 in clinical tissues and adjacent tissues of 20 cases of esophageal squamous cell carcinoma. ns: no significant difference, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.*

3.3. Silencing of VPS9D1-AS1 Expression by siRNA in ESCC Cells

To investigate the specific function of VPS9D1-AS1 in esophageal squamous carcinoma, firstly, the expression of VPS9D1-AS1 was detected in normal esophageal epithelial cell lines (HEEC) and ESCC cell lines (TE13, Eca109) using qPCR, and the results showed that the expression of VPS9D1-AS1 was significantly increased in both TE13 and Eca109 cells compared with HEEC cells ($P < 0.001$, Figure 3A). qPCR assay showed two specific siRNAs (si-VPS9D1-1 and si-VPS9D1-2) designed against the VPS9D1-AS1 sequence, and the interference efficiency of si-VPS9D1-1 was superior to that of si-VPS9D1-2 in TE13 cells (Figure 3B) and Eca109 cells (Figure 3C). Therefore, si-VPS9D1-1 was chosen for subsequent experiments.

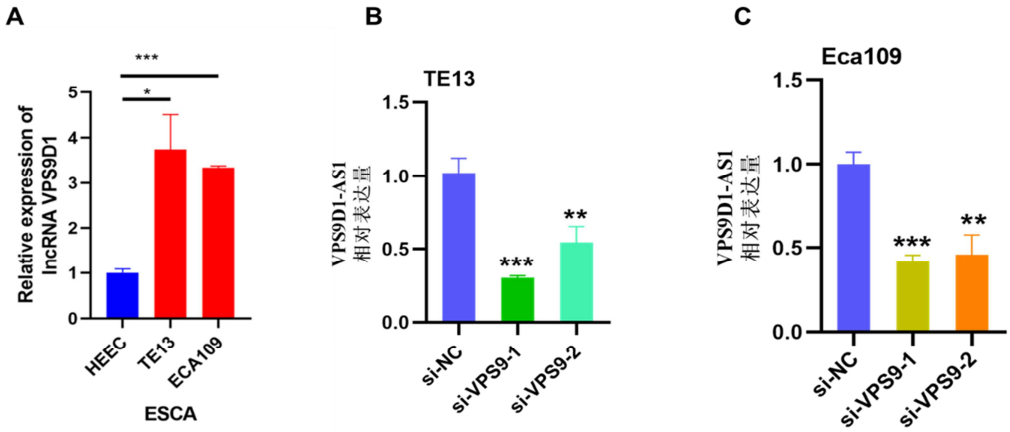

Figure 3. *Expression level of VPS9D1-AS in esophageal squamous cell carcinoma cells transfected with siRNA. (A): The relative content of VPS9D1-AS1 in esophageal squamous cell cells and normal cells; (B): TE-13 cells lines; (C): Eca-109 cells lines, **: P<0.01, ***: P<0.001, compared with si-NC.*

3.4. Silencing of VPS9D1-AS1 Inhibits the Proliferation and Migration of ESCC Cells

CCK-8 assay showed that silencing VPS9D1-AS1 significantly inhibited the proliferation of TE13 and Eca109 cells compared with the si-NC group ($P<0.001$, Figure 4A, B). Transwell migration assay also showed that silencing of VPS9D1-AS1 significantly inhibited the migratory ability of both TE13 and Eca109 cells ($P<0.001$, Figure 4C; $P<0.05$, Figure 4D). The above experiments demonstrated that VPS9D1-AS1 has an important role in the proliferation and

migration of ESCC cells.

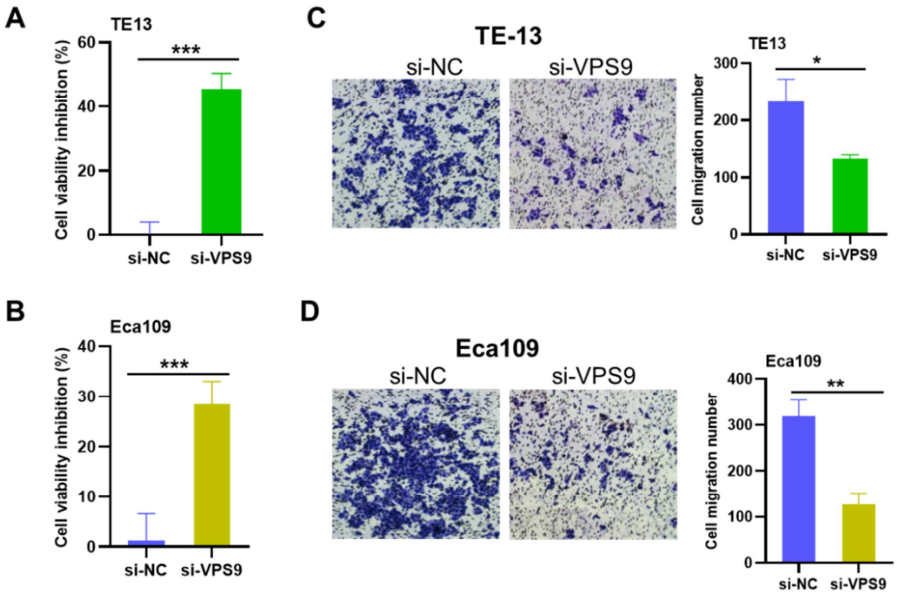

Figure 4. *Inhibition of si-VPS9 on the proliferation and migration ability of esophageal squamous cell carcinoma cells. (A, C): TE13 cell line;(B, D): Eca109 cell line, *: P<0.05, **: P<0.001, ***: P<0.001, compared with si-NC.*

3.5. Silencing of VPS9D1-AS1 Inhibits Tumor Growth of ESCC Cells in Vivo

In recent years, clinical trials of small interfering RNAs (siRNAs) as a new strategy for tumor therapy have been carried out in mesenchymal and pancreatic cancers, in order to explore whether siRNAs targeting VPS9D1-AS1 can exert effective anti-tumor effects *in vivo*. In this study, we designed and synthesized cholesterol-modified si-VPS9D1-AS1 for subcutaneous graft-tumor injection to monitor tumor growth changes in esophageal squamous carcinoma. The results showed that after the 14th day of administration, si-VPS9D1-AS1 significantly inhibited the *in vivo* tumor growth volume of two esophageal squamous carcinoma cells, TE13 and Eca109, compared with the si-NC group (P<0.001; Figure 5A-B, E-F). The mice were weighed after execution and tumor stripping, and the results showed that the tumor weights of mice in the si-VPS9D1-AS1 group were significantly lower than that in the si-NC group (P<0.001, Figure 5C; P<0.05, Figure 5G).

In addition, the expression of VPS9D1-AS1 in the transplanted tumors of nude mice was detected by qRT-PCR, and it was found that the expression of VPS9D1-AS1 in the tumor tissues of both groups injected with si-VPS9D1-AS1 was significantly lower than that of the si-NC group (P<0.0001; Figure 5D, H). It indicates that effective silencing of VPS9D1-AS1 can significantly inhibit the tumor growth of ESCC cells, and that si-VPS9D1-AS1 can be a candidate therapeutic strategy for esophageal squamous carcinoma.

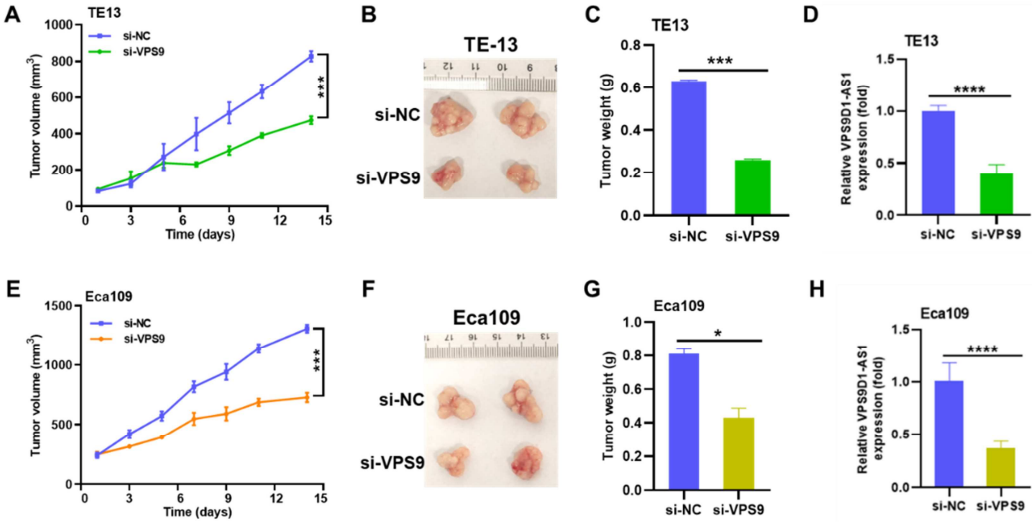

Figure 5. *Inhibition of si-VPS9 on the growth of transplanted tumor in nude mice. (A-D): Nude mice inoculated with TE13 cell line; (E-H): Nude mice inoculated with Eca109 cell line, *: P<0.05, ***: P<0.001, ****: P<0.0001, compared with si-NC.*

3.6. Silencing of VPS9D1-AS1 Promotes Apoptosis in Esophageal Squamous Carcinoma Cells

Western blot detection of the expression of apoptosis-related proteins BCL-2 and BAX in tumor tissues after administration of each group showed that si-VPS9D1-AS1 significantly down-regulated the expression of the anti-apoptotic protein BCL-2 and up-regulated the expression of the pro-apoptotic protein BAX in the transplanted tumor model tissues of both TE13 and Eca109 cells ($P < 0.001$; Figure 6A, B). It is suggested that si-VPS9 exerts its anti-tumor effects *in vivo* by promoting apoptosis of tumor cells.

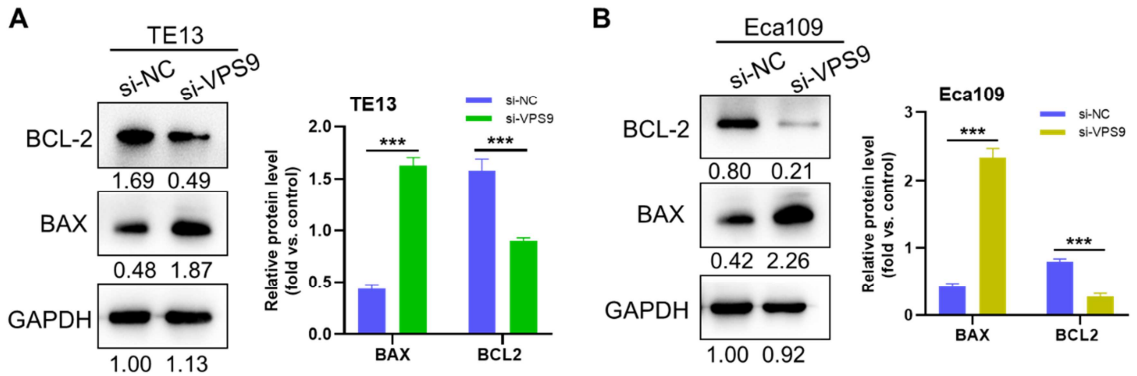

Figure 6. *Western Blot assay to detect the apoptosis-related proteins Bcl-2 and Bax expression after VPS9D1-AS1 knockdown in tumor tissue in the mouse xenografts injected. (A): Nude mice inoculated with TE13 cell line; (B): Nude mice inoculated with Eca109 cell line; ***: P<0.001, compared with si-NC.*

4. Discussion

Esophageal cancer (EC) is a malignant tumor that affects the esophageal epithelium. It can be classified into two subtypes: including esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). ESCC accounts for more than 90% of EC cases in China. ESCC is a fatal disease with a poor prognosis, as indicated by 3-year overall survival (OS) rates ranging from 26.9% to 55.4%. Furthermore, over than 50% of patients experience local or distant recurrence after undergoing concurrent chemoradiotherapy (CRT) [2]. Therefore, a better understanding of the mechanisms of ESCC tumorigenesis and the identification of screening biomarkers are of great significance for the prevention and prognosis of patients with esophageal cancer.

As the correlation between lncRNAs and the development of various cancers continues to be demonstrated [13], long non-coding RNAs (lncRNAs) have emerged as potential prognostic markers for various human cancers [14-16]. Several studies have shown that lncRNAs play important roles in regulating cell proliferation, invasion, and migration. The LINC00707 was highly expressed in ESCC and significantly higher in patients with alcohol consumption, lymph node metastasis, and higher tumor stage. It down-regulated the inhibition of ESCC cell proliferation and metastasis and induced apoptosis of ESCC cells through the activation of the PI3K/Akt signaling pathway in ESCC cells [17]; the lncRNA KDM4A-AS1-encoded peptide may impair ESCC cell viability and migration by regulating fatty acid metabolism and redox processes [18]. Currently, studies on VPS9D1-AS1 associated cancers mainly focus on colorectal cancer [19-21], endometrial cancer [22, 23], non-small-cell lung cancer [24], etc., and VPS9D1-AS1 is still rarely reported in the field of ESCC, and the mechanism of esophageal cancer has not been fully clarified. In this study, we found that the expression of VPS9D1-AS1 in ESCC was significantly higher than that in normal tissues in clinical samples. The *in vitro* activity assay showed that when the expression of VPS9D1-AS1 was down-regulated, the proliferation and migration of ESCC cells were inhibited, suggesting that VPS9D1-AS1, which is differentially expressed in ESCC cells, is likely to be involved in the process of esophageal cancer proliferation and migration, and that VPS9D1-AS1 may also be one of the biomarkers of metastasis in ESCC. Bcl-2 is a key regulatory gene in the family of anti-apoptotic proteins, and its apoptosis inhibitory genes are closely related to its anti-apoptotic effects. The Bax gene, classified as an apoptosis gene, belongs to the same family as Bcl-2, which not only antagonizes the inhibitory effect on Bcl-2, but also promotes cell apoptosis. Further experiments showed that when the expression of VPS9D1-AS1 was down-regulated, the expression level of Bcl-2 was significantly increased while that of Bax was significantly decreased. This suggests that overexpression of VPS9D1-AS1 in ESCC is associated with apoptosis.

Small interfering RNAs (siRNAs) are a class of small, double-stranded RNA molecules that interfere with the expression of specific genes with complementary nucleic acid sequences by post-transcriptional degradation of mRNAs, thus preventing the translation process. With the deepening of research, siRNAs have shown some potential in the field of tumor therapy, and clinical trials on siRNA therapy have been carried out in the fields of solid tumors, mesenchymal and pancreatic cancers [25]. However, due to the susceptibility of siRNA to degradation by nuclease and renal clearance, as well as the negative electronegativity and

large molecular weight of RNA, which make it not easy to penetrate the cellular barrier, siRNA drugs have been limited in the field of tumor-targeted therapy. In this study, in order to improve the stability of siRNA, we chose to modify hydrophobic cholesterol into si-VPS9D1-AS1 to achieve efficient membrane fusion and transmembrane delivery. The results showed that cholesterol-modified si-VPS9D1-AS1 inhibited tumor growth *in vivo*. The tumor model selected for this study was a mouse subcutaneous transplantation tumor model and siRNA drugs were injected directly around ESCC tumors using peritumoral injection, but the targeting of siRNA drugs needs further improved in order to develop clinical therapeutic strategies for siRNA drugs. Antibodies are often used as a means of tumor therapy due to their high specificity and selectivity for tumor cell surface target proteins. Zhili Yu et al [26] designed an antibody-cell-penetrating peptide-substrate peptide-siRNA coupling (Ab-CPP-SP-siRNA) to simultaneously enhance siRNA targeted delivery, site-specific release, and intracellular uptake. Although antibodies have excellent targeting and therapeutic efficacy, they also have limitations such as high immunogenicity and high cost. A nucleic acid aptamer (Aptamer) is a sequence of oligonucleotides with a three-dimensional structure that binds to a target receptor in a manner similar to antigen-antibody-specific binding, targeting a tumor or tumor microenvironment. Nucleic acid aptamers also have the advantages of high stability, relatively low cost of production by chemical synthesis, low toxicity, and low immunogenicity compared with antibodies. Camorani S et al [27] coupled aptamers with siRNA targeting programmed cell death ligand 1 (PD-L1) to achieve effective gene silencing in triple-negative breast cancer. Fang Yang et al [28] developed a self-assembled structure of bivalent aptamer and terminal-free siRNA, which was easy to prepare and effective in inducing apoptosis. The coupling of nucleic acid aptamer and siRNA shows potential in the field of tumor-targeted therapy.

5. Conclusion

The screening of lncRNA VPS9D1-AS1 in this study opens up new avenues and possibilities for the discovery of early diagnostic and prognostic markers related to esophageal cancer or the development of new targets. However, the specific mechanism of VPS9D1-AS1 in affecting the proliferation and migration of esophageal cancer cells remains to be further clarified. In this study, the TE13 and Eca109 cell lines were selected as the research subjects, while the feasibility of VPS9D1-AS1 as a prognostic biomarker in different esophageal cancer subtypes remains to be further investigated. Experimentally, it was demonstrated that cholesterol-modified siRNA VPS9D1-AS1 modified by cholesterol could effectively inhibit tumor growth. Therefore, in order to further expand the therapeutic strategy for esophageal Lynch cancer and enhance the potential of siRNA drugs in the treatment and prognosis of esophageal Lynch cancer, further attempts could be made in the future to add nucleic acid aptamers on the basis of si-VPS9D1-AS1 designed in the present study, to increase the targeting of tumor therapy.

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