Downregulation of VRK1 reduces the expression of BANF1 and suppresses the proliferative and migratory activity of esophageal cancer cells

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Abstract. Esophageal squamous cell carcinoma (ESCC) is a common malignancy worldwide. The disease has a poor prognosis and a low 5-year survival rate. Therefore, it is necessary to identify new strategies to optimize the treatment of ESCC. *Vaccinia-related kinase* (*VRK1*) and *barrier-to-autointegration factor 1* (*BANF1*) are overexpressed in ESCC. In the present study, the roles of *VRK1* and *BANF1* were explored in the development of ESCC. In the present study, the effects of small interfering (si)RNA-induced downregulation of *VRK1* on *BANF1* expression were investigated as well as the effects on proliferative and migratory activity of ESCC cells. Western blot analysis indicated that the protein expression levels of *BANF1* were decreased following siRNA depletion of *VRK1*. Furthermore, the depletion of *VRK1* expression inhibited the proliferation and migration of ESCC cell lines, and flow cytometry analysis indicated that the depletion of *VRK1* triggered cell cycle arrest mainly in the S phase. These results suggested that *VRK1* and *BANF1* may have pivotal roles in the progression of ESCC.

Introduction

Esophageal cancer is one of the most common malignant tumors and ranks as the sixth leading cause of global mortalities, and the third leading cause of death in China (1,2). Esophageal carcinoma mainly includes esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma; ESCC is the predominant subtype of esophageal cancer in developing countries accounting for >90% of all esophageal cancer subtypes in China (3). In 2018, the global cancer observatory reported 572,034 new cases of esophageal cancer and 508,585 deaths from esophageal cancer between males and females combined (2). Despite recent advances in surgical and therapeutic techniques, such as chemotherapy and radiotherapy, extensive metastasis has led to a poor 5-year survival rate of ~15-25% (4). Therefore, there is an urgent need to explore the underlying molecular mechanisms that can be used to improve the diagnosis and treatment of ESCC.

Vaccinia-related kinase (*VRK*) is a member of the serine/threonine kinase family in mammals, which performs pivotal functions by regulating a variety of cellular and physiological activities through phosphorylation reactions (5). *VRK1* encodes a protein of 396 amino acids in length and was originally discovered from a cDNA library enriched in human fetal-specific liver genes (6). *VRK1* is localized to the cell nuclei and is one of the three subtypes of *VRK*; it has exhibited important roles in cell cycle progression, transcriptional activation, chromosome condensation, DNA repair and histone modification (5,7). An increasing number of studies have found that *VRK1* expression regulates the proliferation and survival of cells in normal or malignant tissues (7-9). During embryonic development of hematopoiesis, the development of the mouse liver is accompanied with high expression of VRK1. Similarly, high expression of VRK1 has been demonstrated in regenerated liver and liver cancer, which suggests that its expression is associated with the increase of the number of cells in the early hematopoietic process (10). In addition, VRK1 is highly expressed in high-proliferating cells, such as those found in the testis, thymus and fetal liver (6). Notably, a previous study has shown that *VRK1* induces the G_1/S transition by promoting the expression of *cyclin D1* (*CCND1*) at the G_1/S phase (11). VRK1 expression has been shown to be upregulated in several types of cancer, including glioma, lung carcinomas, hepatocellular carcinoma, breast carcinomas and head and neck squamous cell carcinoma (9,12-14).

Barrier-to-autointegration factor 1 (*BANF1*) is encoded by the *BANF1* gene and is a small, highly conserved DNA-binding protein of 10 kDa in size that is located in the cytoplasm and nuclei of cells (15). *BANF1* serves a crucial role in mitotic nuclear recombination, regulation of the stability of the pre-integration complex of retroviruses and in the regulation of transcriptional function (16). Margalit *et al* (15)

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reported a linkage of genomic DNA with the nuclear envelope in the interphase of mitosis through interactions with the nuclear envelope components (lamin) and *BANF1* protein. Previous studies have also reported that phosphorylation regulates the DNA binding activity of BANF1 and its subcellular localization and dimerization (17,18). It is important to note that Ser-4 is a major phosphorylation site of BANF1 during both the interphase and the mitotic phase (19). The phosphorylation of Ser-4 abrogates the interaction of BANF1 with DNA and reduces its interaction with the LEM domain and thereby disrupts the connection between the DNA and the nuclear envelope, which in turn maintains the normal process of the cell cycle (18). Previous studies (7,20,21) have shown that *VRK1* can catalyze the phosphorylation of *BANF1*, which is a high affinity substrate for *VRK1* protein kinase (22). Nichols *et al* (22) demonstrated that VRK1 regulated the interaction between BANF1 and DNA by phosphorylation of the N-terminus of BANF1. VRK1 participated directly in the regulation of the binding of chromatin to membrane proteins and BANF1 by facilitating the phosphorylation of the latter (7,17). Results from the aforementioned studies led to the aim of the present study, which was to investigate the interaction of *VRK1* and *BANF1* and its association with the physiology of ESCC cancer cells.

VRK1 and *BANF1* expression levels were found to be elevated in ESCC tissues compared with the corresponding levels noted in adjacent non-tumor tissues. In addition, the expression levels of *VRK1* and *BANF1* were significantly associated with the clinical characteristics of patients with esophageal cancer (23). In the present study, the ESCC cell lines EC109 and EC1 were used to examine the interaction between *VRK1* and *BANF1* in ESCC. Small interfering (si) RNA was utilized to downregulate the expression of *VRK1* and the changes in the expression levels of *BANF1* were investigated in ESCC cells. In addition, changes in proliferation and migration of ESCC cells were assessed to explore the potential of this protein in targeted therapy of ESCC. Taken collectively, the evidence in the present study indicated that *VRK1* and *BANF1* may have pivotal roles during ESCC development and progression, and represent potential targets for novel ESCC treatments.

Materials and methods

Cell lines and cell culture. The human ESCC cell lines EC109 and EC1 were purchased from Sangon Biotech Co., Ltd. The cell lines were cultured and maintained in RPMI-1640 (Sangon Biotech Co., Ltd.) supplemented with 10% fetal bovine serum (Sangon Biotech Co., Ltd) at 37˚C in the presence of 5% CO_2.

Cell transfection. The siRNA sequences targeting *VRK1* were constructed by Guangzhou RiboBio Co., Ltd. A total of three siRNA sequences were designed against *VRK1*, and the efficacy of transfection was assessed using western blot analysis. The candidate *VRK1* siRNA sequences were as follows: VRK1-176 (5'-GCAGUUGGAGAGAUAAUAATT-3'), VRK1-571 (5'-GCAGCUAAGCUUAAGAAUUTT-3') and VRK1-862 (5'-CCAAUGGCUUACUGGCCAUTT-3'). The negative control siRNA (siNC) sequence was 5'-UUCUCCGAACGUGUCACGUTT-3'. Downregulation of *VRK1* by siRNA in EC109 and EC1 cells was performed using the riboFECT™ CP Reagent (Guangzhou RiboBio Co. Ltd.,) according to the manufacturer's instructions. Cells underwent transfection when in the logarithmic growth phase. EC109 and EC1 cells were cultured in 6-well plates at a density of $4x10^5$ cells/well at 37˚C, in the presence of 5% CO_2. The transfection was performed at 37˚C for 24 h when the confluency of the cells was ~30-50%. The concentration of siRNAs transfected was 50 nM. Subsequent experimentation was performed 24 h after transfection.

Western blot analysis. The protein expression levels of *VRK1* and *BANF1* were detected by western blotting in ESCC cells following siRNA transfection. Transfected EC109 and EC1 cells were collected following transfection which lasted 24 h and washed three times with ice-cold PBS. The proteins were extracted using RIPA lysis buffer (Wuhan Boster Biological Technology, Ltd.) with PMSF (Boster) and the extracts were centrifuged at 4˚C, at 12,000 x g for 15 min. The total protein concentration was determined using the BCA Protein Assay kit (Boster). Following denaturation of the proteins by boiling for 10 min, equal amount of protein samples (25 μg) were separated by 10% SDS-PAGE (Boster) and subsequently transferred to PVDF membranes (Boster). Subsequently, the membranes were blocked with TBS + Tween 20 (TBST; 0.1% Tween-20) containing 5% non-fat milk (Boster) at room temperature for 1 h and subsequently incubated with primary antibodies against *VRK1* (1:1,000; cat. no. ab211358; Abcam), *BANF1* (1:1,000; cat. no. ab231331; Abcam) and *GAPDH* (1:1,000; cat. no. ab9485; Abcam) overnight at 4˚C. The membranes were rinsed with TBST 3-5 times and incubated with horseradish peroxidase-conjugated secondary antibodies (1:1,000; cat. no. bs-40295G-HRP; BIOSS) for 1 h at room temperature. Following an additional rinse with TBST, the protein bands were visualized using the eECL Western Blot Kit (Beyotime Institute of Biotechnology) and a UVP gel imaging scanning analyzer (GENE). Protein expression analysis was performed using Image J software v1.8.0 (National Institute of Health) to calculate the relative protein expression. GAPDH was used to normalize protein expression.

Cell proliferation assay. EC109 and EC1 cell proliferation was examined by the Cell Counting Kit-8 (CCK-8; Sangon Biotech) assay. A total of $2x10^3$ cells/well were seeded in 96-well plates. In the following 3 days, 10 μl CCK-8 reagent was added in 100 μl fresh medium after culturing for 12, 24, 36, 48, 60 and 72 h and the cells were incubated at 37˚C for 2 h. The absorbance was measured at 450 nm to calculate the number of viable cells. All the assays were repeated three times.

Flow cytometry and cell cycle analysis. Cells were harvested by trypsinization without EDTA and collected by centrifugation at 400 x g in 37˚C for 5 min. The cells were resuspended and washed twice with pre-cooled PBS to obtain a single-cell suspension. The cells were fixed overnight at 4˚C with 500 μl 70% alcohol. The cell suspension concentration was estimated at $1x10^6$ cells/ml. Cell cycle analysis was performed using propidium iodide staining following treatment with RNaseA (cat. no. R1030; Beijing Solarbio Science & Technology Co.,

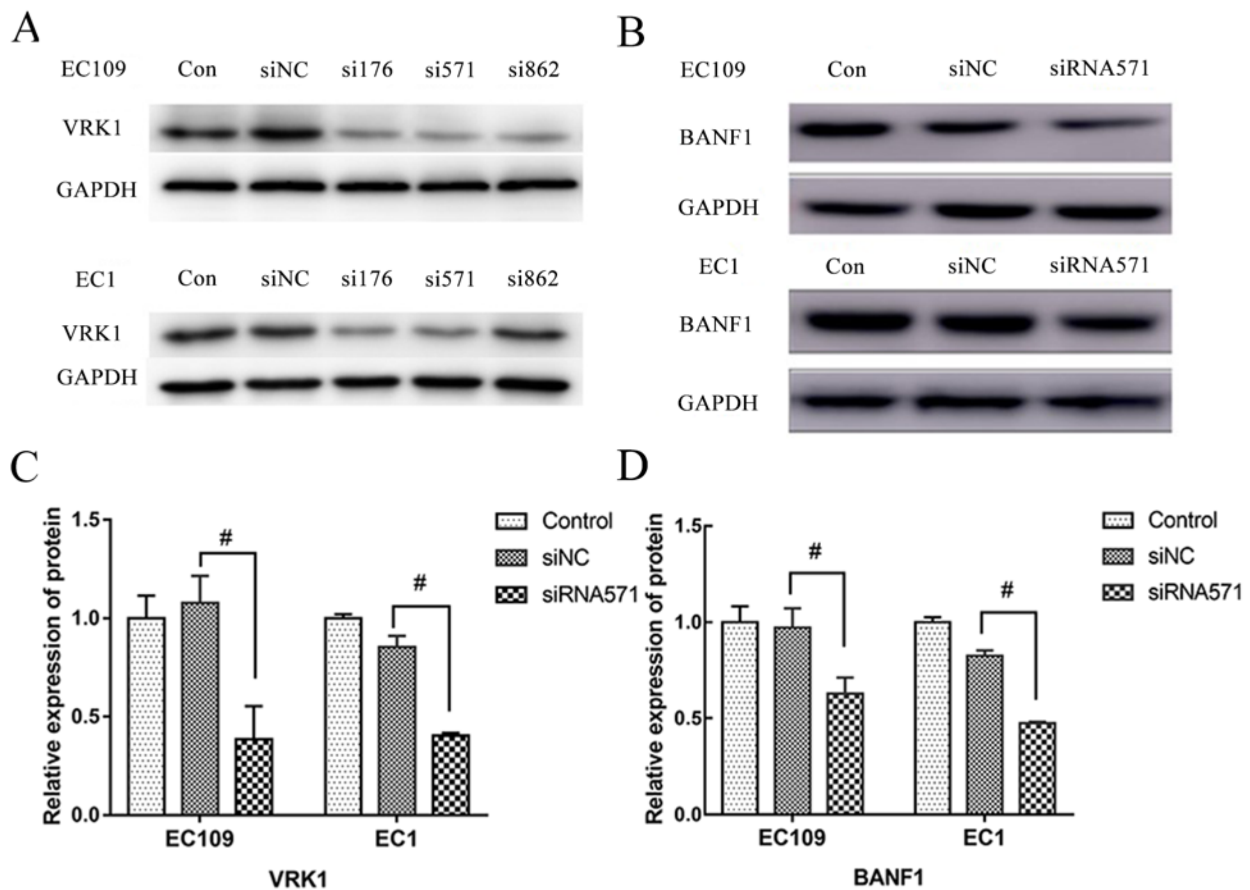

Figure 1. BANF1 expression is reduced following inhibition of the expression of VRK1 in ESCC cell lines EC109 and EC1. (A) Expression of VRK1 following transfection with gene-specific siRNAs, including VRK1-176, VRK1-571 and VRK1-862 in ESCC cell lines EC109 and EC1. In order to select a siRNA with stable transfection effect and eliminate the interference of other factors, 2 siRNAs (VRK1-176 and VRK1-571) were selected for a preliminary experiment. As the status of ESCC cells transfected with siRNA-176 was unstable, VRK1-571 was selected. (B) Expression of BANF1 was reduced after the expression of VRK1 was inhibited, as shown by western blot. (C) Semi-quantification analysis of the expression of VRK1 and BANF1 after transfection with VRK1-siRNA571 from (B). (D) Semi-quantification analysis of the expression of BANF1 after transfection with VRK1-siRNA571 from (B). #P<0.05. BANF1, barrier-to-autointegration factor 1; con, non-transfected control; ESCC, esophageal squamous cell carcinoma; NC, negative control; siRNA, small interfering RNA; VRK1, vaccinia-related kinase.

Ltd.) to remove contaminating RNA. The contents of DNA in each phase (G_0/G_1, S and G_2/M) were detected using a FACSAria flow cytometer (BD Biosciences) and ModFit LT software v.4.0 (Verity Software House, Inc.).

Transwell migration assays. The Transwell migration assay was performed to detect the changes of the migratory ability of ESCC cells following transfection with siRNAs. The migration assay was performed by a Transwell system (8.0 μm pore size; 24-well insert). The cells were seeded at a density of $5x10^4$ cells in FBS-free DMEM (Sangon Biotech Co., Ltd) to the permeable membrane of the insert in the upper chamber. The lower chamber was filled with 600 μl DMEM containing 10% FBS (Sangon Biotech Co., Ltd). Following incubation for 24 h at 37˚C, the cells left on the upper surface of the insert were carefully removed with a cotton swab and the migrated tumor cells on the lower surface were stained by crystal violet for cell number determination.

Statistical analysis. All experimental data were analyzed using the SPSS software package, v21.0 (IBM Corp.). The GraphPad Prism software (v6.0; GraphPad Software, Inc.) was used for graph preparation. Data were analyzed by Student's t-test, χ^2 or ANOVA for multiple groups followed by a Bonferroni's post hoc test. The results are expressed as the mean ± standard deviation, and P<0.05 was considered to indicate a statistically significant difference.

Results

Depletion of VRK1 results in downregulation of BANF1 expression. A total of three siRNA sequences including VRK1-176, VRK1-571 and VRK1-862 were specifically designed and synthesized for transfection into EC109 and EC1 cells, respectively. Following culture for 24 h, siVRK1-571 exhibited the highest reduction efficiency of *VRK1* protein expression and was selected as the siRNA to be used in the subsequent experiments (Fig. 1A). Western blot analysis indicated no significant difference in *VRK1* protein expression between the blank control group and the siNC-transfected group of the EC109 and EC1 cell lines (Fig. 1C). However, the expression levels of *VRK1* in the siVRK1-571 interference group were significantly lower compared with those in the siNC group, with a decrease of 62.40 and 52.14% in the

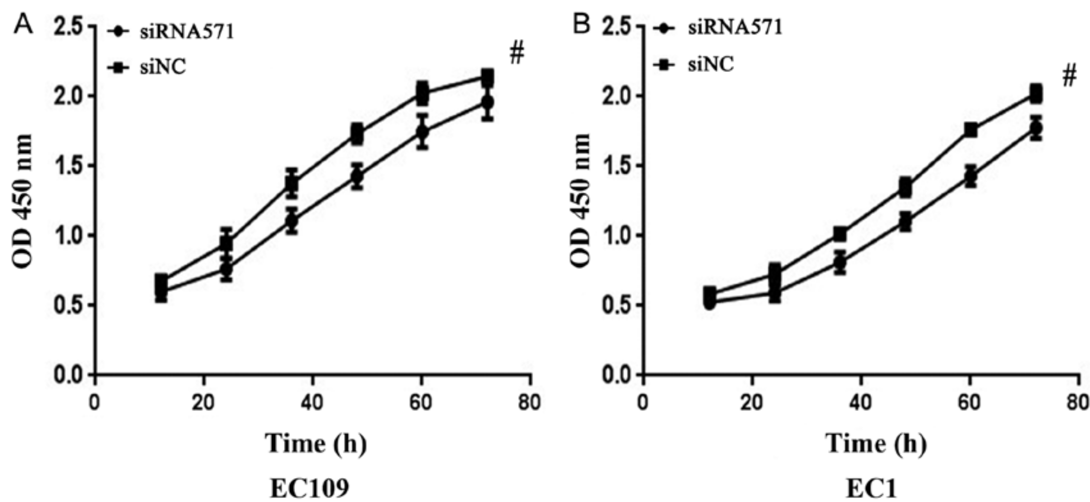

Figure 2. Proliferation of ESCC cells detected via CCK-8 assay. Growth curves of (A) EC109 and (B) EC1 cells transfected with either siNC or siRNA571. #P<0.05 vs. siNC. NC, negative control; siRNA, small interfering RNA.

EC109 and EC1 cell lines, respectively (P<0.05). These results demonstrated that the expression of *VRK1* was downregulated by siRNA specifically and effectively.

Furthermore, western blot analysis demonstrated that the protein expression levels of *BANF1* in siVRK1-571-transfected cells were significantly lower compared with those of the siNC-transfected control group, with a decrease of 24.51 and 52.87% in the EC109 and EC1 cell lines, respectively (P<0.05; Fig. 1B and C). In contrast to these findings, *BANF1* protein expression levels did not reveal a significant difference between the blank and the negative control groups.

Depletion of VRK1 suppresses the proliferation of ESCC cells. CCK-8 cell proliferation analysis was conducted on the EC109 and EC1 cell lines to investigate the effects of *VRK1* on the proliferation of ESCC cells. Following 12 h of transfection, the cells proliferated less effectively and the inhibition rate of EC109 and EC1 cells reached the maximum effect at 48 and 60 h after transfection, respectively (P<0.05; Fig. 2). The results demonstrated that downregulation of *VRK1* significantly inhibited the proliferative ability of EC109 and EC1 cells.

Depletion of VRK1 triggers cell cycle arrest in ESCC cells. To further verify the effect of *VRK1* on ESCC cell proliferation, *VRK1* was depleted and the effects on cell cycle of ESCC cells were examined using flow cytometry. Flow cytometric analysis of EC109 cells indicated that depletion of *VRK1* led to a significantly increased population of cells in the S phase compared with that noted in the negative control group (P=0.003; Fig. 3). The percentage of cells treated with siVRK1-571 at the G_0/G_1 and G_2/M phases was significantly lower compared with that noted in the siNC group ($P_{G0/G1}$=0.005; $P_{G2/M}$=0.001). Similarly, in EC1 cells, the percentage of cells treated with siVRK1-571 at the G_2/M phase was significantly lower compared with that in the negative control group (P=0.022). The percentage of cells treated with siVRK1-571 at the S phase of the cell cycle was higher than that noted in the negative control group (P=0.023; Fig. 3). The results further demonstrated that depletion of *VRK1* induced cell cycle arrest at the S phase, which in turn resulted in inhibition of cell proliferation of ESCC cells.

Depletion of VRK1 inhibits ESCC cell migration. Metastasis is a critical problem during cancer therapy. Therefore, the Transwell assay was used to assess whether loss of *VRK1* could affect tumor migratory activity. The results demonstrated that siRNA-based depletion of *VRK1* significantly reduced the number of migrating cells compared with that noted in the blank control (CON) and the negative control (siNC) groups (Fig. 4). These results indicated that *VRK1* may serve an essential role in the migration of ESCC.

Discussion

Previous reports suggested that *VRK1* has a pivotal role in the regulation of a variety of cellular physiological activities; in various cancers, the expression levels of *VRK1* have been significantly associated with cancer progression and prognosis (9,12,13,24). In addition, *BANF1* has been proven to be a valuable member in regulating the reassembly of the nuclear envelope and maintaining appropriate nuclear architecture during mitosis (15). Certain studies (20,21) have suggested that *BANF1* is an efficient substrate for *VRK1* as *VRK1* phosphorylates the N-terminus of *BANF1* (22). These findings suggested that *VRK1* and *BANF1* were closely associated with cell cycle regulation, which led to the hypothesis that the expression of *BANF1* and *VRK1* may contribute to the development of ESCC. This hypothesis was examined in the present study to provide additional information with regard to the molecular mechanisms of action during ESCC pathogenesis.

In a previous study conducted by our group, the mRNA and protein expression levels of *VRK1* and *BANF1* were higher in tumor tissues compared with those noted in the adjacent non-cancerous tissues, as determined by RT-qPCR and immunohistochemical analyses (23). Furthermore, the expression levels of *VRK1* and *BANF1* were associated with the tumor, node, metastasis (TNM) stage and the differentiation degree of ESCC patients (23). In the present study, *VRK1* expression was depleted by siRNA in ESCC cell lines and, consequently, the expression levels of *BANF1* were significantly downregulated. In addition, in the present study it was demonstrated that the proliferative activity of EC109 and EC1

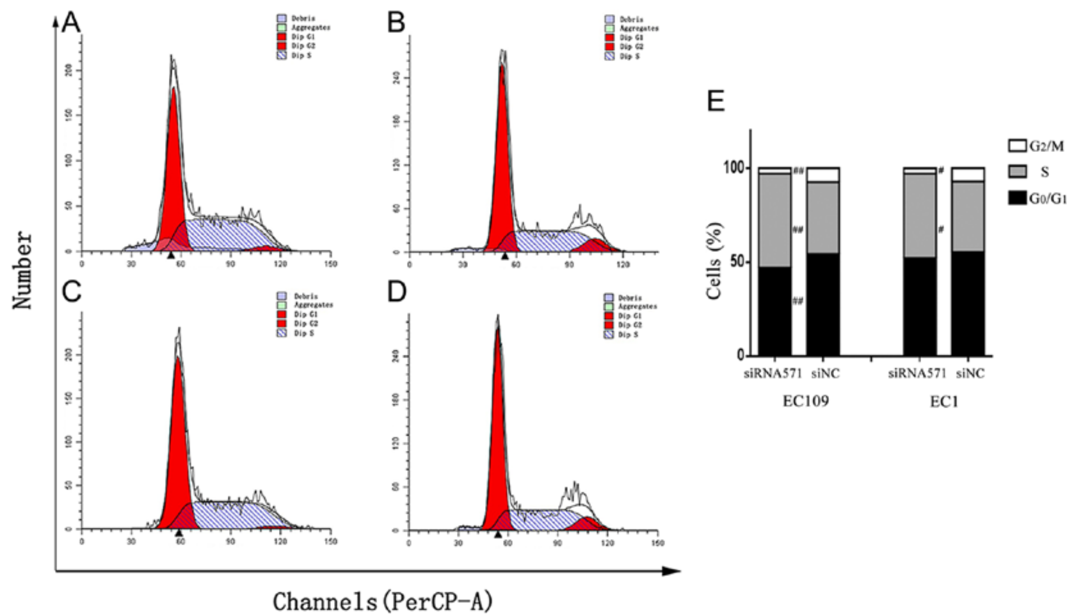

Figure 3. Cell cycle assay. Cell cycle distribution of EC109 and EC1 cells was determined by flow cytometry. (A) EC109-siRNA571, (B) EC109-siNC, (C) EC1-siRNA571 and (D) EC1-siNC, (E) Cell cycle percentage histogram of the G2/M, S and G0/G1 phases of EC109 and EC1 cells. $^{\#}P<0.05$ and $^{\#\#}P<0.01$ vs. siNC. NC, negative control; si, small interfering RNA; VRK1, vaccinia-related kinase 1.

cells were significantly reduced following inhibition of *VRK1* expression. Flow cytometric analysis further revealed that the cell cycle was inhibited mainly at the S phase. Moreover, Transwell assays indicated that *VRK1* depletion significantly suppressed the migratory ability of ESCC cells. In conclusion, depletion of *VRK1* resulted in the downregulation of *BANF1* and suppressed the proliferation and migration of ESCC cells.

A growing body of evidence indicates that *VRK1* has an important role in the development of tumors. In hepatocellular carcinoma, the expression levels of *VRK1* in cancer tissues were higher than those noted in normal and adjacent tissues (12). In breast cancer, although high expression levels of *VRK1* exhibited a protective effect on DNA damage, they also led to poorer disease prognosis (25). Therefore, *VRK1* may represent a potential prognostic indicator for breast cancer (24,25). Further experiments in mammary epithelial cells demonstrated that depletion of *VRK1* inhibits their proliferation and metastasis *in vitro* and *in vivo* (8). *VRK1* has also been suggested as a proliferative marker in head and neck squamous cell carcinoma and as a potential drug target in breast cancer and lung adenocarcinomas (9,14,25). In addition, *VRK1* has an important role in the stress response of DNA damage induced by ionizing radiation and ultraviolet radiation (26,27). In the present study, the increased *VRK1* levels in ESCC were consistent with the results noted in other types of cancer, such as hepatocellular carcinoma and breast cancer (12,13). Therefore, the data suggested that *VRK1* may contribute to the progression of the ESCC. In the present study, the CCK-8 cell proliferation assay demonstrated that the proliferative activity was decreased in EC109 and EC1 cells following depletion of the expression of *VRK1*. Moreover, Transwell experiments indicated that the migratory ability of ESCC cells was also reduced. Given that the reduction of *VRK1* expression could inhibit the proliferation and migration of ESCC cells, it is hypothesized that *VRK1* could represent a potent new target for the treatment of esophageal cancer.

BANF1 has been shown to bind to double-stranded DNA at the LEM domain of transcriptional regulators and at the histone H3 protein, which is localized in the nucleus (16). Previous findings suggested that lamin, LEM-domain proteins and *BANF1* performed essential functions in chromatin organization and cell division (28). *Lamin-A/C*, *lamin-associated polypeptide 2α* and *BANF1* proteins constitute protein complexes that regulate mitotic spindle assembly and localization during mitosis (29). In addition, *BANF1* was directly involved in the formation of the nuclear envelope (NE). These results revealed the essential role of *BANF1* in regulating the mitotic process and normal cell cycle progression. Moreover, *BANF1* has been reported to be a novel biomarker for gastric cancer (30). In one of our previous studies, it was shown that *BANF1* was highly expressed in ESCC and that its expression was associated with the TNM stage and the tumor differentiation degree of ESCC patients (23). The data suggested that the highly expressed levels of *BANF1* may lead to an abnormal mitotic process of ESCC cells through regulation of the interaction of DNA with perinuclear proteins and the assembly and localization of the spindle filaments.

Notably, *BANF1* has also been established as a high-affinity substrate of *VRK1*. In addition, it has been shown that depletion of *VRK1* affects the interaction between *BANF1* and DNA, thereby affecting nuclear membrane structure and mitotic chromosomal dynamics (31). Together, these studies suggested that *VRK1* and *BANF1* were closely associated with the regulation of normal mitosis. The essential role of *VRK1* and *BANF1* in regulating the mitotic nuclear reassembly indicates that the abnormal expression of these two proteins in ESCC may affect the normal cellular functions, such as nuclear organization and cell cycle progression. Based on the high expression of *VRK1* and *BANF1* in ESCC, the present study demonstrated that the

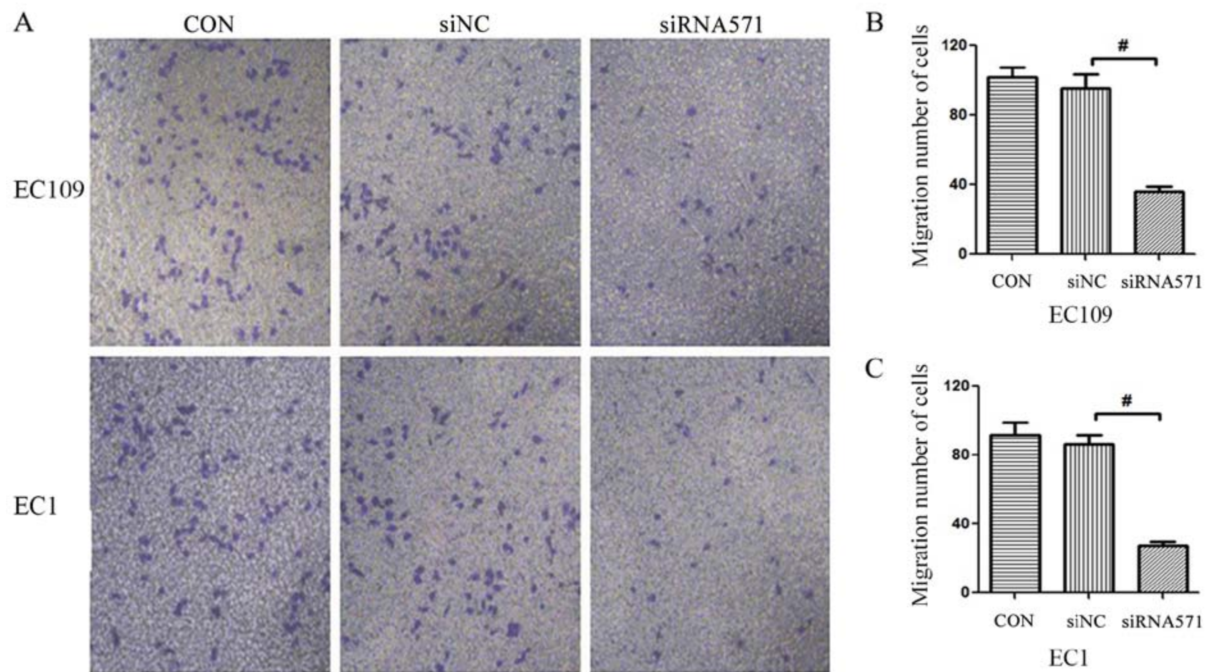

Figure 4. Transwell migration assays. (A) Migratory abilities of EC109 and EC1 cells following transfection and cell culture for 12 h. Comparison of the migratory abilities of (B) EC109 and (C) EC1 cells among different groups; magnification x200. $^{\#}P<0.05$. siNC, siRNA negative control; siRNA571, siRNA targeting VRK1-571.

BANF1 protein was downregulated following depletion of the expression of *VRK1* in ESCC cells. These findings indicated a putative interaction between *VRK1* and *BANF1* in promoting the development of ESCC.

The present study data indicated that the depletion of *VRK1* and the subsequent downregulation of *BANF1* resulted in changes in the cell cycle distribution, which mainly manifested as arrest of the cell cycle at the S phase. The results indicated that the depletion of *VRK1* may affect cell proliferation by blocking cell cycle progression. These results support previous studies (9,11,12). Previous research in head and neck squamous cell carcinoma suggested that VRK1 plays a role in cell cycle regulation and may be a new control mechanism of cell cycle, particularly late in G1-S phase (9). In addition, Valbuena *et al* (11) demonstrated that the elimination of VRK1 by siRNA results in a G1 block in cell division. As a member of the novel *VRK* protein family, *VRK1* can phosphorylate the Thr-18 region of *p53*, a vital tumor suppressor protein (32). The region that becomes phosphorylated comprises the *MDM-2* binding domain and is required for maintaining *p53* stability (33). *VRK1* has been shown to be a key regulator of *p53* and to control cell proliferation. A study by Waters *et al* (34) further revealed that *VRK1* promoted germ cell proliferation by preventing *p53* from triggering abnormal cell cycle arrest. An additional study reported a newly formed autoregulatory loop between *p53* and *VRK1* (35). Therefore, *VRK1* has been regarded as an upstream regulator of *p53*, which participates in the integration of various cell signals by *p53* (36). In addition, *VRK1* can phosphorylate other transcription factors, such as *c-Jun* and *ATF*, which play essential roles in cell cycle regulation (37,38). Valbuena *et al* (11) demonstrated that the loss of *VRK1* led to the block of the cell cycle. Similar findings were presented in the current report highlighting that the downregulation of *VRK1* triggered cell cycle arrest at the G_1 phase. In addition, *VRK1* is a regulator of *cyclin D1* (*CCND1*) expression in the DNA replication period (39). *VRK1* is also known to phosphorylate histone H3 to regulate chromatin condensation (40,41). Collectively, these results suggest that further study of the interaction between VRK1 and transcription factors such as p53 may be a meaningful direction for exploring the mechanism of cell cycle regulation in ESCC. However, further experiments are required to confirm this assumption. *BANF1* was also reported to perform crucial functions in both the mitotic phase and the cell cycle interphase (42). The reduction or loss of *BANF1* expression caused the aberrant cell cycle progression or phenotype. For example, *BANF1*-null *Drosophila* flies present various cell phenotypes that involve cell cycle arrest, chromatin clumping, abnormal lamin distribution and nuclear lamina structure (43). These findings indicated that the depletion of *BANF1* could affect cell cycle progression. In the present study, *BANF1* expression was decreased following depletion of the expression of *VRK1*. Therefore, it was hypothesized that *VRK1* may regulate abnormal cell proliferation by affecting *BANF1* expression, which may be a possible mechanism in the process of esophageal cancer development.

In conclusion, results from the present study indicated that downregulation of *VRK1* suppressed the proliferative and migratory ability of ESCC cells *in vitro* and suggested that *VRK1* may serve as a therapeutic target in the treatment of ESCC. Furthermore, *VRK1* depletion suppressed *BANF1* expression. Taken collectively, the aforementioned findings suggested a potential connection between *VRK1* and *BANF1* in the development of ESCC. The results presented may be used to further examine the interaction between *VRK1* and *BANF1* in the progression of ESCC.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

ZR, JG, CX and XL performed the experiments. ZR wrote the manuscript. ZR, YL and JL analyzed and interpreted the data and revised the manuscript for important intellectual content. HL participated in the design of the research, and was responsible for the guidance in the experimental process and gave the approval for the final version of the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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