Identification of AK4 as a novel therapeutic target for serous ovarian cancer

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Abstract. The present study aimed to assess the expression level of adenylate kinase 4 (AK4) in human serous ovarian cancer (SOC) tissues and investigate the possible involvement of AK4 in SOC progression. Bioinformatics analysis based on The Cancer Genome Atlas (TCGA) database and immunohistochemical (IHC) assays were performed to assess the expression level of AK4 in human SOC tissues. Clinical pathological features of patients with SOC were also evaluated. Colony formation, MTT, wound healing and Transwell assays were conducted to investigate the effects of AK4 on the proliferation, migration, and invasion of SOC cells *in vitro*. Mouse xenograft and lung metastasis models were developed to evaluate the effects of AK4 on tumor growth and metastasis *in vivo*. High expression levels of AK4 were identified in human SOC tissues compared with in normal tissues according to TCGA database and the results of IHC assays. A contribution of AK4 to tumor growth and metastasis of SOC cells *in vivo* was also shown. The present study confirmed the involvement of AK4 in the progression of SOC, and the results indicated that AK4 could serve as a novel therapeutic target for SOC treatment.

Introduction

Ovarian cancer is a common malignancy of the female reproductive system, with the second highest incidence and the highest mortality among cancers worldwide (1-3). Ovarian cancer is asymptomatic in its early stages, and the rapid growth of ovarian cancer makes its diagnosis and treatment challenging (4,5). Serous ovarian cancer (SOC) is the main type of ovarian cancer, which progresses quickly, lacks effective treatment and is prone to bladder metastasis (6). At present, surgery supplemented by chemotherapy remains the main treatment option for advanced stages of SOC, and treatment outcomes are unsatisfactory (7). In recent years, targeted therapy has shown advantages for SOC treatment (8,9). However, in order to treat SOC effectively, more effective therapeutic targets need to be developed (10).

Adenylate kinase 4 (AK4) is a ubiquitous enzyme and a member of adenylate kinases with multiple biological functions on cellular metabolism, such as maintaining the homeostasis of cellular nucleotides and controlling cellular ATP levels by regulating phosphorylation and activation of the energy sensor protein kinase AMPK (11,12). Human AK4 was initially named AK3, due to its 58% homology with the bovine AK3, and was subsequently renamed as AK4 when identified in the mammalian central nervous system (13). Additionally, AK4 contains an N-terminal mitochondrial import sequence which mediates localization to the mitochondrial matrix (14). AK4 is widely expressed in multiple types of tissues such as kidney, heart and liver (15). AK4 was also reported to participate in protection from oxidative stress (16).

The involvement of AK4 in the progression of multiple types of cancer has been investigated (17-21). AK4 has been associated with the clinical features of patients with lung cancer and promoted the metastasis of lung cancer by down-regulating ATF3 (17,18). Another study on lung cancer found that AK4 could modulate oxidative stress and stabilize HIF-1α to promote the metastasis of lung adenocarcinoma (19). Similarly, AK4 contributed to bladder cancer cell proliferation and invasion *in vitro* and *in vivo* (20). In esophageal cancer, microRNA (miR)-199a-3p, regulated the radioresistance of cancer cells by targeting AK4 (21). However, the possible role of AK4 in the progression and metastasis of SOC remains unclear.

In the present study, the expression levels of AK4 were investigated in human SOC tissues to investigate the role of AK4 in the progression of SOC and to evaluate the potential of AK4 as a therapeutic target for SOC treatment.

Materials and methods

Antibodies, primers and short hairpin (sh)RNA plasmids. Rabbit anti-AK4 [cat. no. ab131327; immunohistochemical (IHC) assay dilution, 1:100; western blotting assay

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dilution, 1:1,000; Abcam] and mouse anti-β-actin (dilution, 1:2,000; cat. no. ab8226; Abcam) antibodies were used.

The following primers were used for reverse transcription-quantitative PCR (RT-qPCR): AK4 forward, 5'-AKATGG ACCGTGTGCTGCTGAAGT-3' and reverse, 5'-TCCGAA ACTTCTCTCCTGGCTC-3'; and GAPDH forward, 5'-GAG TCAACGGATTTGGTCGT-3' and reverse, 5'-TTGATTTTG GAGGGATCTCG-3'.

AK4 shRNA plasmids (cat. no. sc-38908-SH) were purchased from the Santa Cruz Biotechnology, Inc. The shRNA sequence specifically targeting AK4 was as follows: Sense, 5'-AACTTTGGTCTCCAGCATCTCTC-3'. The negative control (NC) shRNA plasmids used in the present contained a non-coding shRNA fragment. The NC shRNA sequence was as follows: Sense 5'-UUCUCCGAGCGUGUCACGUTT-3'.

Bioinformatic analysis. Bioinformatic analysis was conducted via Gene Expression Profiling Interactive Analysis (GEPIA; http://gepia.cancer-pku.cn/detail. php?gene=AK4/) to analyze The Cancer Genome Atlas (TCGA; https://www.cancer.gov/about-nci/organiza-tion/ccg/research/structural-genomics/tcga) data with a threshold of $P<0.05$ and Log(fold-change) >1 or <-1 for differential genes. The median of the survival rates was used as the basis for dividing patients into two groups (low and high AK4 expression) for Kaplan-Meier survival analysis.

Human tissue sample collection and analysis. A total of 98 SOC tissues and the corresponding adjacent normal tissues (5 mm from the tumor tissues) in the current study were collected from patients receiving surgical therapy at the Second Hospital of Lianyungang (Lianyungang, China) between July 2017 and June 2019. The clinical-pathological characteristics, such as patient age, gender, tumor stage and grade, lymph node metastasis, recurrence, and vascular invasion are listed in Table I.

To explore the possible association between the expression level of AK4 and SOC progression, IHC assays were performed. Briefly, sections were fixed with 4% paraformaldehyde (PFA) at room temperature for 20 min and embedded into paraffin. Subsequently, human tissue sample sections (5-μm-thick) were deparaffinized at 60°C for 60 min and washed with xylene. Rehydration was performed in a descending alcohol series. Subsequently, sections were blocked with 2% BSA (Sigma-Aldrich; Merck KGaA) for 30 min at room temperature. Slides were incubated with the aforementioned AK4 antibody (cat. no. ab131327; 1:100; Abcam) at room temperature for 2 h. Subsequently the sections were incubated with a biotinylated secondary antibody (cat. no. ab99807; 1:100; Abcam) for another 1.5 h at room temperature, and 3,3'-diaminobenzidine was used as a chromogen substrate. A light microscope (IX71; Zeiss AG) was used for imaging at x100 and x200 magnification.

In brief, the percentage of cells with positive staining was scored as follows: 0, 0% stained cells; 1, 1-20% stained cells; 2, 21-60% stained cells; and 3, 61-100% stained cells. The following scoring was used for staining intensity: 0 (no staining), 1 (low staining), 2 (moderate staining) and 3 (high staining). The expression levels of AK4 were classi-fied based on the following staining index: Score of staining intensity + score of staining cell percentage. Staining index <3

was considered as low expression, while staining index ≥3 was considered as high expression.

Cell culture and transfection. The human SOC cell lines, CAOV3 and OVCAR3, were purchased from American Type Culture Collection. CAOV3 and OVCAR3 cells were incubated in DMEM and RPMI-1640 medium, respectively, supplemented with 10 and 20% FBS (Gibco; Thermo Fisher Scientific, Inc.), respectively, in a 5% CO_2 incubator at 37°C with 100 U/ml penicillin and 0.1 mg/ml streptomycin.

The aforementioned AK4 shRNA plasmids were transfected into both CAOV3 and OVCAR3 cells using Lipofectamine® 2000 (cat. no. 11668019; Invitrogen; Thermo Fisher Scientific, Inc.). AK4 knockdown was confirmed in both CAOV3 and OVCAR3 cells, two of the most commonly used *in vitro* models for ovarian cancer. In 6-well plates, 5 μl transfec-tion reagent and 1.5 μg shRNA plasmids were mixed in 300 μl serum-free DMEM, left to stand for 5 min and then mixed. Following incubation at room temperature for 20 min, the mix was added to serum-starved cells and incubated at 37°C for 4 h. For the control group, the shRNA targeting sequence was nonsense and did not target intracellular RNAs. Only CAOV3 cells were used for the *in vivo* assays, since these cells are used more often *in vivo*, for which the stable AK4-knockdown cells were used. CAOV3 cell line with stable AK4 depletion were screened through shRNA plasmid transfection and used for the xenograft and lung metastasis assays *in vivo*.

RT-qPCR assay. TRIzol® (cat. no. 15596026; Invitrogen; Thermo Fisher Scientific, Inc.) was used to extract total RNA from human CAOV3 and OVCAR3 cells. Total RNA was reverse transcribed into cDNA using a cDNA synthesis system (cat. no. 6110A; Takara Bio, Inc.) at 42°C for 1 h. qPCR was performed using a SYBR Ex Taq kit (cat. no. 638319; Takara Bio, Inc.). The following thermocycling conditions were used for qPCR: Initial denaturation at 95°C for 3 min; followed by 30 cycles of denaturation at 95°C for 30 sec, annealing at 58°C for 30 sec and extension at 72°C for 30 sec. The $2^{-\Delta\Delta Cq}$ method was used to quantify the results (22). AK4 expression levels were normalized to the expression of GAPDH.

Western blotting assays. CAOV3 and OVCAR3 cells or tissues from mice were lysed using RIPA buffer (cat. no. 9800; Cell Signaling Technology, Inc.). The BCA method was used for protein determination. Proteins (20 μg/lane) were sepa-rated via 8% SDS-PAGE and were transferred onto PVDF membranes. After blocking with 5% milk in TBS with 0.5% Tween-20 at room temperature for 2 h, the membranes were incubated at room temperature for 2 h with the aforementioned primary antibodies: Rabbit anti-AK4 (cat. no. ab131327; 1:1,000; Abcam) and mouse anti-β-actin (cat. no. ab8226; 1:3,000; Abcam). Subsequently, the PVDF membranes were incubated with HRP-conjugated goat anti-mouse and anti-rabbit secondary antibodies (1:5,000; cat. nos. ab6789 and ab6721, respectively; Abcam) for 45 min at room temper-ature. Signals were detected using an ECL kit (Novex™ ECL Chemiluminescent Substrate Reagent kit; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The blots were semi-quantified using ImageJ v1.8.0 software (National Institutes of Health).

Table I. Association between AK4 expression level and clinicopathological characteristics in 98 patients with serous ovarian cancer.

Characteristic	Number of patients	AK4 expression Low (n=50)	High (n=48)	χ^2	P-value
Age, years				0.428	0.513
<55	58	28	30		
≥55	40	22	18		
Tumor size, cm				6.862	0.009
<10	46	17	29		
≥10	52	33	19		
Preoperative chemotherapy				2.081	0.149
Yes	44	26	18		
No	54	24	30		
Differentiation				2.506	0.113
Low	32	20	12		
High	66	30	36		
FIGO stage				5.176	0.023
I-II	42	27	15		
III-IV	56	23	33		
Lymph node metastasis				1.440	0.230
Yes	53	30	23		
No	45	20	25		

FIGO, The International Federation of Gynecology and Obstetrics; AK4, adenylate kinase 4.

Colony formation assays. A total of 1,000 CAOV3 and OVCAR3 cells were seeded into a 6-well culture plate and transfected with NC or AK4 shRNA plasmids. For six-well plates, 6 μl transfection reagent and 1.5 μg the plasmids were added into 300 μl of serum-free medium, incubated for 5 min at room temperature and then mixed. After additional incubation at room temperature for 15 min, the mix was added to serum-starved cells for 4 h at 37°C.

The culture medium was replaced with 2 ml fresh medium every 2 days. After 14 days of incubation at 37°C and 5% CO_2, cells in the six-well plate were fixed with 4% PFA for 30 min at room temperature and stained with 0.2% crystal violet at room temperature for 20 min, then washed with PBS. The number of colonies was manually counted.

MTT assay. CAOV3 and OVCAR3 cells were seeded into 96-well plates at a density of ~500 cells/well, transfected with NC or AK4 shRNA plasmids, as aforementioned, and incubated for 24 h. Cells were then incubated with MTT for 4 h at room temperature. Subsequently, the culture medium was removed and the cells were washed with PBS. Then 150 μl DMSO was added into each well to dissolve the purple formazan, and the absorbance value was measured with a microplate reader at a wavelength of 570 nm.

Wound healing assays. The wound healing assay was used to determine cell migration. In brief, CAOV3 and OVCAR3 cells were transfected with the aforementioned plasmids. Subsequently, the cells were wounded at 100% confluence by scraping with a 20-μl pipette tip, followed by washing. Cells were serum-starved prior to and during the assay. Images were captured at 0 and 24 h to evaluate the migration degree of cancer cells. A light microscope (magnification, x20; IX71; Zeiss AG) was used for imaging, and ImageJ software (v1.8.0) was used to calculate the wound area.

Cell invasion assays. Cell invasion was measured using Transwell chambers (8-μm pore size; Corning Inc.) with Matrigel. The chambers were precoated with 20% Matrigel for 30 min at 37°C. After transfection, ~5x10^5 CAOV3 and OVCAR3 cells in serum-free DMEM or RPMI-1640, respectively, were seeded into the upper chamber with 20% Matrigel. The complete medium (DMEM or RPMI-1640 with 10% FBS) was added into the bottom chamber. After 48 h of incubation at 37°C, the cells that invaded into the bottom chamber were fixed in 4% PFA for 20 min, stained with 0.1% crystal violet for 20 min at room temperature and counted under a light microscope (magnification, x50; IX71; Zeiss AG).

Tumor growth assays. All animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the Second People's Hospital of Lianyungang. A total of 22 female BALB/c nude mice (8-week-old; weight, ~20 g) were supplied by Beijing Vital River Laboratory Animal Technology Co., Ltd. Mice were fed with food and water *ad libitum*, and were kept at a Specific Pathogen-Free level at 20°C and a humidity of 60%, alternating between light and dark for 12 h. None of the mice died accidentally during
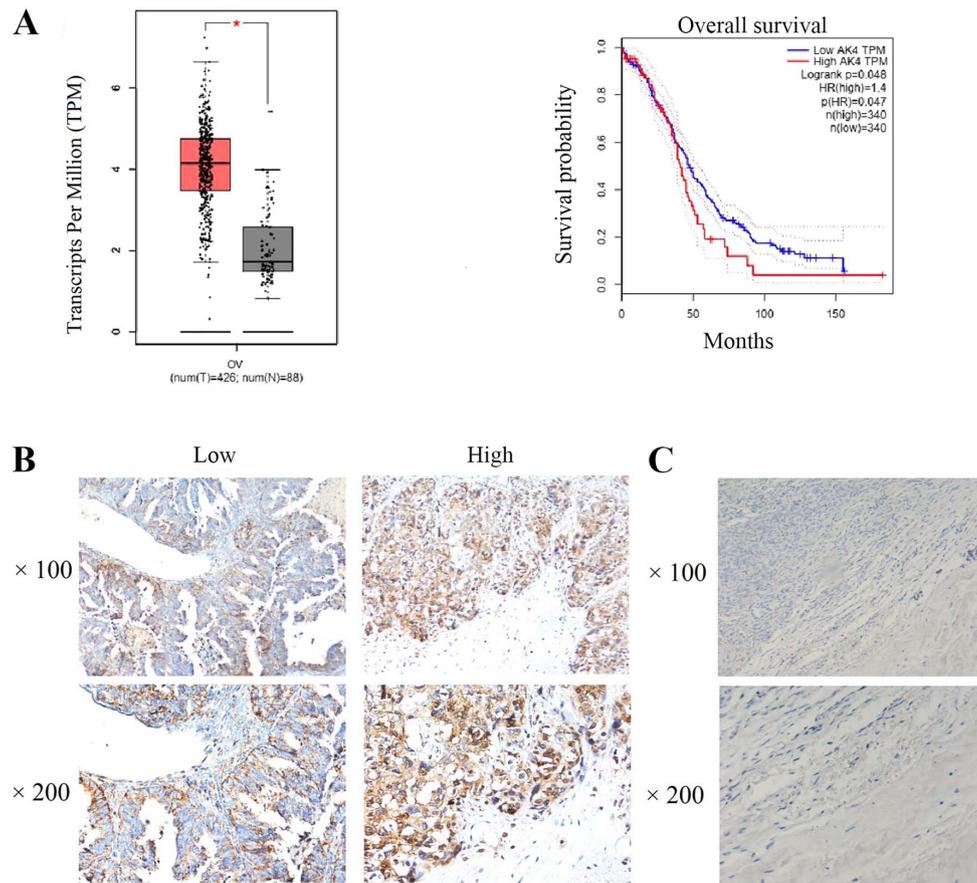
Figure 1. AK4 was highly expressed in human SOC tissues. (A) The GEPIA database was used to analyze the mRNA levels of AK4 in 426 SOC tumor tissues and 88 normal tissues. The overall survival time of patients with SOC with high or low AK4 expression were compared. *P<0.05. (B) IHC assays were performed and representative images of AK4 staining in human SOC tissues were captured (magnification, x100 and x200). (C) IHC assays were used to confirm the low expression levels of AK4 in the corresponding non-tumor tissues (magnification, x100 and x200). IHC, immunohistochemical; AK4, adenylate kinase 4.

the study. The mice were sacrificed by cervical dislocation, and their heartbeat was checked to determine whether they were dead.

The experimental procedure was performed as previously described (22). Briefly, CAOV3 cells were stably transfected with NC or AK4 shRNA lentiviruses, as aforementioned. Subsequently, $\sim10^6$ CAOV3 cells in 200 μl Matrigel (Corning, Inc.) were subcutaneously implanted into the abdomen of 8-week-old BALB/c nude mice. A total of 12 athymic nude mice were included in control (n=6) and AK4 depletion (n=6) groups. From the 14th day after injection, the volume of each tumor was measured every 3 days using a Vernier caliper. After 29 days, all animals were sacrificed and the tumor growth curves were calculated. Tumor volume was calculated as follows: Tumor volume (mm^3)=Tumor length (mm) x Tumor width (mm)^2/2.

Lung metastasis assays. For lung metastasis assays, a total of 10 athymic nude mice were included in control (n=5) and AK4 depletion (n=5) groups. 1x10^6 CAOV3 cells stably infected with NC or AK4 shRNA plasmids (as aforementioned) were resuspended in 100 μl PBS buffer and injected into the tail vein of 8-week-old BALB/c nude mice. The mice were sacrificed after 7 weeks, the lungs of the mice were surgically opened and complete lung tissues were taken out and were observed and photographed using a camera (magnification, x2.5). The tumor lung metastasis degree was measured according to the volume

of metastatic tumor tissue in the lung, which was measured using ImageJ v1.8.0 software.

Statistical analysis. GraphPad Prism 8.0 (GraphPad Software, Inc.) software was used for statistical analysis. All the experiments were repeated 3 times. All results are presented as the mean ± standard deviation. The differences in expression levels of AK4 were analyzed using an unpaired Student's t-test or Mann-Whitney U test, as appropriate. Kaplan-Meier analysis and log-rank test was used to analyze the association between AK4 expression and the survival rate of patients. The association between clinical features and AK4 expression levels was analyzed using χ^2 test. Unpaired student's t-test was used for statistical comparisons between two groups in the *in vitro* and *in vivo* assays. P<0.05 was considered to indicate a statistically significant difference.

Results

AK4 is overexpressed in human SOC tissues. First, bioinformatic analysis was performed to explore the mRNA expression levels of AK4 in SOC and normal tissues at using the GEPIA database. AK4 mRNA expression was increased in 426 cancer tissues compared with 88 normal tissues from different patients (P<0.05; Fig. 1A). Patients with high AK4 expression had a decreased overall survival time compared
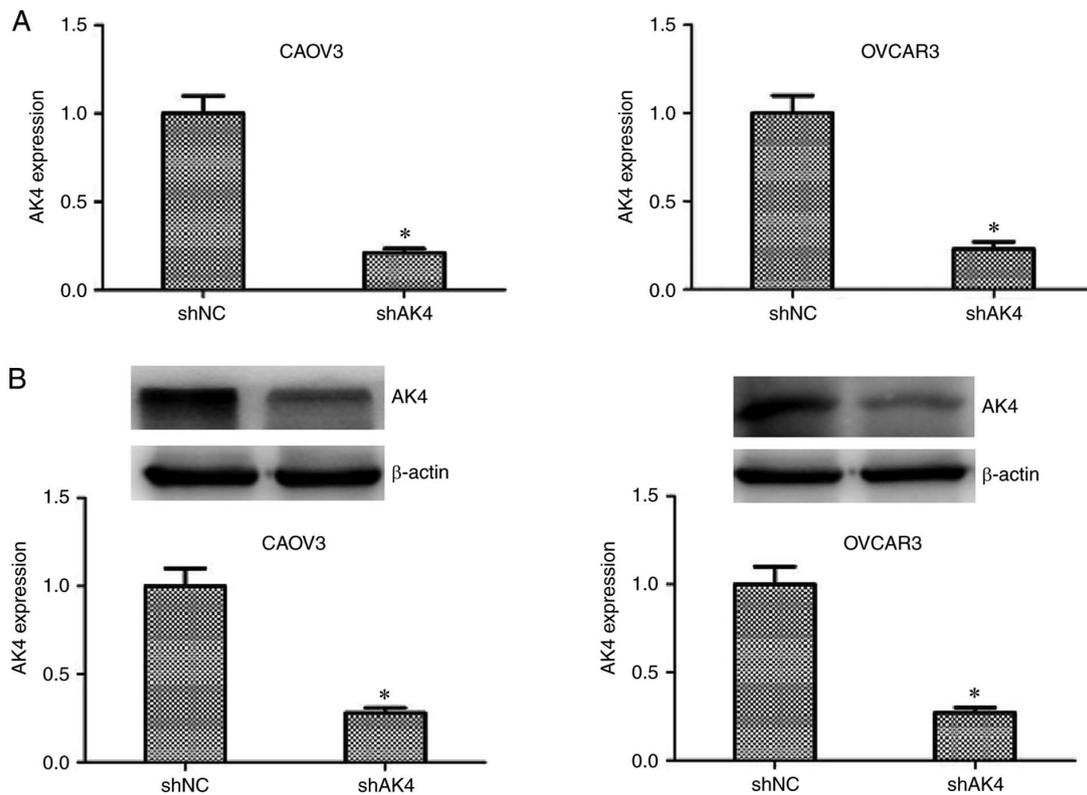
Figure 2. AK4 expression is significantly decreased in CAOV3 and OVCAR3 human SOC cells following transfection with AK4 shRNA plasmids. (A) Quantitative PCR assays revealed a decrease in the mRNA levels of AK4 following transfection with shRNA plasmids in CAOV3 and OVCAR3 cells. (B) Western blotting assays revealed successful inhibition of AK4 expression after transfection with shRNA plasmids in CAOV3 and OVCAR3 cells. The results are presented as the mean ± standard deviation. $^*P<0.05$ vs. shNC. NC, negative control; AK4, adenylate kinase 4; sh, short hairpin RNA.

with patients with low AK4 expression (P=0.048; Fig. 1A). These data suggested that AK4 may be used to predict a poor prognosis in patients with SOC. IHC assays were subsequently performed using SOC tissues and their paired normal tissues collected at the Second Hospital of Lianyungang to detect the expression level of AK4. The staining results indicated that AK4 was markedly expressed in SOC tissues compared with in the adjacent tissues (Fig. 1B and C).

To further investigate the effect of AK4 on SOC progression, SOC tissue samples were divided into AK4 high or low expression groups, according to the staining index. The clinicopathological characteristics, including patient age, tumor size, preoperative chemotherapy, differentiation, FIGO stage, and lymph node metastasis, were compared between high and low AK4 expression groups (Table I). No significant associations were found between AK4 expression and age (P=0.513), preoperative chemotherapy (P=0.149), differentiation (P=0.113) and lymph node metastasis (P=0.230). However, AK4 expression was significantly associated with tumor size (P=0.009) and FIGO stage (P=0.023) (Table I). These data indicated that AK4 may promote the progression of SOC.

Knockdown of AK4 suppresses the proliferation, migration and invasion of SOC cells in vitro. To further investigate the involvement of AK4 in the progression of SOC, shRNA plasmids targeting AK4 or NC shRNA plasmids were transfected into two SOC cell lines, CAOV3 and OVCAR3. The results revealed that AK4 mRNA and protein expression levels were significantly decreased following transfection with AK4 shRNA plasmids in CAOV3 and OVCAR3 cells compared with the respective NC shRNA groups (P<0.05; Fig. 2).

Subsequently, the possible effects of AK4 on the viability of SOC cells were assessed through colony formation and MTT assays. The number of colonies in the AK4 depletion group was significantly decreased compared with the NC shRNA group in CAOV3 and OVCAR3 cells (P<0.05; Fig. 3A). Similarly, the results of MTT assays also revealed that the OD value in the AK4 shRNA group was significantly decreased compared with the NC shRNA group (P<0.05; Fig. 3B). These results demonstrated that AK4 may play a role in the regulation of SOC cell viability.

The possible effects of AK4 on the migration and invasion of SOC cells were evaluated via wound healing and Transwell assays, respectively. An impaired wound healing capacity was observed in the AK4 shRNA group compared with the shNC group in CAOV3 and OVCAR3 cells *in vitro* (P<0.05; Fig. 3C). Additionally, Transwell assays suggested that the knockdown of AK4 efficiently suppressed invasion of CAOV3 and OVCAR3 cells. Collectively, these results indicated that AK4 affected the migration and invasion of SOC cells *in vitro*.

AK4 knockdown inhibits tumor growth and metastasis of SOC cells in vivo. The possibility that AK4 contributes to tumor growth and metastasis of SOC cells was further assessed using a mouse xenograft model and a lung metastasis model.

To confirm the aforementioned *in vitro* results, CAOV3 cells were first infected with AK4 shRNA lentiviruses to stably knock down the expression of AK4, and subcutaneously injected into nude mice. After 14 days, the tumors
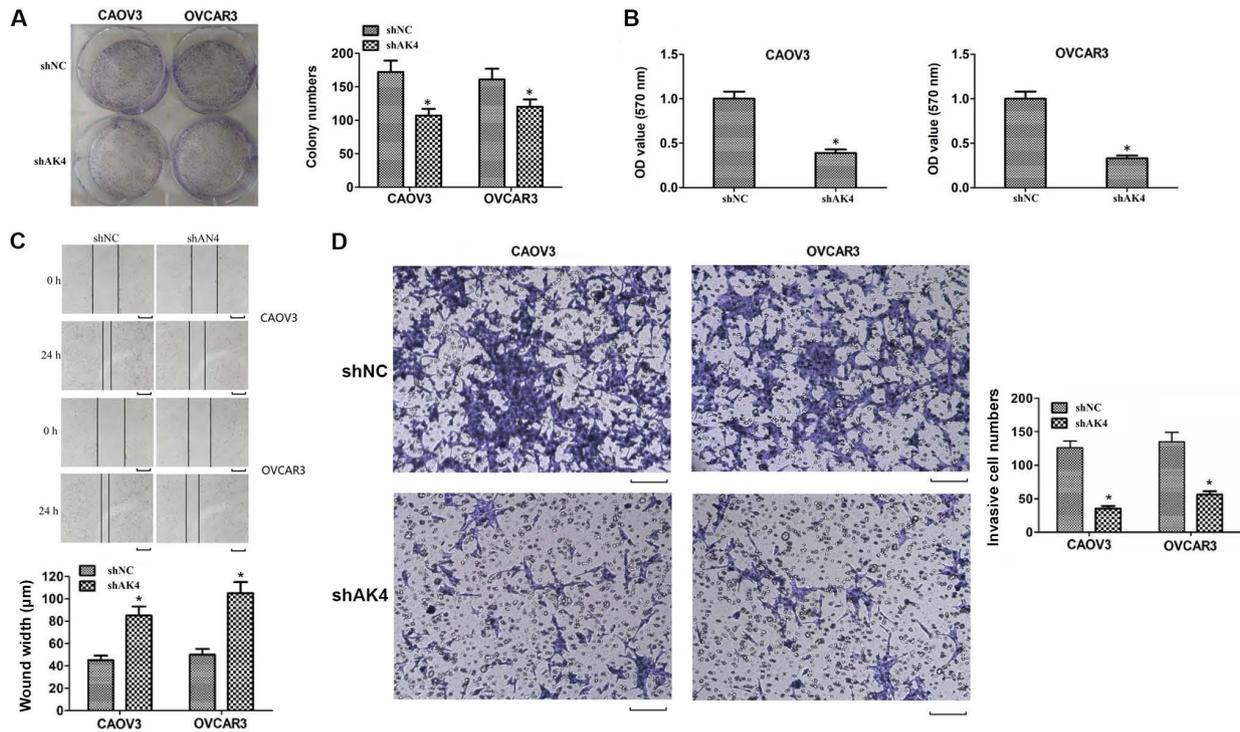
Figure 3. AK4 knockdown inhibits proliferation, migration and invasion of SOC cells *in vitro*. (A) Colony formation assays were conducted using CAOV3 and OVCAR3 cells transfected with NC or AK4 shRNA plasmids and the number of colonies was counted. (B) The results of MTT assays revealed decreased OD values at 570 nm wavelength caused by AK4 depletion. (C) Wound healing assays were performed using CAOV3 and OVCAR3 cells treated with NC or AK4 shRNA plasmids. Representative images and wound width were shown. Scale bar, 1 mm. (D) Transwell assays were conducted using CAOV3 and OVCAR3 cells transfected with NC or AK4 shRNA plasmids, and the number of invasive cells was counted. Scale bar, 100 μm. The results are presented as the mean ± standard deviation. *P<0.05 vs. shNC. NC, negative control; AK4, adenylate kinase 4; sh, short hairpin RNA.
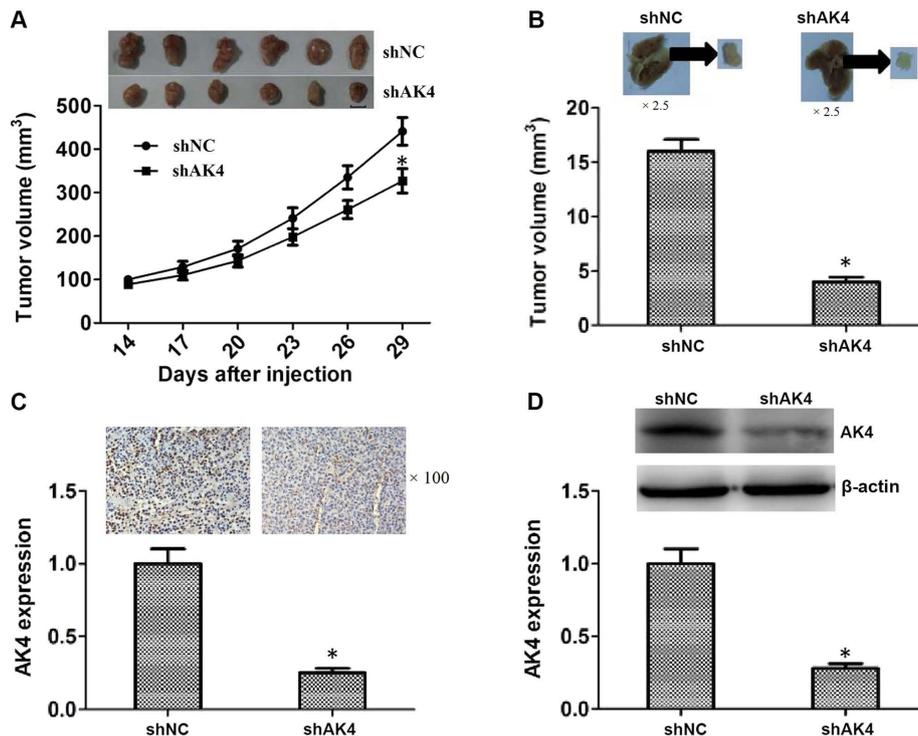
Figure 4. AK4 induces tumor growth and metastasis of SOC cells in mice. (A) CAOV3 cells infected with NC or AK4 shRNA lentiviruses were implanted into nude mice. After 14 days, tumors began to form and the volume of each tumor was measured every 3 days (n=6 in each group). After 29 days, all tumors were isolated and the growth curves were analyzed according to the average volume of 5 tumors in the AK4 and NC shRNA groups. Scale bar, 5 mm. (B) CAOV3 cells were infected with NC or AK4 shRNA lentiviruses, implanted into the tail vein of nude mice. Lung tissues were isolated after 7 weeks, images were captured and the degree of lung metastasis was calculated. Magnification, x2.5. (C) Immunohistochemical assays revealed the expression levels of AK4 in NC and AK4 stable knockdown primary tumors excised from mice. Magnification, x100. (D) Western blotting assays showed the expression levels of AK4 in NC and AK4 shRNA tumors. The results are presented as the mean ± standard deviation. *P<0.05 vs. shNC. NC, negative control; AK4, adenylate kinase 4; sh, short hairpin RNA.

began to form and the volume of tumors was measured every 3 days. After 29 days, all tumors were excised and representative images were shown (P<0.05; Fig. 4A). The growth curves of tumors in NC and AK4 shRNA groups were also shown in Fig. 4A. Tumor volume in the AK4 depletion groups was significantly lower compared with that in the shNC group on day 29 (Fig. 4A). Additionally, an increased lung metastasis degree was observed after 7 weeks of injection compared with the NC group (P<0.05; Fig. 4B) Lung tissues were isolated from mice injected with NC and AK4 shRNA cells, images were captured and individual tissues were analyzed. The tumor volume in the AK4 shRNA group was significantly decreased compared with that in the NC shRNA group (P<0.05; Fig. 4B).

Further, IHC assays were conducted to confirm the effective silencing of AK4 in the primary tumor tissues (Fig. 4C). The expression levels of AK4 were also determined in the primary tumor tissues using western blotting, and the results revealed that AK4 protein expression levels were decreased in the AK4 shRNA group compared with that in the shNC group (Fig. 4D). Overall, the results of the *in vivo* assays indicated the potential involvement of AK4 in tumor growth and metastasis of SOC cells *in vivo*.

Discussion

Surgical adjuvant chemotherapy is the most common therapeutic method for advanced SOC; however, it has little effect on improving patient survival rate (3). For this type of malignant tumor with high metastasis rates and fast progression, targeted therapy would be a suitable treatment method (8). Liposome targeted therapy is an effective strategy for tumors prone to metastasis (23,24). A number of targeted therapy drugs, such as bevacizumab, an anti-angiogenic agent, and olaparib, a poly-ADP ribose polymerase inhibitor, have been tested in clinical trials for SOC or used in the treatment of patients with high-grade SOC (25,26). The current study demonstrated that a member of the adenylate kinase protein family, AK4, could serve as a promising therapeutic target for the treatment of SOC. Analysis of TCGA database data and IHC assays revealed a high expression level of AK4 in human SOC tissues compared with healthy control samples. The present study suggested that AK4 may be involved in SOC progression and serve as a potential therapeutic target. Further research is required to fully elucidate the underlying molecular mechanisms.

In the current study, the protein expression of AK4 was markedly enhanced in human SOC tissues compared with adjacent normal tissues, which is consistent with previous investigations. Jan *et al* (17) reported that AK4 was significantly upregulated when glioma cells were exposed to hypoxia and was associated with hypoxia-inducible factor-1α-mediated invasion and migration in human glioma cells, indicating that AK4 is a possible cancer biomarker (27).

Colony formation and MTT assays revealed an effect of AK4 on SOC cell proliferation. Additionally, wound healing and Transwell assays further showed that AK4 mediated the migration and invasion of SOC cells *in vitro*. Consistent with these data, the subsequent *in vivo* results showed that AK4 promoted tumor growth and lung metastasis of SOC cells

in mice. Therefore, the results of the present study indicated that AK4 may affect the progression of SOC. Previous studies have also confirmed that AK4 played a role in cancer development. AK4 could modulate anticancer drug sensitivity in lung cancer via the regulation of mitochondrial activity (28). AK4 could also affect multi-chemoresistance and radioresistance in osteosarcoma and esophageal cancer, and AK4 expression was regulated by miR-199a-3p (21,29). Another study indicated that AK4 also affected the proliferation and invasion of bladder cancer cells *in vitro* (20). These studies, together with the findings of the present study, indicate that AK4 may play a role in cancer progression.

In the *in vivo* metastasis model used in the present study, AK4 knockdown could significantly inhibit tumor growth and lung metastasis degree following intravenous injection of cancer cells, suggesting that AK4 played a role in promoting tumor growth and metastasis. In a previous study on lung cancer, AK4 expression promoted the invasion step of the invasion-metastasis cascade by repressing ATF3 expression and resulted in relieving the expression of the downstream effector, MMP2 (17). In a mouse model of bladder cancer, AK4 shRNA transfection markedly inhibited tumor growth and metastasis compared with that in a scramble control group (20). AK4 is also a potential oncogene upregulated in metastatic colorectal cancer and exhibiting *in vitro* oncogenic properties (30). In addition, withaferin-A was identified as a potential agent for the treatment of metastatic lung cancer that could regulate AK4-associated gene expression (19).

In conclusion, the present study investigated the possible involvement of AK4 in the progression of SOC. The results revealed high expression levels of AK4 in human SOC tissues and AK4 was associated with the tumor size and FIGO stage of patients with SOC. Furthermore, AK4 affected the proliferation, migration, and invasion of SOC cells *in vitro*, and contributed to tumor growth and metastasis of SOC cells in mice. Therefore, AK4 could serve as a promising therapeutic target for SOC treatment.

Availability of data and materials

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

Authors' contributions

MH and XQ were responsible for study conception and design. MH, XQ, YW and FM performed the experiments. Acquisition of data was performed by MH, XQ and YW. Analysis and interpretation of data, writing, review and/or revision of the manuscript, and administrative and technical support were performed by MH and XQ. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Institutional Animal Care and Use Committee of the Second People's Hospital of Lianyungang (Lianyungang, China) and the Human Ethics Committee of the Second People's Hospital of Lianyungang (Lianyungang, China). All patients signed informed consent forms prior to surgery.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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