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Silencing of STX4 inhibits the proliferation, migration and invasion of ovarian cancer cells via EMT/MMP2/ CCND1 signaling pathway

Wenfeng Ye[1,2*], Chunyan Xue[2], Linlin Chen[3], Xiangnan Chen[4] and Dachuan Zhang[5]

Abstract

Background Ovarian cancer (OC) is one of the most common malignant tumors of the female reproductive system and 55–75% of patients relapse after surgery and standard postoperative chemotherapy and radiotherapy. Syntaxin4 (STX4) is localized in the plasma membrane and plays a role in the occurrence, development, invasion and metastasis of cancer cells.

Objective To investigate the changes in the biological behavior and effects of STX4 gene silencing on the invasion and metastasis of OC cell lines.

Methods The proliferation, migration and invasion abilities of two groups of OC cell lines SK-OV-3 and CAOV-3 constructed with an interfering plasmid (pLVX-shRNA1-STX4-shRNA) and a negative control plasmid (pLVX-shRNA1-nonspecific-shRNA), were examined via Cell Counting Kit-8, Transwell and scratch assays. The EMT markers vimentin and E-cadherin, MMPs (MMP1, MMP2 and MMP9) and CCND1 were used to explore the possible molecular mechanism of STX4 by which STX4 affects OC cells behavior, after which the effect of STX4 gene silencing on the proliferation of OC cells in *vivo* was tested.

Results After STX4 silencing, the biological behaviors of ovarian cancer cells including proliferation, migration and invasion, were significantly weakened. The results revealed that the E-cadherin, MMP2 and CCND1 levels of both OC cell lines were decreased after STX4 gene silencing. Animal models of STX4 gene silencing showed the tumorigenicity of tumor cells was reduced.

Conclusion We demonstrated for the first time that STX4, an important regulator of OC progression, was associated with the growth and metastasis of OC cells through correlations with EMT, MMP2, and CCND1, suggesting its potential as a new therapeutic target for OC.

Keywords STX4, Ovarian cancer, Matrix metalloproteinase, Epithelial mesenchymal transition, Cyclin Dl, Invasion and metastasis

*Correspondence:
Wenfeng Ye
yewenfengcz@163.com
[1]Department of Obstetrics and Gynecology, Shanghai Pudong New Area People's Hospital, Pudong, Shanghai 201299, China
[2]Department of Obstetrics and Gynecology, The Third Affiliated Hospital, Soochow University, Changzhou 213003, Jiangsu, China
[3]Department of Obstetrics and Gynecology, Taizhou Second people's Hospital of Jiangsu Province, Taizhou 225511, Jiangsu, China
[4]Department of Obstetrics and Gynecology, Nantong First people's Hospital of Jiangsu Province, Nantong 226001, Jiangsu, China
[5]Department of Pathology, The Third Affiliated Hospital, Soochow University, Changzhou 213003, Jiangsu, China

Introduction

Ovarian cancer (OC) is one of the most common female malignancies and the third most common cancer related to the female reproductive system after cervical cancer and uterine body cancer with an incidence rate of 10–15 per 100,000 [1]. It was reported that there were 20,000 new cases of OC and 13,000 OC-related deaths in the United States in 2022 [2]. In China, the total number of gynecological cancer cases increased from 177,839 to 241,800, with the number of OC cases increasing by 2.4% from 2007 to 2016. From 2017 to 2030, the number of projected gynecological cancer cases changed from 246,581 to 408,314 and the number of OC cases slightly increased [3]. The temporal trends of cancer deaths and mortality rates were similar to those of cancer cases and incidence rates from 2007–2030 [3].

The majority of patients with OC are diagnosed at an advanced stage because of insidious anatomical structure of the ovary 、 atypical clinical symptoms and lack of effective screening biomarkers [4]. Standard treatment for OC includes complete cytoreductive operation and postoperative adjuvant chemotherapy with platinum as the main drug. A thorough operation is the basis of successful treatment, which can improve the response for satisfactory chemotherapy, reduce disease-related symptoms, and improve the immune capacity of the host by removing immunosuppressive cytokines [5]. Despite the provision of satisfactory chemotherapy regimens, the 5-year survival rates for OC have not improved significantly over the past 20 years [6, 7]. This is partly due to the resistance of platinum and paclitaxel to classical chemotherapy regimens [8, 9]. The occurrence of drug resistance is an important cause of patient recurrence and death, but there is no effective treatment for patients with platinum drug resistance [10].

In addition to the above standard regimen, an increasing number of explorations and treatments have been conducted. For example, microbial treatment for eubiosis [11], hyperthermic intraperitoneal perfusion chemotherapy, etc [12]., but no obviously beneficial evidence has shown that these treatments also significantly benefit OC patients. Recently, bevacizumab or PARP inhibitors (PARPis) have shown efficacy in prolonging progression-free survival (PFS) for maintenance therapy but not overall survival (OS), indicating that more effective maintenance therapy is needed [13–15]. Moreover, a new situation has emerged again. once recurrence is diagnosed by imaging or tumor indicators after the use of a PARPi, the tumor burden is much greater than that after traditional surgery or chemotherapy [16]. Therefore, the individualized treatment of OC is receiving increasing attention. With the progress of immunology and molecular biology, the correlation between various signaling pathways and the tumors has attracted the attention of scholars. More clinical trials are focused on targeted approaches and immunotherapy for the treatment of ovarian cancer [17, 18].

Syntaxin4 (STX4) is a member of the syntaxin family and one of the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) component proteins. STX4 is abnormally expressed in a variety of malignancies, including increasing breast tumor invasion by mediating invadopodium development [19] and promoting cancer role in Clear Cell Renal Cell Carcinoma [20]. Studies have shown that the STX family is abnormally expressed in a variety of tumor cells and participates in the development and metastasis of tumor cells by regulating the transport of α5β1 and α3β1 integrin vesicles [21].

The epithelial-mesenchymal transition (EMT) is involved in tumor progression and metastatic expansion [22]. EMT involves epithelial cells losing their original cell surface marking and obtaining features of mesenchymal cells, which cause the tumor to break away from its original location to distant regions. A number of studies have shown that EMT is associated with ovarian cancer progression [23].

We found that the expression level of STX4 in OC are significantly increased [24]. There are many types of OC, among which serous epithelial OC accounts for the largest proportion, and our results also revealed that the STX4 level was significantly higher in serous OC than in mucinous OC. However, it is not clear whether STX4 can be used as a potential target to regulate the growth, invasion and metastasis of OC cells, therefore we aimed to use gene silencing technology to downregulate the expression level of STX4, and observe its influence and mechanism on the biological behavior of OC cells and its relation with EMT. Through these experiments, we hope to provide a new strategy for the clinical diagnosis and treatment of OC.

Materials and methods

Reagents and cell line culture

The experiment mainly targeted cells and animals, so clinical trial number was not applicable. The monoclonal antibodies used were as follows: STX4 (ab77037, Abcam, CO, United Kingdom), other antibodies including E-cadherin (ab231303), matrix metalloproteinases (MMPs) including MMP1 (ab134184), MMP2 (ab92536), MMP9 (ab76003) and Cyclin D1 (CCND1) (ab16663),which were purchased from Abcam (Abcam, CO, USA), and vimentin (#3295, Cell Signaling Technology CO, USA). The PrimeScript RT-PCR Kit II was from TakaRa (A160847A, Japan). OC cell lines included SK-OV-3 and CAOV-3. SK-OV-3 cancer cells were obtained from the laboratory of the Third Affiliated Hospital of Jiangsu University, and CAOV-3 cells were obtained from the Shanghai Cell

Bank of the Chinese Academy of Sciences. SK-OV-3 cells were cultured in complete culture Dulbecco's modified Eagle's medium (DMEM) without pyruvate, and CAOV-3 cells were cultured with DMEM containing pyruvate. The cells were cultured at 37 °C in the presence of 5% CO_2 in DMEM supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) 10,000 units penicillin/streptomycin. The growth rate of the SKOV3 cell line was relatively fast, and the medium was replaced 24–48 h later. The growth rate of the CAOV-3 cell line was relatively slow, so the culture medium was generally changed 1–2 times a week depending on the degree of cell adherence.

Quantitative reverse transcription PCR (qRT-PCR)

OC cells cultured for three generations were washed three times with PBS, the supernatant was discarded, and 1mL of TRIzol was added to the full cell lysate until it was clarified for RNA extraction. The RNA was then reverse transcribed into cDNA via the PrimeScript RT-PCR Kit II. Real-time PCR was conducted on an ABI 7500 system (Applied Biosystems, USA) on the basis of the manufacturer's instructions, and SYBR Green was used as a DNA specific fluorescent dye. *GAPDH* was selected as a housekeeping gene. The Primer sequences for both *GAPDH* and the target gene *STX4* were as follows:

GAPDH forward primer 5'-GAACATCATCCCTGCC TCTACT-3',

Reverse primer 5'-CCTGCTTCACCACCTTCTTG-3',

STX4 Forward primer 5'-ATGCGGGACAGGACCCA – 3',

Reverse primer 5'-TTATCCAACCACTGTGACGC-3'.

Gene expression was determined via a one-step RT-PCR protocol in a 20-μL reaction system consisting of 10 μL 2×One-Step TB Green RT-PCR Buffer 4, 0.8 μL PrimeScript 1 Step Enzyme Mix 2, 0.8 μL forward primer (10 μM), 0.8 μL reverse primer (10 μM), 0.4 μL Rox Reference Dye II (50×) and 7.2 μL OC cell RNA template (1:10 dilution). The detailed steps, including reverse transcription and amplification, followed the manufacturer's instructions. The experiment was conducted with three technical replicates for each sample. The relative expression level of *STX4* was normalized to that of *GAPDH* and calculated via the $2^{-\Delta\Delta T}$ method.

Western blot analysis

Total proteins from OC cells were isolated via lysis buffer. Then, a Western blot analysis was carried out. The protein content was determined via the BCA method. In brief, proteins were separated on a 10% SDS-PAGE gel, transferred onto PVDF membranes and incubated with diluted primary antibodies against STX4 (1:1500), MMP1 (1:1000), MMP2 (1:1000), MMP9 (1:1500), CCND1(1:2500), E-caherin (1:2000), and Vimentin (1:1000) overnight at 4℃.The samples were then incubated with secondary antibody at room temperature according to the manufacturer's instructions.

Immunohistochemistry

The primary antibodies used for immunohistochemistry, including antibodies against STX4, E-cadherin and vimentin, are commercially available. Formalin-fixed, paraffin-embedded consecutive Sect. (4-μm thick) were heated at 85℃ for 2 h and then cooled at room temperature for 20 min. The slides were immersed in dimethylbenzene for deparaffinization, hydrated in ethanol and subjected to antigen retrieval in 2% EDTA-citrate antigen retrieval solution in a pressure cooker. Subsequently, the slides were rinsed with PBS, immersed in hydrogen peroxide at room temperature to block endogenous peroxidase activity and incubated with 3% BSA to block nonspecific binding. Next, the slides were incubated with primary antibodies against STX4 (1:300), E-cadherin (1:400) and vimentin(1:600) at 4℃ for 14 h respectively. The secondary antibodies were used according to the manufacturer's instructions. The evaluation criteria were as follows: A: according to the cell staining intensity score, no positive staining (negative) was given 0 points, light yellow (weakly positive) was given 1 points, brown yellow (positive) given 2 points, and brown (strongly positive) was given 3 points; B: according to the percentage of positive cells, the score was 4 points, ≤ 25% counts was given 1 points, 26-50% was given 2 points, 51-75% was given 3 points, and > 75% was given 4 points. The two ratings are multiplied to obtain the final rating.

Construction of a lentiviralus vector for STX4 gene silencing

DH5α from *escherichia coli* was obtained from Invitrogen, and the eukaryotic expression plasmid plvx-U6-PGK-puro was designed by Shanghai Tingzhou Pharmaceutical Technology. The plasmid plvx-U6-PGK-puro contains a human U6 promoter encoding the green fluorescent protein (GFP) reporter gene. The target fragment was inserted with the *Bam*H I and *Eco*R I which are regulated by the U6 promoter, and the lentiviral vector expressed of puromycin resistance gene regulated by the PGK promoter. The shSTX4 interference sequence is shown below:

Top strand(63 bp): 5'-gatccCCGTCAACACA AGAATGAGAATTCAAGAGATTCTCATTCT TGTGTTGACGGTTTTTTg-----3'.

Bottom strand(63 bp): 5'-aattcAAAAAACC GTCAACACAAGAATGAGAATCTCTTGA ATTCTCATTCTTGTGTTGACGGg-----3'.

The scramble siRNA used was: 5'-TTCTCCCCGAAC AACAACGTGTCACCACCACGT-3'.

Two groups of OC cell lines SK-OV-3 and CAOV-3 were constructed with interfering plasmid vector

(pLVX-shRNA1-STX4-shRNA) and negative control plasmid vector (pLVX-shRNA1-nonspecific-shRNA) to establish stably transfected cell lines after STX4 gene silencing. Virus packaging was performed in 293T cells via Lipofectamine 3000.

Cell proliferation assay (CCK8 assay)

A 100μL cell suspension with a density of 2×10^4 cells / mL was added to 96-well plates. The petri dishes were precultured in a 5% CO_2 incubator for 24 h (37℃). A CCK-8 kit was used for the cell proliferation assay following the manufacturer's instructions. Ten microliters of CCK-8 reagent (CCK-8, Beyotime, c0037, Beijing) was directly added to the culture medium of the cells to be tested. The absorbance was measured at 450 nm by using a spectrophotometer (Tecan Sunrise, Switzerland).

Wound healing assay

A marker pen was used to draw horizontal lines on the back of the 24-well plate with a ruler. The cell density was subsequently adjusted to 1×10^5/mL. After monolayer cells were cultured, a 200μL disposable suction tip was used to make scratches on the single layer of cultured cells at the bottom of each 24 well plates. The shape of the scratch should be "1". Then the cells were placed in an incubator ay 37℃ with 5% of the CO_2, the shed cancer cells were washed away at 0 h, 24 h, and 48 h with PBS, and images were taken with an inverted microscope. The relative distance of cell migration was measured in the scratch area under an inverted microscope. The relative distance of cell migration was measured in the scratch area under an inverted microscope to calculate the actual migration distance according to the original cell scratch area distance.

Migration and invasion assays

A 24-well transwell was used for the cell migration and invasion assays. For the invasion assay, the upper surface of the transwell chamber was coated with the precooled Matrigel and then dried into a gel. DMEM was added to hydrate the basilar membrane, and the cell suspension was prepared at 5×105/mL The SK-OV-3 and CAOV-3 cells in the silenced group and the control group were added to the chamber at approximately 1×10^5 cells/well. Then, the cells were cultured for 24–48 h, the transwell chambers were removed, the cells were fixed with 4% methanol, and photos were taken with an optical microscope. For the migration assay, transwells were coated with 10 μg/mL FN under the chamber filtration membrane and properly air-dried. SK-OV-3 and CAOV-3 cells from the silenced group and control group were added to the chambers. After routine culture, the transwell chamber was removed and washed with PBS. The cells in the upper layer of the microporous membrane were gently wiped with a wet cotton swab, fixed with methanol and stained with crystal purple. The cell numbers were then counted and compared.

Establishment and analysis of xenograft nude mouse model

The animal experiments were approved by the ethics committee (2022-D-36). BALB/c NOD mice (8-week-old females) weighing approximately 20 ± 2 g were obtained from the Slack Laboratory Animal Center in Shanghai. BALB/c nude mice were subcutaneously injected with lentivirus stably transfected with skov-3 cells (lv-shSTX4) or negative control sk-ov-3 cells (lv scramble). When tumor diameter was less than 20 millimeters or the tumor tissue of the mice was severely ulcerated and the mice could not eat and drink on their own, the mice reached the humane end, and the mice were killed. Tumor growth curves were plotted, and tumor volume and weight were compared between the two groups. IHC was used to detect the expression of STX4, E-cadherin and vimentin in tumor tissues.

Statistical analysis

SPSS 22 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. A t test verification for comparison between 2 groups; one-way analysis of variance with Tukey's post hoc test for comparisons of multiple groups; In this study, $P<0.05$ was considered statistically significant. In this study, $P<0.05$ was considered statistically significant.

Results

STX4 Silencing in OC cell lines inhibited the proliferation、migration and invasion of OC cells

To analyze the function of OC cells, cell lines SK-OV-3 which originated from high-grade serous carcinoma ([https://www.atcc.org] for SK-OV-3), the most comm on subtype of ovarian cancer and CAOV-3 which originated from ovarian papillary cystadenocarcinoma ([https://www.atcc.org] for CAOV-3), often classified as hig h-grade serous carcinoma, were used. The expression of STX4 gene in two cell lines were evaluated respectively, and SK-OV-3 presented relatively high expression levels. Therefore, follow-up experiments involving gene silencing could be conducted in both groups. After STX4 gene was silenced in the OC cell lines SK-OV-3 and CAOV-3, PCR and Western Blot were used to determine the transfection effect. After transfection, shSTX4 reduced STX4 mRNA by 70% ($P<0.01$) and protein levels by 75%($P<0.01$) as shown in Fig. 1A. The effect of STX4 silencing on the proliferation of OC cells was analyzed via a CCK8 assay with a growth curve. CCK8 assays revealed that the proliferation and growth of the OC cell lines SK-OV-3 and CAOV-3 in the two groups significantly

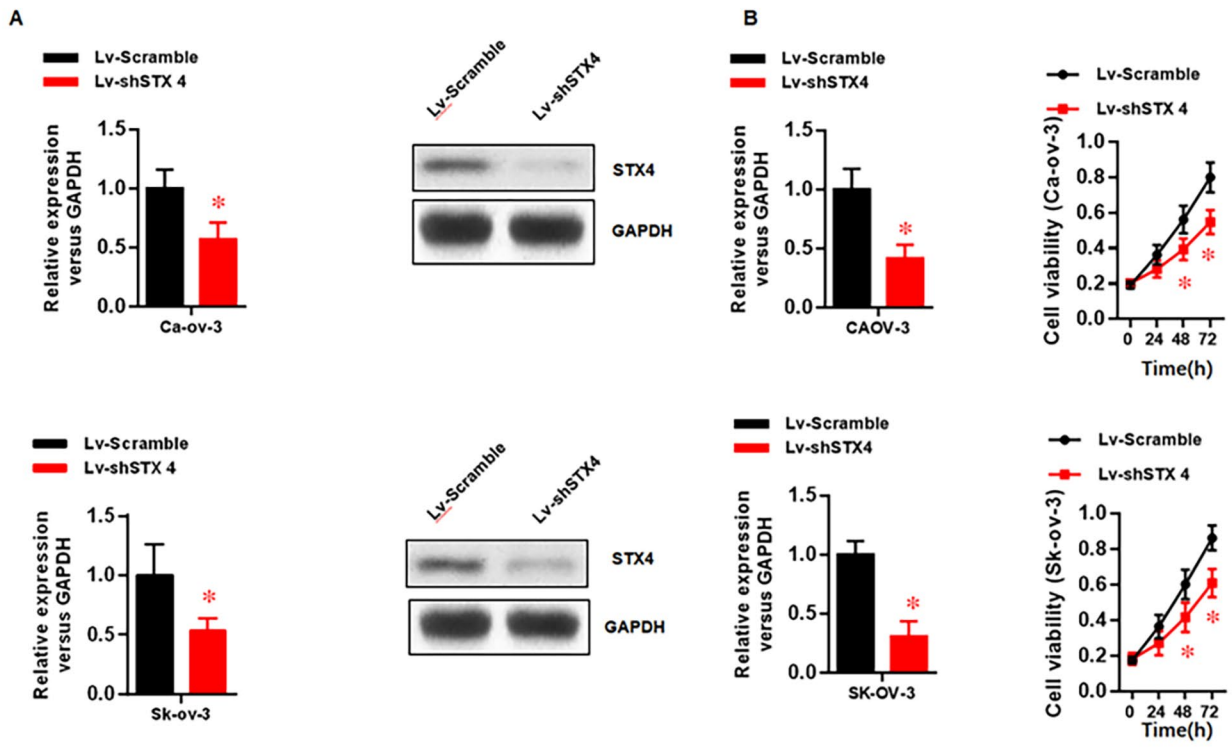

Fig. 1 **A** Effect analysis of OC cell lines after treatment with STX4shRNA: up: mRNA and protein level (WB assay) changes in CAOV-3 cells; down: Changes in the mRNA and protein levels(WB assay) in SK-OV-3 cells. **B** CCK8 for the cell proliferation assay: up: CAOV-3 cells; down: SK-OV-3 cells. *$P < 0.05$, t-test

decreased after STX4 gene silencing($P < 0.05$)(Fig. 1B). Wound healing assays revealed that the migration and healing rates of cancer cells in the gene silenced group were significantly lower than those in the blank control group ($P < 0.05$)(Fig. 2B). Transwell chamber invasion and migration assays of OC cells revealed that compared with that in the blank control group, the number of cancer cells invading through the transwell matrix was also significantly lower in the gene-silenced group ($P < 0.05$) (Fig. 2A).

The mechanism by which STX4 affects the malignant behavior of OC cell lines *in vitro*

The STX4 gene-silencing plasmid and negative control plasmid were transfected into two groups of OC lines, SK-OV-3 and CAOV-3. Western blotting was used to detect changes in the expression levels of EMT-related proteins, including the epithelial marker E-cadherin and the mesenchymal marker vimentin, in the two groups of cells, as well changes in the expression of MMP1, MMP2 and MMP9, indicating invasion and metastasis ability. Western blotting was used to detect changes in the level of CCND1. These experiments revealed that E-cadherin expression was increased, whereas vimentin expression was significantly downregulated in the two groups of OC cell lines after STX4 silencing. There was a significant difference in the change in vimentin ($P < 0.05$) (Fig. 3). MMPs also changed after STX4 gene silencing. In both groups of OC cells, MMP2 was significantly downregulated, whereas MMP1 was not changed. MMP9 was decreased only in CAOV-3 cells. Moreover, the CCND1 level in OC cells in both groups was downregulated after STX4 gene silencing ($P < 0.05$) (Fig. 3).

Effects of STX4 gene silencing on the proliferation of OC cells in vivo

OC cell transplantation models were constructed in the abdominal wall of mice via lentivirus stably transfected SK-OV-3 cells (lv-shSTX4) and negative control SK-OV-3 cells (lv-scramble). IHC was used to detect the expression of STX4, E-cadherin and vimentin in tumor tissues. Compared with those in the negative control group, the tumor volume and weight of the tumor-bearing mice in the STX4 gene-silenced group were significantly lower. The growth curve of the STX4 gene silenced group decreased significantly beginning on the 20th day. The results of the immunohistochemical study revealed that STX4 expression in tumor tissues in the STX4 gene silenced group was significantly decreased, whereas E-cadherin expression was significantly increased and vimentin expression was significantly decreased ($P < 0.05$) (Fig. 4).

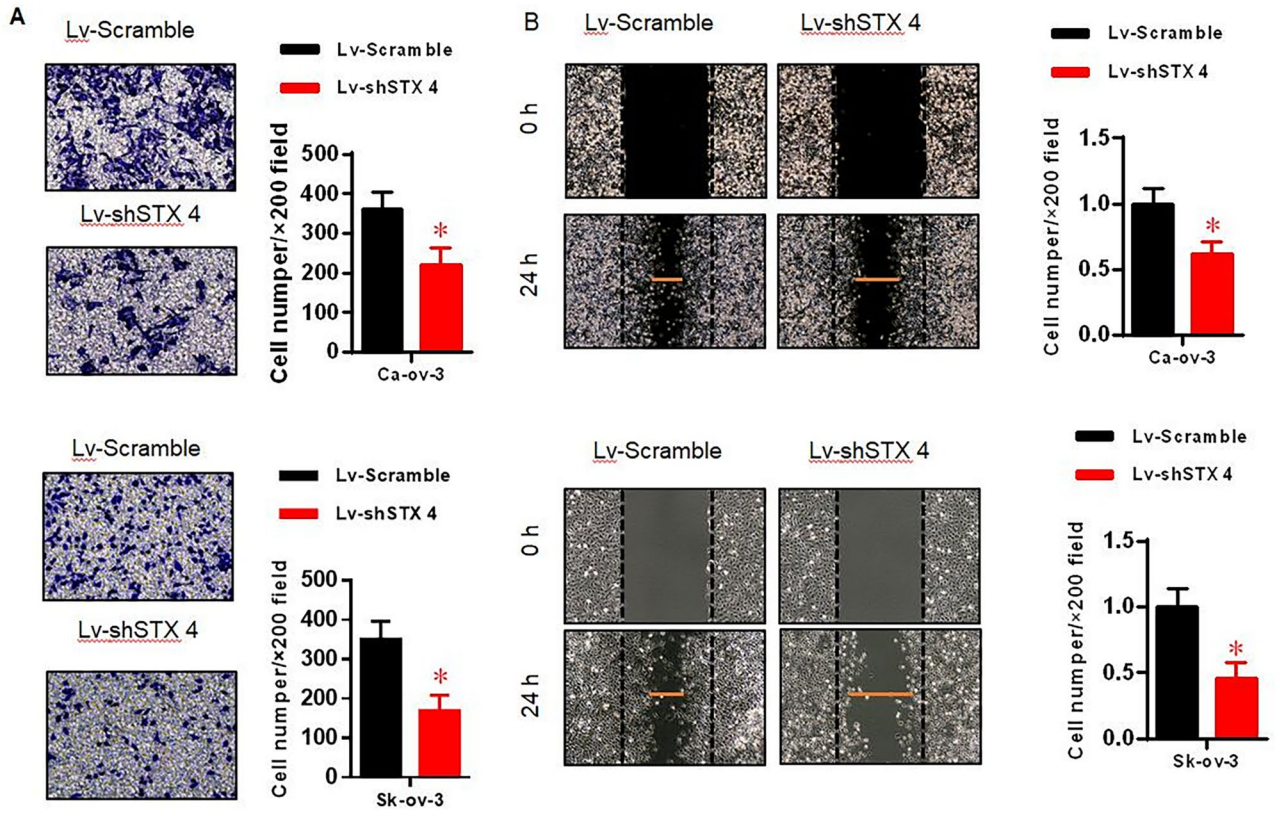

Fig. 2 **A** Transwell chamber invasion and migration assay of OC cells in both groups. up: CAOV-3 cells; down: SK-OV-3 cells. **B** Wound healing assay of OC cells in both group>. up: CAOV-3 cells; down: SK-OV-3 cells. *$P < 0.05$,t-test

Discussion

The extensive metastasis of OC is the main reason for its high mortality. Tumor combination therapy is a new direction for OC therapy. Many researchers believes that further empirical trials using traditional drug combinations are likely to produce only modest incremental improvements in the outcomes of OC. At present, despite the increasing use of active combined therapies, transient chemotherapy sensitivity and stagnation in long-term survival still exist [25–27]. Given the heterogeneity of OC, future treatments could improve long-term survival by translating research findings at the molecular and cellular levels into personalized treatment strategies and optimizing early detection. The cause of most cancer deaths is metastasis, not the primary tumor. To prevent these deaths, improvements in the treatment of metastatic disease are needed. Therefore, the need for new therapeutic drugs that are effective without drug-resistance is an urgent problem for clinicians. Currently, the development of biological treatments for tumors, such as antiangiogenic drugs, has initially become a focus of targeted OC therapy [28]. However, not all the treatments are clinically effective. Therefore, the development of new and effective immunotherapies and biotherapies has become the focus of targeted therapy.

Studies have indicated that STX4 increased breast tumor invasion by mediating invadopodium development [19] and contributed to cancer progression by enhancing AKT expression and stimulating the activation of VEGF signaling pathways in Clear cell renal cell carcinoma [29]. Our cell experiments confirmed that high expression of STX4 can promote the growth, invasion and migration of OC cells. This finding was consistent with the clinical data and in vitro experimental results that we analyzed. These finding also confirmed that STX4 may promote the invasion and migration of OC cells and participate in the progression of this disease. Similarly, we wanted to investigate the mechanism by which STX4 affects the development of OC through OC cell lines. Our study focused on changes in EMT markers after STX4 gene silencing. After STX4 gene silencing, the epithelial marker E-cadherin was increased in SK-OV-3 cells, while the mesenchymal marker vimentin was significantly downregulated, indicating that STX4 could affect the behavior of ovarian cells by affecting EMT markers in cancer cells. Our results revealed a statistical difference in the downregulation of vimentin, while E-cadherin showed an increasing trend, but the difference was not statistically significant. We speculated that the changes in various markers may not be completely synchronized in the

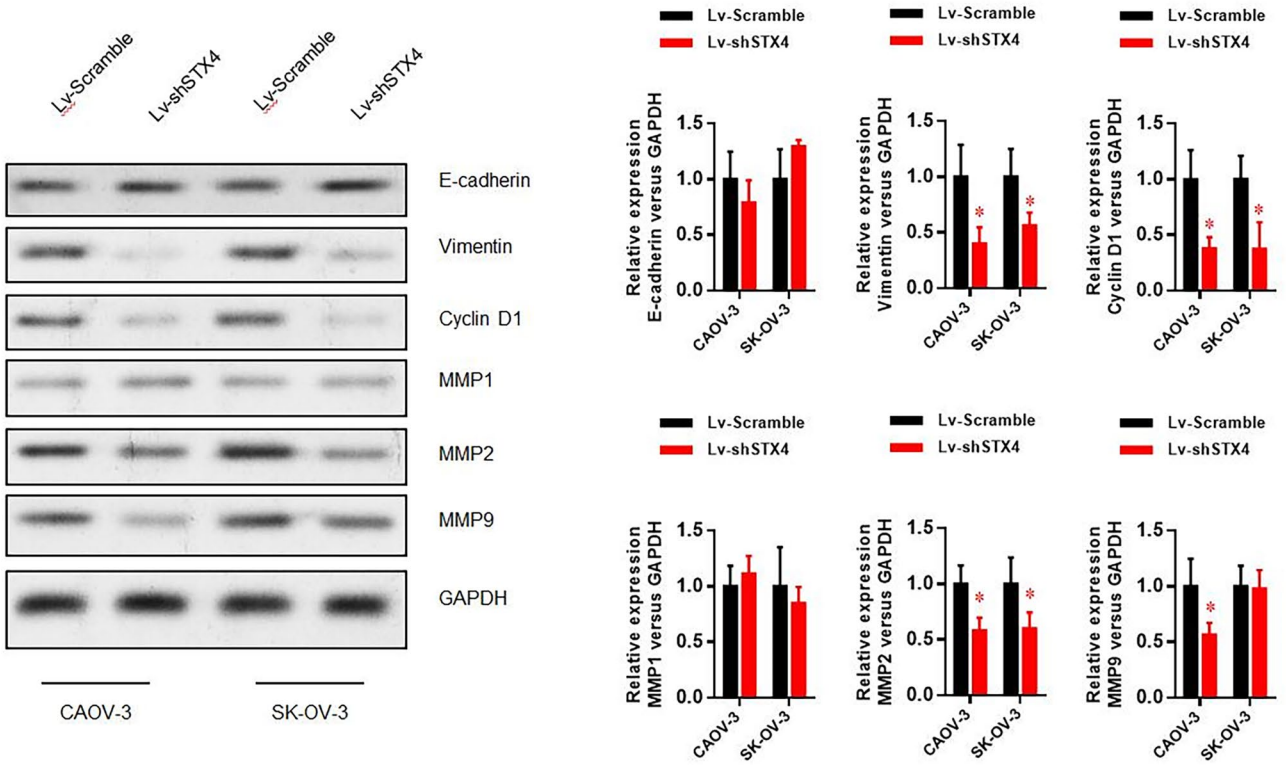

Fig. 3 Protein expression results of STX4 gene silencing via WB analysis of EMT markers (E-cadherin, vimentin), MMPs (MMP1, MMP2, and MMP9) and CCND1 in OC cell lines. *$P < 0.05$,one-way ANOVA

process of EMT. But we have not conducted experiments on overexpression for multi-faceted functional verification, this would be the direction for our further exploration in the future. EMT refers to the process of reversible transformation from epithelial cells into mesenchymal cells, which is essentially involves cytoskeletal recombination, decreased adhesion and loss of polarity. EMT is accompanied by changes in matrix-degrading proteins, which facilitate cell motility and invasion [30]. Studies have shown that EMT is an important mechanism for the occurrence and development of malignant tumors. Studies have shown that "partial EMT" in a variety of tumor cells, such as hepatocellular carcinoma, breast cancer, non-small cell lung cancer, is associated with chemotherapy resistance [31–33].

In addition, the expression of MMPs, which are markers of invasion and metastasis, also changed after STX4 gene silencing. MMP2 was significantly downregulated in both types of OC cells, while MMP9 was decreased only in CAOV-3 cells, and MMP1 was not significantly changed in either group of cells, indicating that the biological characteristics of different OC cells were not identical. The invasion and metastasis of malignant tumors are complex pathological processes, and the degradation of the extracellular matrix and basement membrane is the key link between the invasion and metastasis of malignant tumors [34]. MMPs are known for their role in mediating the tumor microenvironment during tumor progression [35]. MMPs enable the degradation of barriers, including the extracellular matrix and basement membrane, facilitating the metastasis of tumor cells [36]. Moreover, our study revealed that after STX4 gene silencing, CCND1 tended a decrease in both groups of OC cells, and a variety of studies have shown that CCND1 expression tends to increase in ovarian malignant tumors. CCND1 has also been reported to be a cell cycle regulatory protein that plays a critical role during cycle phase transition and cell proliferation. Studies have shown that the OS and PFS of OC patients with high CCND1 expression are significantly shorter than those of patients with low CCND1 expression [37].

In vivo experiments from our study revealed that the growth rate of OC cells significantly decreased after STX4 gene silencing in tumor-bearing mice, and the tumor volume and weight were also significantly reduced, which further confirmed the role of STX4 in the occurrence and development of OC and provided new evidence for the clinical treatment of OC.

However, our research also has certain limitation. We only conducted cell silencing experiments, overexpression experiments for multiple verifications did not carry out. In our experiment, after STX4 gene silencing downregulation of vimentinl was obvious, while E-cadherin showed an upward trend, the specific mechanism was

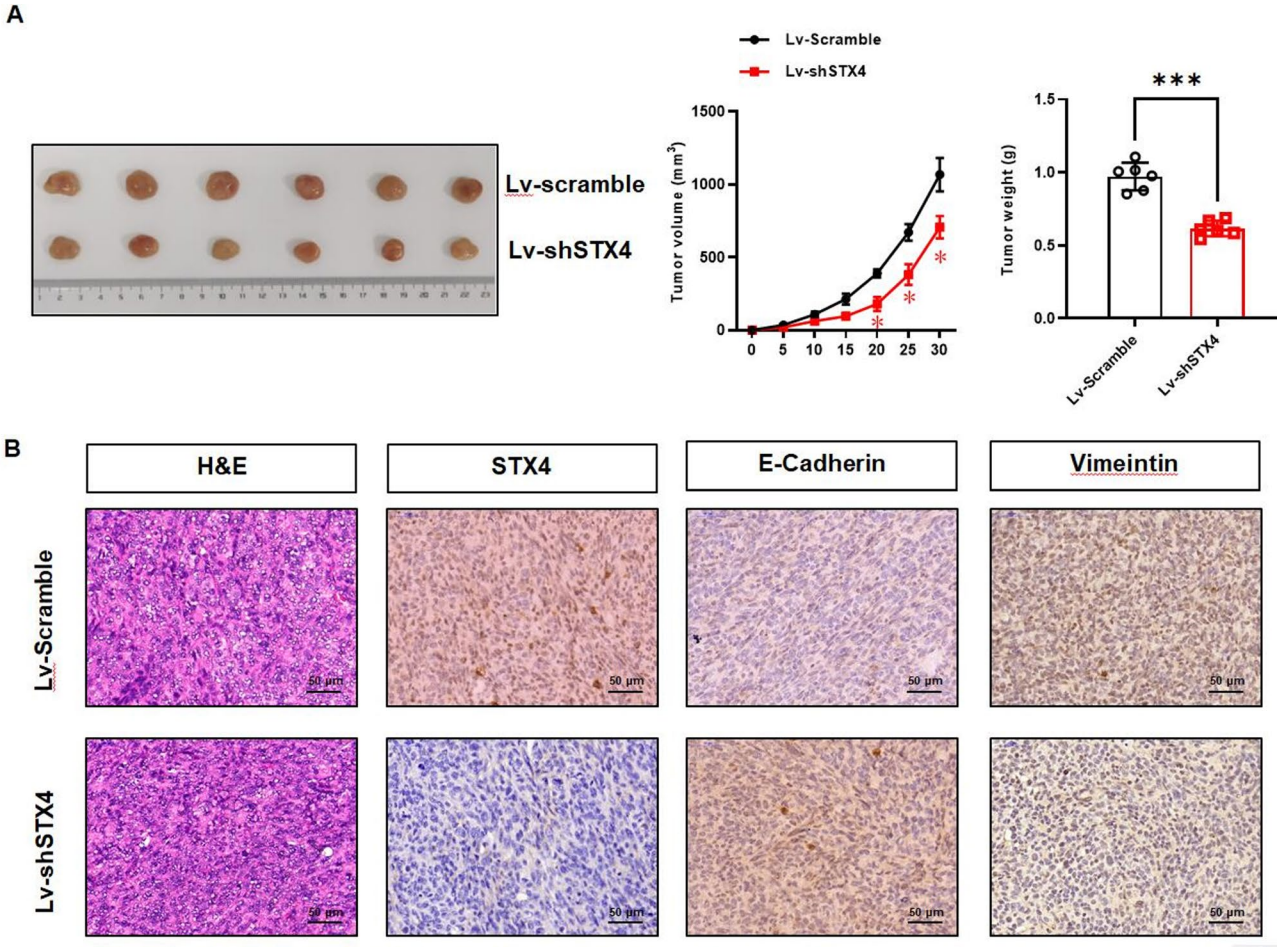

Fig. 4 **A** OC tissue volume and quality between two groups of tumor-bearing mice. **B** Immunohistochemical staining with STX4, E-cadherin and Vimeintin of two groups of tumor-bearing mice. *$P < 0.05$, t-test

still unclear to us. We will take further research in the future for explore the mechanism of STX4 in the development and treatment of OC.

Conclusion

In summary, our data demonstrate that STX4 was associated with the growth, invasion and metastasis of ovarian cancer cells by regulating EMT, MMP2 and CCND1. It may become a new treatment option for ovarian cancer. However, the specific mechanism targeting cell invasion needs to be verified in future studies. STX4 may be a novel therapeutic target in patients with OC.

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Author contributions
W.Y made designed the work and drafted manuscript preparationX.C revised it critically for important intellectual content W.Y, L. C and X. C had data collectionC. X, W. Y and D. Z had the analysis and interpretation of resultsAll authors reviewed the results and approved the final version of the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Competing interests
The authors declare no competing interests.

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