

Siva 1 inhibits proliferation, migration and invasion by phosphorylating Stathmin in ovarian cancer cells

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Abstract. Ovarian cancer is one of the most common types of gynecologic malignant tumor, with high incidence and high mortality rates. It is difficult to diagnose ovarian cancer early due to the complex structure and function of the ovaries. Siva 1 is a well-known pro-apoptosis protein that functions in multiple types of cancer cells: There are several studies demonstrating that Siva 1 arrests apoptosis and facilitates cancer development in osteosarcoma and non-small cell lung cancer. Whether Siva 1 functions in ovarian cancer remains unknown. In the present study, it was established that Siva 1 was stably overexpressed in ovarian cancer cell lines, and demonstrated that the overexpression of Siva 1 inhibited proliferation, promoted apoptosis and suppressed migration and invasion by facilitating phosphorylation of Stathmin and polymerization of α -tubulin in ovarian cancer cells. These data provide specific novel insights into the molecular mechanism of ovarian cancer, and may be of significance for the clinical diagnosis and therapy of ovarian cancer.

Introduction

Ovarian cancer is one of the most common types of gynecologic malignant tumor, whose incidence rate ranks third in cancers of the female reproductive system, and mortality ranks first (1). The etiology of ovarian cancer remains unknown, and may be associated with a number of internal and external factors. Early diagnosis of ovarian cancer is difficult due to the complicated structure and function of the ovary. In addition, late-stage tumors often recur and metastasize subsequent to surgery, and eventually evolve resistance to chemotherapy. The five-year survival rate of patients with ovarian cancer is

<30% (1,2). Therefore, it is necessary to identify molecules that function in the occurrence, development and metastasis of ovarian cancer, and to develop more effective diagnosis and therapy in clinic.

Siva 1 is a pro-apoptotic protein first obtained by Prasad *et al* from a HeLa cell library via yeast two-hybrid screening with CD27, a member of the tumor necrosis factor receptor superfamily, as the bait (3). Siva 1 exists in a wide variety of tissues and cells, and serves a crucial role in certain extrinsic and intrinsic apoptosis signaling pathways, for example: Siva 1 interacts with CD27 and induces apoptosis via the caspase-independent mitochondrial pathway in T lymphocytes (4); Siva 1 maybe stimulated by thromboxane receptor and aggravates apoptosis of HeLa cells induced by cisplatin (5). Siva 1 binds to B-cell lymphoma-extra large (Bcl-XL) and inhibits Bcl-XL-mediated apoptosis protection from UV radiation in breast cancer cells (6). Additionally, Siva 1 participates in virus infection-associated apoptosis: It has been demonstrated that Siva 1 promotes apoptosis of A549 cells induced by influenza A virus and sensitizes CD4⁺ cells to HIV-I envelope-induced apoptosis in a caspase-dependent manner (7,8).

However, Siva 1 has been demonstrated to serve opposite roles in other previous studies. In KRAS^{G12D}-derived non-small cell lung cancer (NSCLC), Siva 1 is highly expressed and facilitates tumorigenesis by regulating metabolism and autophagy (9); the knockdown of Siva 1 inhibits human fetal lung cell proliferation and induces cell cycle arrest via the alternative reading frame tumor protein (p) 53 pathway (10); Siva 1 destabilizes p53 and promotes its degradation, suppresses p53-mediated apoptosis in osteosarcoma cells, and the knockdown of Siva 1 inhibits tumorigenesis (11). In addition, Li *et al* revealed that Siva 1 phosphorylated Stathmin at Ser16, attenuated its microtubule-destabilizing activity and suppressed epithelial-mesenchymal transition and metastasis of breast cancer cells (12). Stathmin is a microtubule destabilizer, participating in cell mitosis and migration by regulating microtubule stability. Additionally, it is highly expressed in a number of malignant tumors, including ovarian cancer (13), suggesting a cancer-promoting effect.

In conclusion, the effect of Siva 1 is different in a number of cell types, which is a result of the combined action of multiple signaling pathways. At present, Siva 1 is pro-apoptotic

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and carcinostatic in colorectal (14), cervical (5) and breast cancer (12) and acute leukemia (15), but anti-apoptotic and carcinogenic in osteosarcoma (11) and NSCLC (9). However, it remains unknown whether Siva 1 functions in ovarian cancer. In the present study, the effect of Siva 1 on ovarian cancer cells and the underlying mechanism of its action in these cells were investigated.

Materials and methods

Cell culture, transfection and establishment of stable cell line. Ovarian cancer SKOV3 and OVCAR-3 cell lines were purchased from the Shanghai Cell Bank of Chinese Academy of Sciences and cultured with RPMI-1640 (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA USA) supplemented with 10% fetal bovine serum (FBS; Hyclone; GE Healthcare; Logan, UT, USA) at 37°C in 5% CO₂. A2780 cells were purchased from Cell Preservation Center of Wuhan University and cultured with Dulbecco's modified Eagle's medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented 10% FBS at 37°C in 5% CO₂. Siva 1 overexpression plasmid pCMV3-Siva 1 or the control pCMV3 [China National Pharmaceutical Group (CNPGC); Sinopharm, Beijing, China] were transfected into A2780 cells with lipofectamine reagent (Invitrogen; Thermo Fisher Scientific, Inc.). A total of 24 h after transfection, 200 µg/ml G418 (Invitrogen; Thermo Fisher Scientific, Inc.) was added to the medium, and the culture medium was renewed every 2 days. Approximately 1 week later, monoclonal cell clusters were observed and selected for culture. Thereafter, the Siva 1 stably expressed A2780 cell line and its control were used for subsequent experiments.

RNA isolation and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was isolated from the cells by total RNA rapid isolation kit (BioTeke Corporation, Beijing, China) according to the manufacturer's protocol. The concentration was measured by the OD₂₆₀/OD₂₈₀ ratio using a NanoDrop 2000 (Thermo Fisher Scientific, Inc., Wilmington, DE, USA). The RNA was reverse transcribed with Moloney Murine leukemia virus (M-MLV) reverse transcriptase (BioTeke Corporation), with oligo-deoxy-thymidine nucleotides and random primers (Sangon Biotech Co., Ltd., Shanghai, China) as the RT primers. The negative control was the RNA in pCMV3-transfected cells. For the RT-PCR, the following items were added with RNA samples into each tube: 1 µg RNA, 1 µl oligo (dT) primers, 1 µl random primers, 2 µl dNTP and ddH₂O to make a final reaction volume of 14.5 µl. Each tube was heated at 70°C for 5 min, cooled on ice for 2 min, and the following items were added: 4 µl 5X buffer (250 mM Tris-HCl (pH 8.3), 375 mM KCl, 15 mM MgCl₂, 50 mM DTT; Dongsheng Biotech, Guangzhou, China), 0.5 µl RNasin ribonuclease inhibitor (Tiandz, Inc., Beijing, China) and 1 µl M-MLV reverse transcriptase. The thermocycler conditions were as follows: 25°C for 10 min, then at 42°C for 50 min, heated at 95°C for 5 min, and finally cooled on ice for several min. There was 20 µl cDNA sample in each reaction. All the instruments used in this step were treated with Surface RNase Erasol (Tiandz, Inc.) and all the reagents used in this protocol were RNase-free.

The produced cDNA (1 µg) was used for qPCR by Taq PCR MasterMix (BioTeke Corporation) supplemented with

SYBR Green (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) to detect Siva 1 or Stathmin using the following procedure: 95°C for 10 min, 40 cycles of 95°C for 10 sec, 60°C for 20 sec and 72°C for 30 sec, and finally 4°C for 5 min, with β-actin as the internal control. The primer sequences are provided in Table I. The data were analyzed by the 2^{-ΔΔCq} method (16), with β-actin as the reference gene.

Western blot analysis. Protein samples from SKOV3, OVCAR-3 and A2780 cell lines were extracted using a total protein extraction kit (Wanleibio Co., Ltd., Shenyang, China) according to the manufacturer's protocol. Subsequent to quantification of the content using a bicinchoninic acid protein quantification kit (Wanleibio Co., Ltd.), the protein was isolated by SDS-PAGE using 40 µg protein in each lane and transferred to polyvinylidene fluoride (PVDF) membrane (EMD Millipore, Billerica, MA, USA). Subsequent to blocking with 5% skim milk (YILI, Hohhot, Inner Mongolia, China) at room temperature for 1 h, diluted by TBS Tween (TBST), the PVDF membrane was incubated with the following antibodies at 4°C overnight: rabbit anti-human anti-Siva 1 polyclonal antibody (dilution, 1:200; Santa Cruz Biotechnology, Inc., Dallas, TX, USA, cat. no., sc-48767), rabbit anti-human anti-cleaved caspase-3 polyclonal antibody (dilution, 1:1,000; Abcam, Cambridge, UK, cat. no., ab2302), goat anti-human anti-cleaved caspase-9 polyclonal antibody (dilution, 1:200; Santa Cruz Biotechnology, Inc., cat. no., sc-22182), rabbit anti-human anti-Bcl-2-like protein 4 (Bax) polyclonal antibody (dilution, 1:400; Boster, Hubei, Wuhan, China, cat. no., BA0315), rabbit anti-human anti-Bcl-2 polyclonal antibody (dilution, 1:400; Boster, cat. no., BA0412), rabbit anti-human anti-Stathmin polyclonal antibody (dilution, 1:500; Bioss Antibodies, Beijing, China, cat. no., bs-1902R), rabbit anti-human anti-phosphorylated Stathmin polyclonal antibody (dilution, 1:500; Bioss Antibodies, cat. no., bs-3431R), rabbit anti-human anti-α-tubulin polyclonal antibody (dilution, 1:1,000; Abcam, cat. no., ab178484) or mouse anti-human anti-acetyl-α-tubulin monoclonal antibody (dilution, 1:1,000; Abcam, cat. no., ab24610). Subsequent to washing with TBST 4 times for 5 min each, the PVDF membrane was incubated with goat anti-rabbit polyclonal IgG-horseradish peroxidase (HRP; dilution, 1:5,000; Wanleibio Co., Ltd., cat. no., WLA023), goat anti-mouse polyclonal IgG-HRP (dilution, 1:5,000; Wanleibio Co., Ltd., cat. no., WLA024) or donkey anti-goat polyclonal IgG-HRP (dilution, 1:5,000; Beyotime Institute of Biotechnology, Haimen, Jiangsu, China, cat. no., A0181) at 37°C for 45 min, followed treatment with a ECL reagent (Wanleibio Co., Ltd.). Subsequent to the removal of antibodies by stripping buffer (Wanleibio Co., Ltd.), the PVDF membrane was incubated with rabbit anti-human anti-β-actin antibody (dilution, 1:1,000; Santa Cruz Biotechnology, Inc., cat. no., sc-47778) and goat anti-rabbit polyclonal IgG-HRP (dilution, 1:5,000; Wanleibio Co., Ltd.) to detect the internal control, β-actin. The bands were analyzed with Gel-Pro-Analyzer software (version 4, Media Cybernetics, Silver Spring, MD, USA), and experiments were performed in triplicate.

Immunofluorescence. A2780 cells were seeded onto glass slides prior to this protocol. When cell confluence reached 70-80%, the cells were fixed in 4% paraformaldehyde

Table I. Sequences of real-time PCR primers.

Name	Sequence (5' to 3')
Siva 1	F: CCAAGCGACTCCTGTTCCTC R: CCAATCAGCATCTGCCCAC
Stathmin	F: TCGCTTGTCTTCTATTACCA R: CTTCTTTCTCGTGCTCTCGTT
β -actin	F: CTTAGTTGCGTTACACCCTTCTTG R: TGTCACCTTCACCGTTCCAGTTT

F, forward; R, reverse.

(CNPGC; Sinopharm) at room temperature for 15 min, and permeated with 0.1% TritonX-100 (Amresco, LLC., Solon, OH, USA) for 30 min. Subsequent to blocking with goat serum (Beijing Solarbio Science & Technology Co., Ltd., cat. no., SL2-10) for 15 min, the cells were incubated with rabbit anti-human anti-Siva 1 polyclonal antibody (dilution, 1:50; Santa Cruz Biotechnology, Inc., cat. no., sc-48767) at 4°C overnight. Subsequent to rinsing with PBS, the cells were incubated with goat anti-rabbit polyclonal IgG-Cy3 (1:200) (Beyotime Institute of Biotechnology, cat. no., A0516) for 60 min, stained with DAPI (Biosharp, Hefei, China) for 5 min, and mounted with an anti-fade solution (Beijing Solarbio Science & Technology Co., Ltd.) in the dark. The slides were observed and images were captured with a fluorescence microscope (Olympus Corporation, Tokyo, Japan) at magnification, x400, and analyzed with Image pro-plus v6.0 (IPP) software (Media Cybernetics, Rockville, MD, USA).

CCK-8 assay. A2780 cells were seeded in a 96-well plate with 3×10^3 cells/well. Cell viability was detected at 24, 48, 72, 96 and 120 h after seeding. The CCK-8 reagent (Wanleibio Co., Ltd.) was added into the culture medium at $10 \mu\text{l}/100 \mu\text{l}$ medium/well, and incubated at 37°C for 1 h. Then, the optical density at 450 nm of culture medium was determined by microplate reader (Biotek Instruments, Inc., Winooski, VT, USA).

Colony formation. A2780 cells were seeded onto 35-mm petri dishes with 500 cells per dish, and cultured at 37°C in 5% CO₂. Approximately 2 weeks later, the majority of the clones were able to be seen by the naked eye. The cells were fixed with 4% paraformaldehyde (CNPGC; Sinopharm) at room temperature for 20 min and stained with Wright-Giemsa stain reagent (Nanjing Jiancheng Bioengineering Research Institute, Nanjing, China) for 5-8 min. Subsequent to rinsing with PBS 2-3 times, cell clones were counted and the clone formation rate was calculated as follows: clone number/number of cells seeded. Clones with >50 cells were considered positive.

Hoechst staining. A2780 cells were seeded on microscope slides in a 12-well plate with 3×10^5 per well. When cell confluence reached 60-70%, the cells were fixed with 4% paraformaldehyde (CNPGC; Sinopharm) at room temperature for 20 min and stained with Hoechst 33258 stain reagent (Wanleibio Co., Ltd.) for 5 min in the dark. Cells were blocked

with Antifade Mounting Medium (Beyotime Institute of Biotechnology) and observed with fluorescence microscopy (Olympus Corporation) at magnification, x400, and analyzed with CellSens version 1.6 (Olympus Corporation).

Wound healing assay. A wound healing assay was performed to measure cell migration ability. A2780 cells were cultured with serum-free medium supplemented with $1 \mu\text{g}/\text{ml}$ mitomycin C (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) for 1 h. The wound was caused by $200 \mu\text{l}$ pipette tip, and images of the wound sizes 0, 24 and 48 h subsequent to wounding were captured with an inverted phase contrast microscope (Motic, Xiamen, Fujian, China) at magnification, x100. The cell migration rate at 24 h was calculated as (wound size at 0 h-wound size at 24 h)/wound size at 0 h; the cell migration rate at 48 h was calculated as (wound size at 0 h-wound size at 48 h)/wound size at 0 h.

Transwell assay. A2780 cell invasion was detected by Transwell assay supplemented with Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) *in vitro*, with A2780 cells transfected with pCMV3 vector used as the negative control. All experiments were performed in triplicate. A total of $40 \mu\text{l}$ 2.5 mg/ml Matrigel was added into the Transwell chamber (Corning Incorporated, Corning, NY, USA) and preheated at 37°C to solidify. A total of $200 \mu\text{l}$ serum-free cell suspension with 1×10^4 cells were seeded into the upper chamber, and $800 \mu\text{l}$ medium with 20% FBS (Hyclone; GE Healthcare; Logan, UT, USA) was added into the lower chamber. Subsequent to culturing for 24 h, the Transwell chamber was removed and the cells in upper chamber were wiped away. The cells in lower chamber were fixed with 4% paraformaldehyde (CNPGC; Sinopharm) at room temperature for 20 min and stained with 0.5% crystal violet reagent (Amresco, LLC) for 5 min. Subsequent to rinsing with water, the cells in the lower chambers were counted with an inverted phase contrast microscope (Motic, Xiamen, Fujian, China) at magnification, x200.

Flow cytometry. A2780 cells were cultured in 6-well plates until the cell confluence reached 90%. The cells were collected and treated with Annexin V-fluorescein isothiocyanate/propidium iodide apoptosis detection kit (Wanleibio Co., Ltd.) according to the manufacturer's protocol, and detected by flow cytometry (BD Biosciences).

Statistical analysis. The data in the present study are presented as the mean \pm standard deviation, with three individual experiments, and were analyzed with one-way analysis of variance. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Establishment of Siva 1 stably overexpressed cell line. In order to study the roles of Siva 1 in ovarian cancer cells, Siva 1 expression level was first detected in three ovarian cancer cell lines, A2780, OVCAR-3 and SKOV3. The western blot analyses results demonstrated that the expression level of Siva 1 in A2780 cells was markedly lower compared with OVCAR-3 cells and SKOV3 cells (Fig. 1A and B). The overexpression

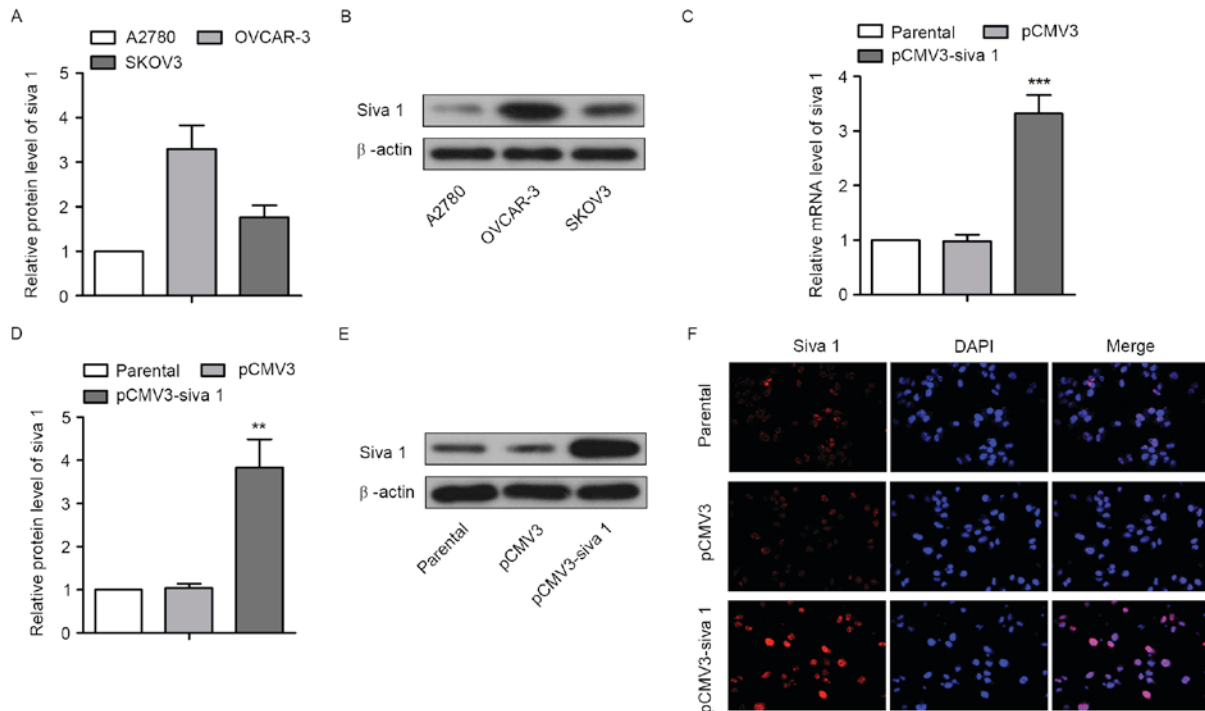


Figure 1. Establishment of the cell line with stable Siva 1 overexpression. (A and B) Expression level of Siva 1 in A2780, OVCAR-3 and SKOV3 cells detected by western blot analysis. (C) Siva 1 mRNA level in stably overexpressed cells detected by real-time PCR. (D and E) Protein expression levels of Siva 1 in the cells with stable overexpressed detected by western blot analysis. (F) Distribution of Siva 1 in cells with stable overexpression detected by immunofluorescence. ** $P < 0.01$, *** $P < 0.001$ vs. pCMV3.

plasmid pCMV3-Siva 1 was then constructed and transfected into A2780 cells. By screening with G418, a monoclonal cell line was obtained, with A2780 cells transfected with pCMV3 as the control and untreated A2780 cells as the parental line. qPCR and western blot analyses showed that mRNA and protein levels of Siva 1 in pCMV3-Siva 1 cells increased 3.39-fold ($P = 0.0004$) and 3.68-fold ($P = 0.0019$), respectively, compared with pCMV3 (Fig. 1C-E). The immunofluorescence assay results demonstrated the same increase, and Siva 1 was distributed in the cytoplasm and in the nuclei (Fig. 1F).

Siva 1 inhibits the growth and proliferation of ovarian cancer cells. CCK-8 and colony formation assays were performed to detect cell viability and colony formation abilities of the cell lines. As shown in Fig. 2A, Siva 1 impaired cell viability by 21 ($P = 0.0056$), 25 ($P = 0.0005$), 30 ($P < 0.0001$), 32 ($P = 0.0001$) and 31% ($P < 0.0001$) in 24, 48, 72, 96 and 120 h, respectively. The colony formation assay indicated that Siva 1 decreased the colony formation ability of A2780 cells by 44% ($P = 0.0025$) (Fig. 2B). Therefore, these data suggest that overexpression of Siva 1 inhibited the proliferation of ovarian cancer cells.

Siva 1 promotes apoptosis of ovarian cancer cells. To determine the effect of Siva 1 on apoptosis of ovarian cancer cells, flow cytometry and Hoechst staining were performed. Flow cytometry detection demonstrated that Siva 1 increased the apoptosis rate by 4.55-fold ($P < 0.0001$) (Fig. 3A and B). Using Hoechst staining, bright nuclei in were observed in the pCMV3-Siva 1 cells (Fig. 3C), which indicates chromosome breakage and nucleus shrinkage in apoptosis progress. In addition, there are a number of signaling molecules participating

in intrinsic apoptosis, which may be detected as apoptosis markers. In the present study, the expression levels of executioners of apoptosis, cleaved caspase-3 and cleaved caspase-9, pro-apoptotic protein Bax and anti-apoptotic protein Bcl-2 were detected. The western blot analyses results demonstrated that in cells that expressed stable levels of Siva 1, the protein levels of cleaved caspase-3 increased 1.96-fold ($P = 0.0016$), cleaved caspase-9 increased 2.03-fold ($P = 0.0002$), Bax increased 2.02-fold ($P = 0.0005$) and Bcl-2 decreased by 44% ($P = 0.0004$) (Fig. 3D and E). These data indicate that Siva 1 promoted the apoptosis of ovarian cancer cells.

Siva 1 suppresses the migration and invasion of ovarian cancer cells. Since migration and invasion are other vital aspects of cancer cell malignancies, the migration and invasion potential of ovarian cancer cells was measured. The wound healing assay demonstrated that subsequent to Siva 1 overexpression, migration rate decreased by 41% ($P = 0.0052$) and 49% ($P = 0.001$) after 24 and 48 h, respectively (Fig. 4A and B). The Transwell assay with Matrigel found that Siva 1 decreased the invasion potential of ovarian cancer cells by 35% ($P < 0.0001$) (Fig. 4C and D).

Siva 1 enhances the phosphorylation of Stathmin. The effects of Siva 1 on Stathmin in ovarian cancer cells were then investigated. qPCR and western blot analyses results demonstrated that overexpression of Siva 1 did not increase Stathmin expression, but promoted its phosphorylation (Fig. 5A-C). As the polymerized tubulin was acetylated, the level of acetyl- α -tubulin was also measured to determine whether the microtubule-destabilizing activity of

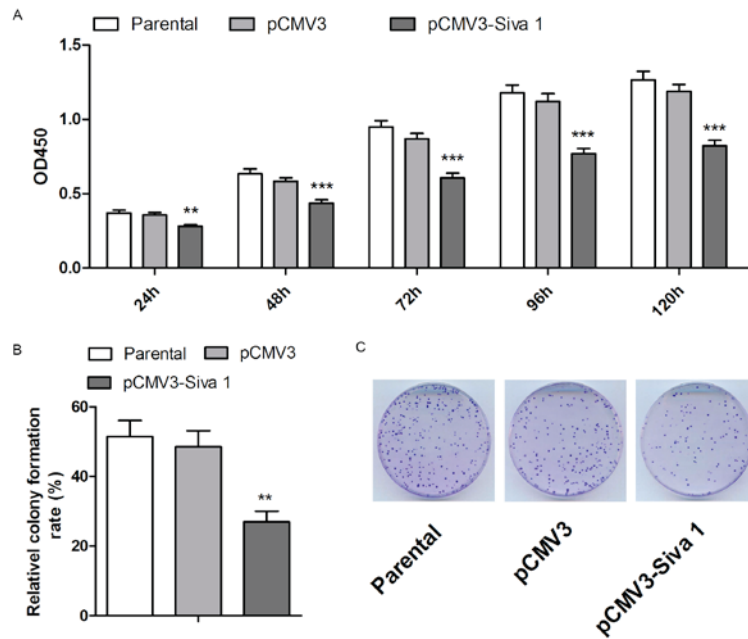


Figure 2. Siva 1 inhibits proliferation of ovarian cancer cells. (A) Overexpression of Siva 1 inhibited A2780 cell viability as detected by CCK-8 assay. (B and C) Overexpression of Siva 1 represses colony formation of A2780 cells as detected by plate colony formation assay. **P<0.01 vs. pCMV group; ***P<0.001 vs. pCMV group; OD, optical density.

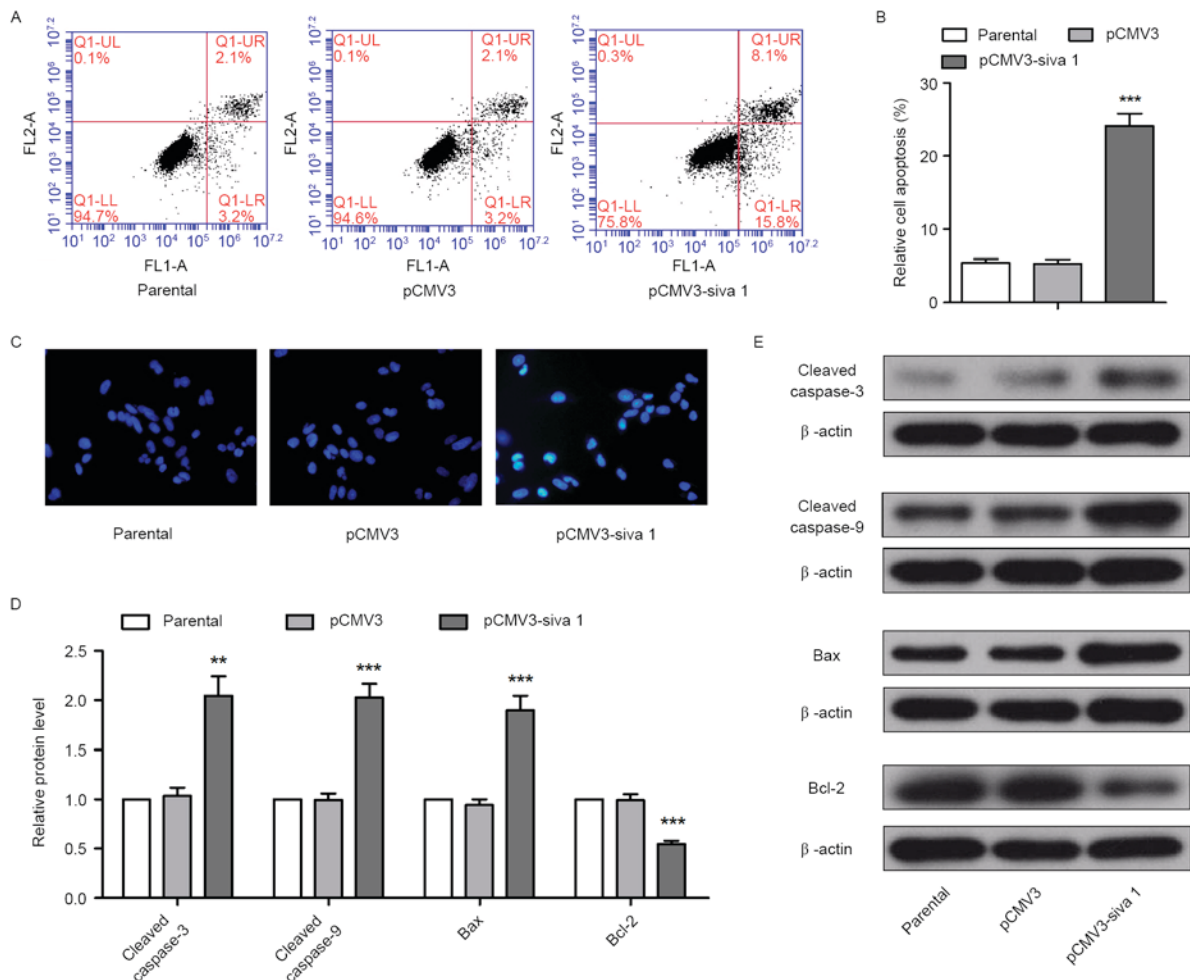


Figure 3. Siva 1 promotes apoptosis of ovarian cancer cells. (A and B) Expression of Siva 1 promotes apoptosis of A2780 cells detected by flow cytometry. (C) Apoptosis of A2780 cells detected by Hoechst staining. (D and E) Expression level of apoptosis-associated proteins, cleaved caspase-3, cleaved caspase-9, Bax and Bcl-2 detected by western blot. **P<0.01 vs. pCMV group; ***P<0.001 vs. pCMV group; Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-like protein 4.

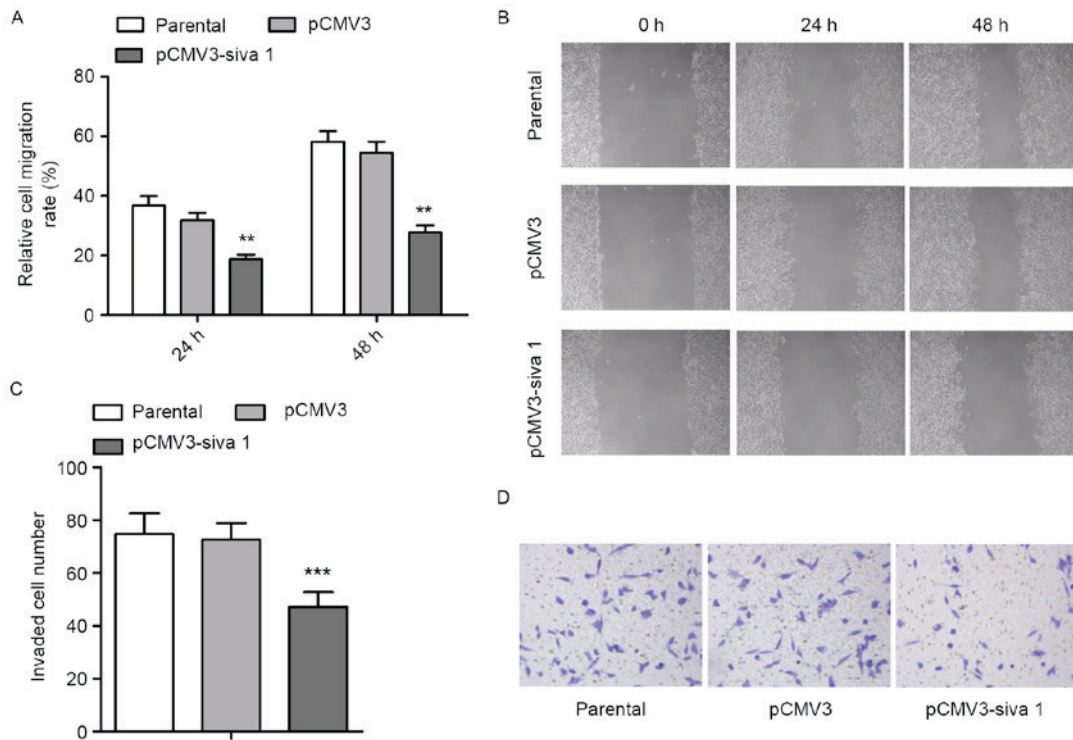


Figure 4. Siva 1 suppresses migration and invasion of ovarian cancer cells. (A and B) Overexpression of Siva 1 suppresses the migration of A2780 cells, as detected by wound healing assay. (C and D) Overexpression of Siva 1 inhibits the invasion of A2780 cells, as detected by Transwell assay with Matrigel. **P<0.01 vs. pCMV group; ***P<0.001 vs. pCMV group.

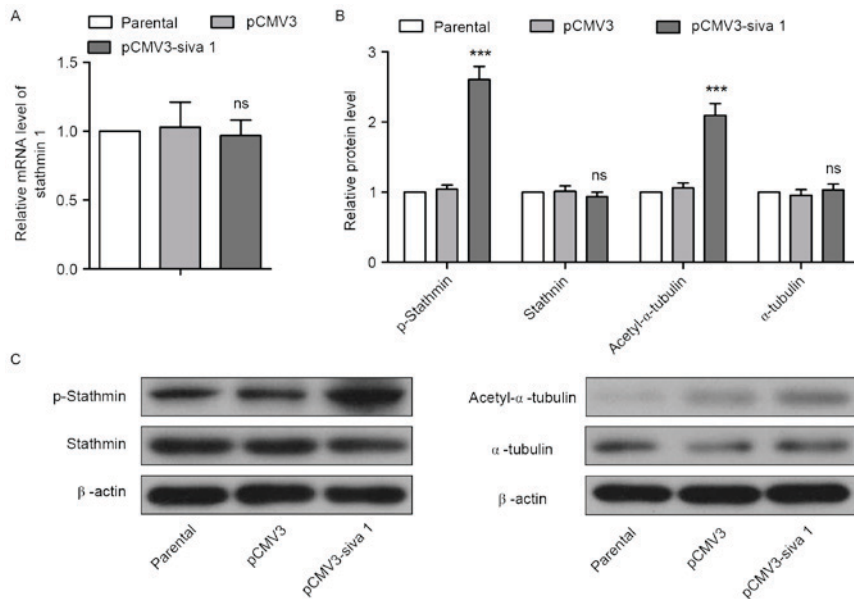


Figure 5. Siva 1 enhances phosphorylation of Stathmin. (A) Siva 1 does not affect Stathmin mRNA level. (B and C) Overexpression of Siva 1 enhances phosphorylation of Stathmin and acetylation of α-tubulin, but did not markedly affect the expression level of Stathmin and α-tubulin detected by western blot. ***P<0.001 vs. pCMV group; ns, no significance.

Stathmin was impaired. Western blot analyses revealed that acetyl-α-tubulin levels were elevated, but the levels of α-tubulin were not in cells with an overexpression of Siva 1, which indicates that Siva 1 stabilized microtubules by inhibiting Stathmin activity.

Taken together, these data suggest that Siva 1 inhibited cell growth and proliferation, promoted apoptosis and suppressed

migration and invasion by promoting Stathmin phosphorylation and stabilizing microtubules in ovarian cancer cells.

Discussion

There have been several studies demonstrating that Siva 1 facilitated or suppressed apoptosis in different types of cancer

cells previously. In the present study, it was revealed that Siva 1 promoted intrinsic apoptosis and inhibited the proliferation of ovarian cancer cells. In this process, the levels of apoptosis executors activated caspase-3, caspase-9 and pro-apoptosis protein Bax increased, and the level of the anti-apoptosis protein Bcl-2 decreased. Additionally, the phosphorylation of Stathmin and acetylation of α -tubulin increased, which represented an increase in the number of microtubules produced.

Stathmin, also known as oncoprotein 18, is a microtubule destabilizing protein. It binds to tubulin and maintains the dynamic process of continuous microtubule synthesis and degradation (17). Microtubules, composed of α - and β -tubulin, constitute cytoskeleton, spindle, cilia and flagella, and participate in support, material transport, cell movement, mitosis and other functions. During the mitosis process, tubulin is rapidly depolymerized and re-polymerized into spindle fibers. Once Stathmin was phosphorylated and inactivated, cell proliferation was inhibited (18,19). In the present study, Siva 1 inhibited cell proliferation and promoted apoptosis by facilitating the phosphorylation of Stathmin and decreasing its activity.

Metastasis is another important contributor to the high mortality rates and difficulty incurring malignant tumors. Stathmin was highly expressed in a number of malignant tumors (20). It reduced the stability of microtubules and provided tumor cells with improved motility. Stathmin has been used as a therapeutic target and prognostic indicator in prostate and cervical cancer, oral squamous cell carcinoma and medulloblastoma (21-24). In the present study, it was revealed that Siva 1 promoted the phosphorylation of Stathmin, increased the level of acetylated α -tubulin, which was present only in polymerized tubulin (25), and therefore inhibited the migration and invasion of ovarian cancer cells.

Siva 1 serves different roles in various cells, and the mechanism of action also relies on the specific cell types. In the present study, Siva 1 was overexpressed in the ovarian cancer A2780 cell line, the changes in cell phenotype were detected, and the potential mechanism was studied. The results of the present study demonstrated that Siva 1 inhibited proliferation, promoted apoptosis, suppressed migration and invasion by facilitating phosphorylation of Stathmin and polymerization of tubulin in ovarian cancer cells. These data provide novel insights into the molecular mechanism of ovarian cancer pathogenesis, and provide a basis for additional studies investigating the clinical diagnosis and treatment methods of ovarian cancer.

References

- Siegel RL, Miller KD and Jemal A: Cancer statistics, 2015. *CA Cancer J Clin* 65: 5-29, 2015.
- Yeung TL, Leung CS, Li F, Wong SS and Mok SC: Targeting stromal-cancer cell crosstalk networks in ovarian cancer treatment. *Biomolecules* 6: 3, 2016.
- Prasad KV, Ao Z, Yoon Y, Wu MX, Rizk M, Jacquot S and Schlossman SF: CD27, a member of the tumor necrosis factor receptor family, induces apoptosis and binds to Siva, a proapoptotic protein. *Proc Natl Acad Sci USA* 94: 6346-6351, 1997.
- Py B, Slomianny C, Auberger P, Petit PX and Benichou S: Siva-1 and an alternative splice form lacking the death domain, Siva-2, similarly induce apoptosis in T lymphocytes via a caspase-dependent mitochondrial pathway. *J Immunol* 172: 4008-4017, 2004.
- Iorio-Morin C, Germain P, Roy S, Genier S, Labrecque P and Parent JL: Thromboxane A2 modulates cisplatin-induced apoptosis through a Siva1-dependent mechanism. *Cell Death Differ* 19: 1347-1357, 2012.
- Gudi R, Barking J, Hawkins S, Chu F, Manicassamy S, Sun Z, Duke-Cohan JS and Prasad KV: Siva-1 negatively regulates NF-kappaB activity: Effect on T-cell receptor-mediated activation-induced cell death (AICD). *Oncogene* 25: 3458-3462, 2006.
- Shiozaki T, Iwai A, Kawaoka Y, Takada A, Kida H and Miyazaki T: Requirement for Siva-1 for replication of influenza A virus through apoptosis induction. *J Gen Virol* 92: 315-325, 2011.
- Py B, Bouchet J, Jacquot G, Sol-Foulon N, Basmaciogullari S, Schwartz O, Biard-Piechaczyk M and Benichou S: The Siva protein is a novel intracellular ligand of the CD4 receptor that promotes HIV-1 envelope-induced apoptosis in T-lymphoid cells. *Apoptosis* 12: 1879-1892, 2007.
- Van Nostrand JL, Brisac A, Mello SS, Jacobs SB, Luong R and Attardi LD: The p53 target gene siva enables non-small cell lung cancer development. *Cancer Discov* 5: 622-635, 2015.
- Wang X, Zha M, Zhao X, Jiang P, Du W, Tam AY, Mei Y and Wu M: Siva1 inhibits p53 function by acting as an ARF E3 ubiquitin ligase. *Nat Commun* 4: 1551, 2013.
- Du W, Jiang P, Li N, Mei Y, Wang X, Wen L, Yang X and Wu M: Suppression of p53 activity by Siva1. *Cell Death Differ* 16: 1493-1504, 2009.
- Li N, Jiang P, Du W, Wu Z, Li C, Qiao M, Yang X and Wu M: Siva1 suppresses epithelial-mesenchymal transition and metastasis of tumor cells by inhibiting stathmin and stabilizing microtubules. *Proc Natl Acad Sci USA* 108: 12851-12856, 2011.
- Wei SH, Lin F, Wang X, Gao P and Zhang HZ: Prognostic significance of stathmin expression in correlation with metastasis and clinicopathological characteristics in human ovarian carcinoma. *Acta Histochem* 110: 59-65, 2008.
- Ray RM, Bhattacharya S and Johnson LR: Mdm2 inhibition induces apoptosis in p53 deficient human colon cancer cells by activating p73- and E2F1-mediated expression of PUMA and Siva-1. *Apoptosis* 16: 35-44, 2011.
- Machado-Neto JA, Lazarini M, Favaro P, de Melo Campos P, Scopim-Ribeiro R, Franchi Junior GC, Nowill AE, Lima PR, Costa FF, Benichou S, *et al*: ANKHD1 silencing inhibits Stathmin 1 activity, cell proliferation and migration of leukemia cells. *Biochim Biophys Acta* 1853: 583-593, 2015.
- Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25: 402-408, 2001.
- Gupta KK, Li C, Duan A, Alberico EO, Kim OV, Alber MS and Goodson HV: Mechanism for the catastrophe-promoting activity of the microtubule destabilizer Op18/stathmin. *Proc Natl Acad Sci USA* 110: 20449-20454, 2013.
- Alli E, Yang JM and Hait WN: Silencing of stathmin induces tumor-suppressor function in breast cancer cell lines harboring mutant p53. *Oncogene* 26: 1003-1012, 2007.
- Price DK, Ball JR, Bahrani-Mostafavi Z, Vachris JC, Kaufman JS, Naumann RW, Higgins RV and Hall JB: The phosphoprotein Op18/stathmin is differentially expressed in ovarian cancer. *Cancer Invest* 18: 722-730, 2000.
- Roos G, Brattsand G, Landberg G, Marklund U and Gullberg M: Expression of oncoprotein 18 in human leukemias and lymphomas. *Leukemia* 7: 1538-1546, 1993.
- Mistry SJ, Bank A and Atweh GF: Targeting stathmin in prostate cancer. *Mol Cancer Ther* 4: 1821-1829, 2005.
- Kouzu Y, Uzawa K, Koike H, Saito K, Nakashima D, Higo M, Endo Y, Kasamatsu A, Shiiba M, Bukawa H, *et al*: Overexpression of stathmin in oral squamous-cell carcinoma: Correlation with tumour progression and poor prognosis. *Br J Cancer* 94: 717-723, 2006.
- Xi W, Rui W, Fang L, Ke D, Ping G and Hui-Zhong Z: Expression of stathmin/op18 as a significant prognostic factor for cervical carcinoma patients. *J Cancer Res Clin Oncol* 135: 837-846, 2009.
- Kuo MF, Wang HS, Kuo QT, Shun CT, Hsu HC, Yang SH and Yuan RH: High expression of stathmin protein predicts a fulminant course in medulloblastoma. *J Neurosurg Pediatr* 4: 74-80, 2009.
- Schulze E, Asai DJ, Bulinski JC and Kirschner M: Posttranslational modification and microtubule stability. *J Cell Biol* 105: 2167-2177, 1987.