

Downregulation of *SASH1* correlates with tumor progression and poor prognosis in ovarian carcinoma

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Abstract. SAM- and SH3-domain containing 1 (*SASH1*) is a recently identified tumor suppressor gene that is required in the tumorigenesis of breast and other solid carcinomas. The *SASH1* protein contains SH3 and SAM domains, indicating that it may serve an important role in intracellular signal transduction. The purpose of the present study was to investigate the expression of *SASH1* in ovarian carcinoma and the correlation between its expression with clinical pathological features and clinical significance, and the effect of *SASH1* on cell proliferation, apoptosis and migration of ovarian SKOV3 cells. The human ovarian carcinoma tissues and adjacent normal tissues were collected following surgery. Reverse transcription-quantitative polymerase chain reaction and western blot analysis were used to detect the expression levels of *SASH1* mRNA and protein, respectively. The expression levels of *SASH1* mRNA and protein in ovarian carcinoma tissues were significantly lower than that observed in adjacent normal tissues ($P<0.05$). The expression levels of *SASH1* in samples from patients without lymph nodes metastasis and patients with early FIGO stage was lower than those with lymph nodes metastasis and patients with advanced FIGO stage ($P<0.05$). Flow cytometry analysis and Transwell invasion chamber experiments were used to investigate the effect of *SASH1* on the cell proliferation, apoptosis and migration of SKOV3 cells. The recombinant plasmid pcDNA3.1-*SASH1* was constructed and transfected into SKOV3 cells. In addition, the SKOV3 cells in the pcDNA3.1-*SASH1* group exhibited

significantly reduced cell growth, proliferation, and migration ability compared to the empty vector group and normal group ($P<0.01$). There were a greater number of apoptotic cells in the pcDNA3.1-*SASH1* group compared to the empty vector group and normal group ($P<0.01$). Taken together, these results indicated that *SASH1* may be a tumor suppressor gene in ovarian carcinoma, and *SASH1* expression inhibited growth, proliferation and migration, and enhanced apoptosis of SKOV3 cells.

Introduction

In industrialized countries, including the USA, Japan and UK, ovarian carcinoma is one of the most common gynecological malignancies diseases and the leading cause of gynecological cancer mortality (1,2). There are multiple reasons for this, one cause may be that ovarian carcinoma is generally detected late, almost 70% of all patients present with advanced stage III and IV cancer, and a number of patients are misdiagnosed (3-5). The majority of patients suffer from abdominal, gastrointestinal, urinary, or pelvic pain, which rarely lead to timely definitive diagnosis, leading to the generally late detection of ovarian carcinomas (6,7).

Ovarian carcinoma is relatively asymptomatic at its early stages, and this results in a low chance of early detection (8,9). The majority of ovarian carcinoma patients already have tumor cells throughout the abdomen [International Federation of Gynaecologists and Obstetricians (FIGO) stages III-IV] and there is a low 5-year overall survival (OS) rate (10). Multiple genetic changes are involved in ovarian carcinoma development, which are not well characterized. To understand the pathogenesis of ovarian carcinoma is an important challenge, involving the identification of novel oncogenes and tumor suppressor genes (11,12).

In 2003, Zeller *et al* (13) discovered SAM and SH3- domain containing 1 (*SASH1*), a potential target gene on chromosome 6q24.3, through systematic comparison of candidate expressed sequence tags with genomic sequences from the genomic interval 6q23-25 (13). It has been demonstrated that *SASH1* is down-regulated in the majority (74%) of breast tumors in comparison with the corresponding normal breast epithelial tissues. The *SASH1* gene encodes one signal adapter protein

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consisting of several protein-protein interaction domains (14). The SAM domain can exhibit more complex functions in these protein-protein interaction domains (15). The SAM domain mediates protein-protein interactions through homologous and heterologous oligomerization with the SAM domains of other proteins, and it can mediate Smaug protein and mRNA binding to facilitate transcriptional regulation (16,17). SASH1 is a member of a recently described family of SH3/SAM adapter molecules according to its domain structure, thus suggesting a role in signaling pathways (18).

The carcinogenesis and tumor progression of ovarian carcinoma is a complex process with multiple factors and stages (19). The activation of oncogenes and mutation or deletion of tumor suppressor genes are the leading causes of ovarian carcinoma (7,20,21). Therefore, the study of suppressor genes and apoptosis-related genes in carcinogenesis and tumor progression in ovarian carcinoma has drawn increasing attention. A previous study indicated that *SASH1* is a tumor suppressor gene, located on chromosome 6q24.3 (22). Zeller *et al* (13) demonstrated reduced or absent *SASH1* expression in 6 breast cancer cell lines, which exhibit a chromosome 6q24.3 deletion. These results indicated that the down-regulation of *SASH1* is at least in part due to gene deletion (13). Down-regulation of *SASH1* expression was closely correlated with tumor invasion, metastasis, and poor prognosis (22-24). However, the specific role of *SASH1* in ovarian carcinoma has not yet been reported in the literature. In the present study, the expression of *SASH1* in ovarian carcinoma tissues was determined and its correlation to the clinical pathology of ovarian carcinoma by using reverse transcription-quantitative polymerase chain reaction (RT-qPCR) or western blot analysis. In addition, SKOV3 ovarian carcinoma cells were transfected with a eukaryotic expression vector expressing the full-length *SASH1* cDNA, and the changes in SKOV3 cell viability, proliferation, apoptosis and migration were assessed. These data might provide information for the prediction of ovarian carcinoma prognosis and the establishment of targeted therapies.

Materials and methods

Specimens. Fresh resection tissue specimens were collected from 79 patients with ovarian carcinoma at the Maternal and Child Health Care Hospital of Nantong (Nantong, China) from June 2004 to December 2013. All patients agreed to the procedure and signed informed consent forms. The samples were preserved in liquid nitrogen immediately, stored for analysis, and made anonymous according to the ethical and legal standard. No patients had received prior chemotherapy, radiotherapy or other preoperative treatments, and none had any other associated inflammatory disease. All tumor tissue and the adjacent normal ovarian tissue from 79 ovarian carcinoma cases were pathologically verified by the Department of Pathology of the Maternal and Child Health Care Hospital of Nantong. The clinicopathological characteristics of 79 ovarian patients were collected, including age at diagnosis, FIGO stage (25), histological type, and lymph node status. Histological type was classified according to the World Health Organization (WHO) criteria (26). The patient characteristics are summarized in Table I.

Reagents. Ovarian carcinoma SKOV3 cells were obtained from the American Type Culture Collection (Rockville, MD, USA). Fetal bovine serum, RPMI 1640 and cell culture plates and cell culture dishes were purchased from Corning Incorporated (New York, NY, USA).

TRIzol reagent was obtained from Thermo Fisher Scientific, Inc. (Waltham, MA, USA). FastStart Universal SYBR Green Master (Rox), Annexin V-FITC and propidium iodide were purchased from Roche Diagnostics (Basel, Switzerland). First Strand cDNA Synthesis Kit was purchased from Qiagen, Inc. (Valencia, CA, USA). Lipofectamine 2000, pcDNA3.1 vector, and pGEM-T vector were purchased from Invitrogen (Thermo Fisher Scientific, Inc.). Trypsin and PBS were purchased from Sigma-Aldrich, Inc. (Shanghai, China). Rabbit anti-SASH1 polyclonal antibody and mouse anti-human β -actin monoclonal antibody were purchased from Abcam Corporation (Cambridge, UK). ReadyPrep™ Protein Extraction kit and Quick Start™ Bradford Protein assay were purchased from Bio-Rad Laboratories (Richmond, CA, USA). Restriction endonuclease *Bam*HI and *Xho*I and DNA marker were obtained from Takara Corporation (Dalian, Liaoning, China). T4 DNA ligation was purchased from Promega Corporation (Beijing, China). Taq DNA polymerase and the prestaining protein ladder were purchased from Fermentas, Inc. (Glen Burnie, MD, USA).

Transwell invasion chamber was purchased from Corning Corporation (Midland, MI, USA). Matrigel was obtained from Collaborative Biomedical Products (Bedford, MA, USA). IRDye 800 conjugated affinity purified goat anti-mouse IgG and IRDye 800 conjugated affinity purified goat anti-rabbit IgG were purchased from Li-COR Biotechnology (Lincoln, NE, USA). Hoechst 33342 dye was purchased from Beyotime Institute of Technology, Inc. (Haimen, China).

RT-qPCR for detecting the expression levels of SASH1. RT-qPCR for *SASH1* was used to detect the expression levels of *SASH1* in 79 ovarian carcinoma tissues and adjacent normal tissues. *SASH1* (GenBank code: NM_015278.3) forward primer P1: 5'-ATACCTCGGCTTGACATT-3', reverse primer P2: 5'-ATACCTCGGCTTGACATT-3'. Ki-67 (GenBank code: AJ567756.1) forward primer P1: 5'-ACTTGCCTCCTAATACGC-3', reverse primer P2: 5'-CAGGTTGCCACTCTTTCT-3'. Internal marker gene *GAPDH* (GenBank code: NM_002046) forward primer P1: 5'-CCACAGTCCATGCCATCACT-3', reverse primer P2: 5'-TCCACCACCCTGTTGCTGTAG-3'. All of the above primers were synthesized and provided by Shanghai Invitrogen Biotechnology Co. Ltd (Shanghai, China).

Total RNA was extracted from tissue samples using TRIzol reagent according to the manufacturer's protocol. Each tissue sample (100 mg) was added, and the sample was homogenized and the total RNA was extracted from the tissue samples. Next, the reverse transcriptase (RT) reactions were performed with a First Strand cDNA Synthesis kit and the first-strand cDNA was synthesized and stored at -20°C in small aliquots.

The synthetic primers for *SASH1*, Ki-67 and *GAPDH* from Shanghai Invitrogen Biotechnology Company were dissolved with ddH₂O and stored in small aliquots at -20°C for later use. PCR amplification was initiated with one cycle of 95°C for 10 min, followed by 40 cycles of 94°C for 15 sec and 60°C for 60 sec on a StepOne™ Real-Time PCR system

Table I. Association between clinicopathological character and *SASH1* expression in patients with ovarian cancer.

Clinical feature	Cases	<i>SASH1</i> mRNA positive rate (%)	P
Normal tissue	79	79 (100.0)	0.000 ^a
Carcinoma tissue	79	48 (60.8)	
Age (year)			0.953
<50	36	22 (61.1)	
≥50	43	26 (60.5)	
Histological type			0.344
Serous	63	36 (57.1)	
Non-serous	26	12 (46.2)	
Residual tumor			0.127
<1 cm	41	21 (51.2)	
≥1 cm	38	13 (34.2)	
FIGO stage			0.016 ^a
I-II	46	32 (69.6)	
III-IV	33	14 (42.4)	
Lymph nodes metastasis			0.021 ^a
Negative	42	30 (71.4)	
Positive	37	17 (46.0)	

Statistical analyses were performed using the pearson χ^2 test. ^aP<0.05 was considered to indicate a significant difference. *SASH1*, SAM- and SH3-domain containing 1; FIGO, International Federation of Gynaecologists and Obstetricians.

(Thermo Fisher Scientific, Inc.). The *GAPDH* was amplified as an internal control. The relative quantification of *SASH1* expression was evaluated using the comparative quantification cycle (Cq) method. The raw data were presented as the relative quantity of *SASH1*, normalized with respect to *GAPDH*. Each sample was examined in triplicate. Mean normalized gene expression \pm standard deviation (SD) were calculated from independent experiments.

Western blot for detecting the expression levels of *SASH1*. In each experiment, 100 mg of tissue sample preserved in liquid nitrogen was homogenized with a protein extraction kit. The homogenate was centrifuged at 16,000 x g for 30 min. The supernatant was saved, and its protein concentration was determined with a protein quantification kit. A 6% stacking and 12% separation SDS-PAGE gel were prepared, 50 μ g of total protein was applied to each lane, and electrophoresis was performed. The proteins were transferred from the gel to a PVDF membrane and then the PVDF membrane was blocked with 5% non-fat dry milk in TBST buffer, and incubated with the rabbit anti-*SASH1* polyclonal antibody (1:500 dilution), and mouse anti-human monoclonal β -actin antibody (1:1,000 dilution), respectively, followed by overnight incubation at 4°C. The membrane was washed with TBST buffer, and further incubated with secondary antibody: the secondary antibodies with the corresponding IRDye 800 labeling (1:2,000 dilution in PBS) at room temperature for 2 h. After TBST washing, film scanning was performed with the Odyssey Infrared Imaging System (LI-COR Biotechnology). The relative expression levels of *SASH1* were represented by the grayscale ratio of

SASH1/ β -actin. The grayscale density was analyzed with QuantityOne version 4.62 software (Bio-Rad Laboratories, Hercules, CA, USA).

Plasmid construction. Primer Premier 5 software (Premier Biosoft, Palo Alto, CA, USA) was used to design for Primer on the flank of gene *SASH1* ORF and the restriction enzyme analysis. The forward primer, 5'-CGGGATCCATGGAGGACG CGGGAGCAGC-3', contained the *Bam*HI restriction enzyme site; the reverse primer, 5'-CCCTCGAGCATGGCCTCAGG CCTGGCG-3', contained the *Xho*I restriction enzyme site. All primers were synthesized by Shanghai Invitrogen Corporation.

The fragment of gene *SASH1* ORF was amplified by PCR with the primers for the *SASH1* gene. The products of PCR were cloned into the pGEM-T vector. The correct recombinant plasmid pGEM-*SASH1* was identified with restriction endonuclease, and sequenced. The vector pcDNA3.1 and the recombinant plasmid pGEM-*SASH1* were simultaneously digested with restriction endonucleases *Bam*HI and *Xho*I. The targeted fragments were ligated by T4 DNA ligase, and the recombinant plasmid pcDNA3.1- *SASH1* were transformed into DH5 α competent cells (Sangon Biotech Co., Ltd., Shanghai, China).

Cell culture and transfection. SKOV3 cells were cultured in RPMI-1640 medium, containing 5% fetal bovine serum and penicillin (100 U/ml)/streptomycin (100 μ g/ml) (Thermo Fisher Scientific, Inc.) at 37°C, 5% CO₂. Cell growth was observed by an inverted phase contrast microscope. When cell growth reached ~80% confluence, the cells were digested

with 0.25% trypsin and passaged. The culture medium was changed each day, and the cells were passaged every 2 to 3 days. Cells in the exponential growth phase were selected for experiments.

SKOV3 cells that were cultured under normal conditions were inoculated uniformly into 6-well culture plates at a density of 3×10^5 cells/ml. According to the operating instructions for Lipofectamine 2000, transfections were conducted with 4 μ g empty vector (pcDNA3.1) as a control or recombinant expression plasmid pcDNA3.1-SASH1. The normal (untransfected) control group was also established. RPMI-1640 medium without serum was used to dilute the plasmids, and 250 μ l Lipofectamine 2000 was added to the medium. After being mixed mildly, the mixture was incubated under room temperature for 20 min, then added to the SKOV3 cell culture medium. After 5 h, the culture medium was switched to RPMI-1640 medium containing 5% fetal bovine serum, the mixture was incubated for another 48 h. Western blot analysis was used to determine the expression of SASH1.

Determination of cell cycle by FCM. The effect of SASH1 expression on the cell cycle of SKOV3 cells was investigated with FCM. SKOV3 cells were cultured at a density of 3×10^5 cells/ml in a 6-well plate in a volume of 1,000 μ l. The transfection methods and grouping were the same as the above. A total of 48 h after transfection, the cells were treated with trypsin, washed twice in PBS, and fixed in 70% cold ethanol overnight at -20°C . The next day, after being washed with PBS, the SKOV3 cells were incubated with RNase solution (100 μ g/ml; Promega Corporation, Madison, WI, USA) for 30 min at 37°C . Finally, the SKOV3 cells were incubated in propidium iodide (PI) solution (100 μ g/ml in PBS) in the dark at 4°C overnight. The PI fluorescence of individual nuclei was measured with a FCM machine (BD FACScalibur, BD Bioscience, San Jose, CA, USA).

Growth curve assay. After cell transfection for 24, 48, 72 or 96 h, SKOV3 cells were stained with Hoechst 33342 dye (5 μ g/ml). Stained cells were observed under a fluorescence microscope (DM IL LED; Leica Microsystems, Wetzlar, Germany), and cell numbers of the total population were counted with the aid of an Image Pro Plus 6.0 software (Media Cybernetics, Inc., Rockville, MD, USA). The cell counting was performed using 10 visual fields in 3 wells.

FCM detection of the effects of SASH1 on the cellular apoptosis. The transfection methods and grouping were the same as above. A total of 48 h after transfection, the cells were digested by trypsin, washed twice in PBS, and resuspended in 195 μ l Annexin V-FITC binding buffer (Roche Diagnostics). A total of 5 μ l Annexin V-FITC was added and mixed gently, and the SKOV3 cells were incubated at room temperature in the dark for 10 min. Then, the SKOV3 cells were centrifuged at $1,000 \times g$ for 5 min and gently resuspended in 190 μ l of Annexin V-FITC binding buffer, 10 μ l PI solution was added and mixed gently, and the cells were kept on ice in the dark and immediately subjected to FCM using (BD FACScalibur; BD Biosciences, Franklin Lakes, NJ, USA). Cell Quest and Macquit FCM software (BD Biosciences) were used to analyze the data. The experiment was repeated three times.

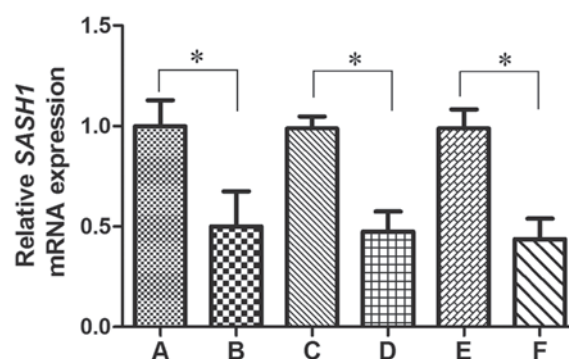


Figure 1. SASH1 mRNA expression levels were analyzed in ovarian cancer tissues by reverse transcription-quantitative polymerase chain reaction. (A) Normal surrounding ovarian tissues; (B) ovarian cancer tissues; (C) negative lymph node metastasis; (D) positive lymph node metastasis; (E) patients in FIGO stages (I, II); (F) patients in FIGO stages (III, IV). * $P < 0.05$ was considered to indicate a significant difference. SASH1, SAM- and SH3-domain containing 1; FIGO, International Federation of Gynaecologists and Obstetricians.

Transwell detection of the effects of SASH1 on the cellular migration. The number of cells that migrated through a polycarbonate membrane was calculated to show the migration ability of SKOV3 cells. Post-transfection, SKOV3 cells were plated on the upper side of a polycarbonate membrane of Transwell chamber in medium without serum. The cells were washed twice with PBS and stained with Hoechst 33342 dye after being cultured under normal conditions for 48 h. The number of cells migrating through the Transwell polycarbonate membrane was calculated under a fluorescence microscope (Leica DM IL LED); 10 randomly selected fields were examined. The results are presented as the mean \pm SD, with three repeated experiments for each group.

Statistical analysis. Stata 7.0 (StataCorp LP, College Station, TX, USA) software was used for statistical analysis using the χ^2 test, t test and one-way analysis of variance. The threshold for statistical significance was $P < 0.05$.

Results

The expression of SASH1 in ovarian carcinoma tissues. The mRNA and protein levels of ovarian carcinoma tissues and adjacent normal tissues were evaluated and compared using RT-qPCR and western blotting analysis, respectively. The SASH1 mRNA expression rate was 60.8% in the ovarian carcinoma tissues, which was significantly lower than that in adjacent normal tissues (100.0%) ($P = 0.000$) (Fig. 1). The SASH1 mRNA expression level decreased in the ovarian carcinoma tissues with increasing FIGO stage ($P = 0.016$) (Fig. 1). The SASH1 mRNA expression level in the ovarian carcinoma tissues from patients with lymph nodes metastasis (46.0%) was significantly lower than that from patients with negative lymph nodes metastasis (71.4%) ($P = 0.021$) (Fig. 1). However, the expression of SASH1 mRNA in ovarian tissues was independent of the patient's age, histological type or tumor size ($P = 0.953, 0.344, 0.127$, respectively), as shown in Table I. The protein levels of SASH1 in ovarian carcinoma tissues and adjacent normal tissues were similar to the mRNA levels of SASH1 (Fig. 2).

Table II. Correlation between *SASH1* and Ki-67 mRNA expression levels in ovarian cancer tissues.

Ki-67 mRNA	<i>SASH1</i> mRNA expression		r	P
	Negative	Positive		
Negative	8	28	-0.3189	0.005 ^a
Positive	23	20		

Statistical analyses were performed using the pearson χ^2 test. ^aP<0.05 was considered to indicate a significant difference. *SASH1*, SAM- and SH3-domain containing.

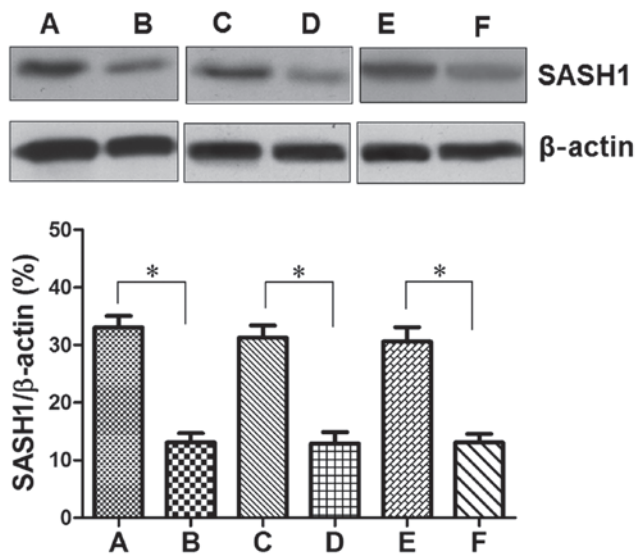


Figure 2. *SASH1* protein expression was analyzed in ovarian cancer tissues by western blotting. (A) Relatively normal surrounding ovarian tissues; (B) ovarian cancer tissues; (C) negative lymph node metastasis; (D) positive lymph node metastasis; (E) patients in FIGO stages (I, II); (F) patients in FIGO stages (III, IV). *P<0.05 was considered to indicate a significant difference. *SASH1*, SAM- and SH3-domain containing 1; FIGO, International Federation of Gynaecologists and Obstetricians.

Regarding the correlation of *SASH1* mRNA expression levels with Ki-67 mRNA expression, 20/48 patients with *SASH1* mRNA-positive expression were also positive for mRNA expression of Ki-67. The mRNA expression levels of *SASH1* and Ki-67 in ovarian carcinoma were negatively correlated ($r=-0.3189$, $P=0.005$) (Table II).

The effect of *SASH1* on SKOV3 cell proliferation. To analyze the effect of *SASH1* expression on the biological characteristics of SKOV3 cells, recombinant expression plasmid pcDNA3.1-*SASH1* was constructed. After SKOV3 cells were transfected with recombinant plasmid pcDNA3.1-*SASH1* or empty vector 48 h, western blot was used to detect the expression of *SASH1*. The result showed that the *SASH1* protein level in the pcDNA3.1-*SASH1* transfection group was significantly higher than that observed in the normal control group ($P<0.01$) or the empty vector (pcDNA3.1) control group ($P<0.01$) (Fig. 3).

The effect of *SASH1* on SKOV3 cell cycle was analyzed by FCM. FCM analysis showed that the percentage of S-phase in the pcDNA3.1-*SASH1* transfected group was lower compared

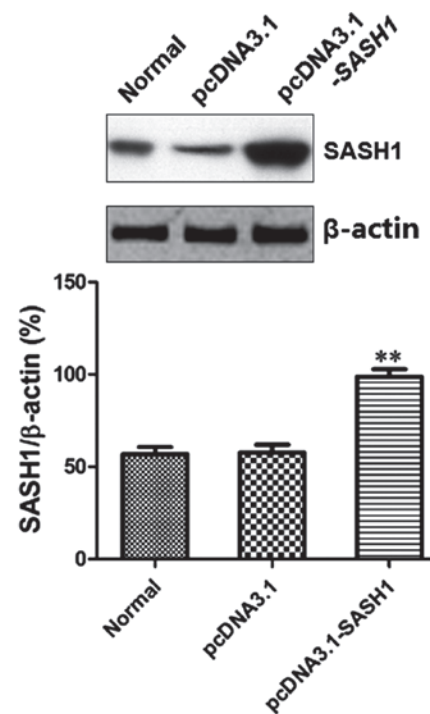


Figure 3. The *SASH1* protein expression in SKOV3 cells was assessed by western blotting after transfection. *P<0.01 vs. normal (untransfected) control group. *SASH1*, SAM- and SH3-domain containing 1.

to the normal control group ($P<0.01$) or the empty vector control group ($P<0.01$) (Fig. 4). The S-phase fraction (%) did not differ between the normal control group and the empty vector control group.

A cell growth curve was used to observe cell growth of SKOV3 cells transfected with pcDNA3.1-*SASH1*. Cell growth was reduced in SKOV3 cells transfected with pcDNA3.1-*SASH1* for 48 h, 72 h or 96 h, compared with cells transfected with empty vector or normal (control) ($P<0.05$) (Fig. 5). These results indicated that *SASH1* may inhibit SKOV3 cell proliferation.

The effect of *SASH1* on SKOV3 cell apoptosis. FCM analysis of cell apoptosis levels showed that the percentage of apoptotic cells in the normal control group and the empty vector control group was significantly lower than that in the pcDNA3.1-*SASH1* transfection group ($P<0.01$) (Fig. 6). The percentage of apoptotic cells did not differ significantly between the normal control group and the empty vector control group. These data

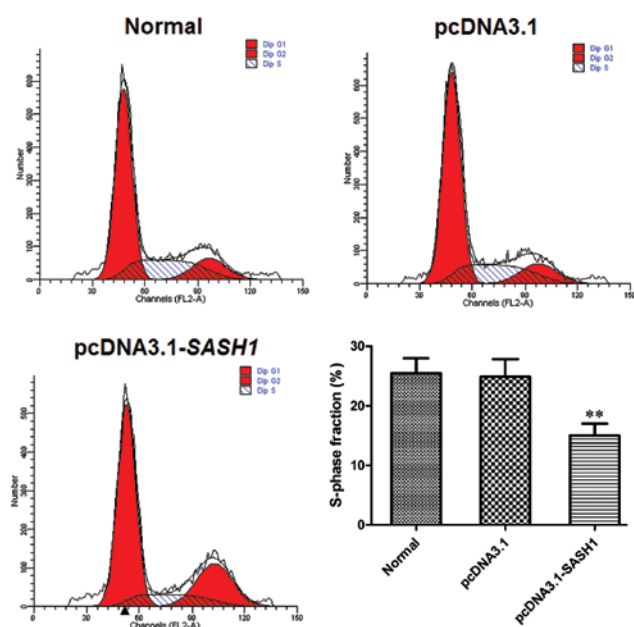


Figure 4. The effect of SASH1 on SKOV3 cell proliferation was analyzed by flow cytometry. ** $P < 0.01$ vs. normal (untransfected) control group. SASH1, SAM- and SH3-domain containing 1.

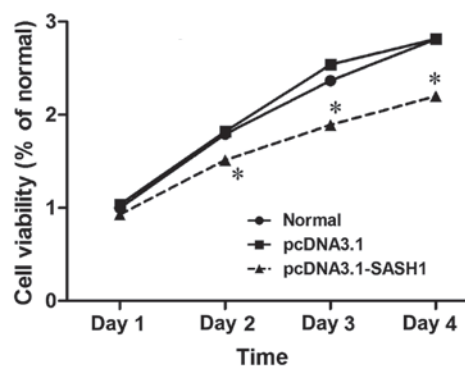


Figure 5. Effect of SASH1 on SKOV3 cell growth. The cell growth curve of SKOV3 cells that were transfected with recombinant vector pcDNA3.1-SASH1 or empty vector pcDNA3.1. The cell number was assessed by microscopically cell counting after Hoechst 33342 dye staining. * $P < 0.05$ vs. normal (untransfected) control group. SASH1, SAM- and SH3-domain containing 1.

indicated that the overexpression of SASH1 may enhance SKOV3 cell apoptosis.

The effect of SASH1 on SKOV3 cell migration. The Transwell invasion chamber system was used to evaluate the invasive ability of transfected cells. The number of cells in the pcDNA3.1-SASH1 transfection group that passed through the polycarbonate membrane was decreased significantly ($P < 0.01$) compared to the normal control group and the empty vector control group (Fig. 7). These results suggested that SASH1 overexpression could suppress SKOV3 cell migration.

Discussion

Ovarian carcinoma is one of the most common gynecological tumors, with reported ~14,000 deaths in 2009 (8,27). Due to the late detection of this disease, ~30% of patients with

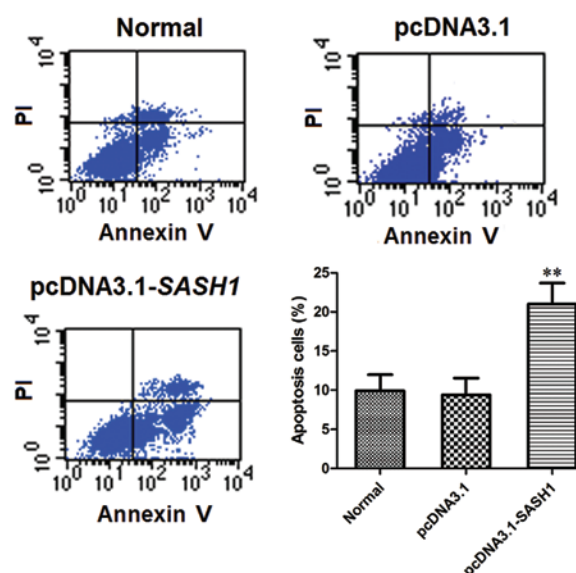


Figure 6. The effect of SASH1 on SKOV3 cells apoptosis was analyzed by flow cytometry. ** $P < 0.01$ vs. normal (untransfected) control group. SASH1, SAM- and SH3-domain containing 1.

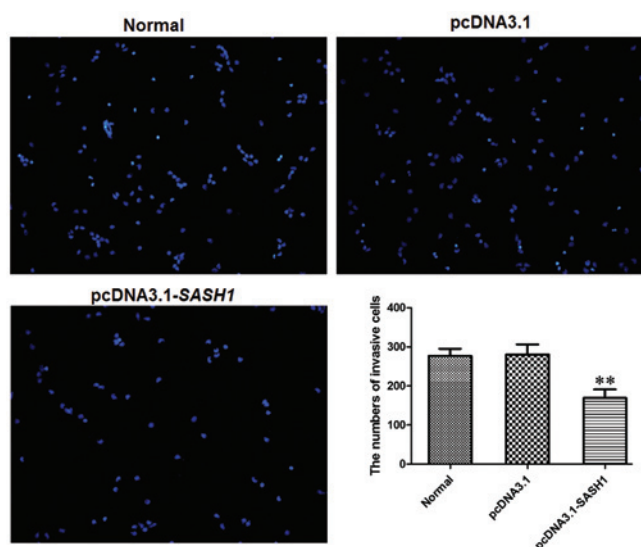


Figure 7. The effect of SASH1 on SKOV3 cells invasion was analyzed by Transwell invasion chamber. The cell number that passed through the polycarbonate membrane was assessed by microscopically cell counting after Hoechst 33342 staining. ** $P < 0.01$ vs. normal (untransfected) control group. SASH1, SAM- and SH3-domain containing 1.

peritoneal metastasis at the time of diagnosis had a five-year survival rate of ~26.9% (28,29). The most common staging criteria, an ovarian carcinoma staging system, was developed by FIGO (30,31). Although previous research has attempted to explain the specific causes for ovarian carcinoma, the molecular mechanism of occurrence and development of this disease remains unclear. Therefore, to clarify the molecular mechanism of metastasis of ovarian carcinoma cells is a serious challenge for the clinical treatment and research of ovarian tumor in the future.

SASH1 is located on 6q24.3, and includes 22 exons and 21 introns, and has two important structural domains: SH3- and SAM-domains. Both domains can mediate protein-protein

interactions (13). However, the SAM domains can serve a more complex function in the way that they mediate protein-protein interactions through homologous and heterologous oligomerization with the SAM regions on other proteins (18). Meanwhile, the SAM domains can mediate binding between Smaug proteins and mRNAs to regulate transcription (13). SASH1 has been suggested as a candidate tumor suppressor. The reduction or absence SASH1 is closely related to tumor growth, invasion, metastasis, and poor prognosis (13,14,23,24). Zeller *et al* (13) reported that *SASH1* mRNA expression was significantly reduced or completely absent in 6 breast cancer cell lines, and that it was also significantly decreased in primary thyroid cancers. They also showed that the down-regulation of *SASH1* expression was correlated with the degree of malignancy (13). These data suggested that the down-regulation of *SASH1* may serve an important role in tumor occurrence, development, and evolution. However, the role of *SASH1* in ovarian carcinoma remains unclear.

Ovarian carcinomas originate from the ovary, and can be divided into various histopathological subtypes (32,33). They differ in their biological behavior and response to current treatment modalities (34). Despite improvements in the application of aggressive cytoreductive surgery and combination chemotherapy, and that the five year survival rate of ovarian carcinoma has not improved, ovarian carcinoma has the most unfavorable total prognosis and tendency to develop chemotherapy resistance (27,35,36). Therefore, investigation of novel genetic or molecular biomarkers for early diagnosis, survival prediction, or therapeutic targets is needed. In the present study, the expression of *SASH1* in ovarian carcinoma was investigated by RT-qPCR and western blotting and the correlation between its expression with clinical pathological features and clinical significance. The results indicated that the expression of *SASH1* in ovarian carcinoma tissue was significantly lower than that in adjacent normal tissue. So, we hypothesize that *SASH1* may serve an important role in inhibiting ovarian carcinoma cell proliferation, which may be an explanation for the fact that *SASH1* demonstrated down-regulation in ovarian carcinoma tissues.

In order to analyze effects of *SASH1* on SKOV3 cell biological characteristics, a recombinant expression vector of *SASH1* was constructed. *SASH1* was overexpressed in SKOV3 cells transfected with pcDNA3.1-*SASH1*, and a low expression level was observed in the empty vector group and normal control group. So, an overexpression cell model of *SASH1* was successfully established. The effects of *SASH1* on SKOV3 cell growth or proliferation by using cell growth curve or FCM. FCM analysis showed that the percentage of cells in S-phase in the pcDNA3.1-*SASH1* transfection group was lower than that in the normal group or empty vector group. The number of SKOV3 pcDNA3.1-*SASH1* transfected cells decreased at all time intervals. These results indicated that *SASH1* may inhibit SKOV3 growth and proliferation. These results demonstrated that *SASH1* may be identified as a tumor suppressor gene by inhibiting tumor cell growth and proliferation.

However, the exact mechanism remains to be determined. FCM was used to detect the effect of *SASH1* on SKOV3 apoptosis by Annexin V and PI double staining. FCM analysis of cell apoptosis levels showed that the percentage of apoptotic cells in the pcDNA3.1-*SASH1* transfection group was

significantly higher than that in the normal group and the empty vector group. The result suggested that *SASH1* also enhanced apoptosis and may inhibit tumor cell growth and proliferation through its role as a tumor inhibitor gene.

Lymph nodes are involved in ~50-70% of cases of advanced ovarian carcinoma (37-39). Lymphatic metastasis is an important malignant progression factor in ovarian carcinoma (40,41). In advanced disease (FIGO stages III-IV) particularly, nodal metastases to the pelvic and para-aortic lymph nodes are common (25). *SASH1* expression in ovarian carcinoma tissues from patients with lymph nodes metastases was significantly lower than that in ovarian carcinoma tissues with negative lymph nodes metastases. Moreover, *SASH1* expression decreased as the FIGO stages increased. These data suggested that *SASH1* may be involved in the invasion and metastasis of ovarian carcinoma, and *SASH1* may also serve an important role in suppressing metastasis processes. Therefore, the present study investigated the effect of *SASH1* expression on the invasion ability of SKOV3 cells by Transwell migration assays. The number of cells in pcDNA3.1-*SASH1* transfection group that passed through the polycarbonate membrane was significantly reduced compared to the normal group and the empty vector control group. *SASH1* overexpression suppressed the cell migration of SKOV3 cells. The results indicated that *SASH1* may be involved in invasion and metastasis-associated molecular pathway.

In conclusion, the results of the present study indicated that the loss or down-regulation of *SASH1* expression may serve an important role in tumor occurrence, invasion and metastasis of ovarian carcinoma. The specific mechanisms underlying the effects of *SASH1* on the tumor occurrence, progression, and invasion of ovarian carcinoma require further research. These studies may provide novel strategies and targets for the treatment of ovarian carcinoma.

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