Long non-coding RNA CASC2 inhibits progression and predicts favorable prognosis in epithelial ovarian cancer

ZHUOWEI XUE, XIAOLU ZHU and YINCHENG TENG

Department of Obstetrics and Gynaecology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai 200233, P.R. China

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Abstract. Epithelial ovarian cancer (EOC) is one of the leading causes of cancer-associated mortality in women. At present, the overall 5-year survival rate of patients with EOC remains poor despite advancements in diagnosis and treatment. Long non-coding RNAs (lncRNAs) have attracted increasing attention in recent years for their extensive roles in tumorigenesis and cancer development. The lncRNA cancer susceptibility candidate 2 (CASC2) was originally identified as a downregulated gene in endometrial cancer, and subsequent studies revealed that CASC2 was able to act as a tumor suppressor gene in various types of cancer. The present study is the first, to the best of the authors' knowledge, to identify the clinical significance and potential role of CASC2 in EOC. The results demonstrated that CASC2 was downregulated in EOC cell lines and tissues. Analysis of association between clinicopathological features and CASC2 expression levels suggested that low CASC2 expression is associated with the serous histological subtype (P<0.001), lymph node metastasis (P=0.038), poor histological grade (P<0.001) and large tumor size (P=0.001) in EOC. Furthermore, low CASC2 expression predicted poor overall survival (P<0.001) and progression-free survival (P<0.001). Functional assays, including Cell Counting kit-8 assays, colony formation assays, and Transwell and Matrigel assays, confirmed that silencing of CASC2 promoted the proliferation, migration and invasion of EOC cells; whereas, ectopic overexpression of CASC2 suppressed the proliferation, migration and invasion of EOC cells. In addition, in the analysis of the risk factors for poor prognosis, low CASC2 expression was identified as an independent risk factor for reduced overall survival [hazard ratio (HR)=0.417; 95% confidence interval (CI)=0.251-0.693; P=0.001] and progression-free survival (HR=0.426; 95% CI=0.260-0.699; P=0.001) in patients with EOC. In conclusion, CASC2 is downregulated in EOC, and it may suppress EOC progression and is an independent risk factor for poor prognosis. CASC2 may be a promising prognostic marker and therapeutic target in EOC.

Correspondence to: Professor Yincheng Teng, Department of Obstetrics and Gynaecology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, 600 Yishan Road, Shanghai 200233, P.R. China
E-mail: ycteng@sjtu.edu.cn

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Introduction

Epithelial ovarian cancer (EOC) is the fifth leading cause of cancer-associated mortality among women worldwide (1). During the past decades, the early detection rate and prognosis of EOC have been substantially improved (2). However, the prognosis for the advanced stages of the disease has not improved significantly in more than two decades (3). Further investigations on the mechanisms of EOC progression are required to aid the development of novel diagnostic markers and therapeutic targets.

Only 2% of the human genome accounts for protein-coding regions, yet >70% of the human genome is transcribed into RNAs that do not encode proteins; these RNAs are called non-coding RNAs (4). Non-coding RNAs are generally divided into two classes according to their size. Long non-coding RNAs (lncRNAs) are defined as a class of non-coding RNAs with a length >200 base pairs. In recent years, numerous lncRNAs have been discovered, and the pivotal roles of lncRNAs in almost every aspect of cell biology have been gradually revealed, such as epigenetic regulation via molecular scaffolding, regulation of mRNA processing, molecular decoying and lncRNA-derived peptides (5).

Cancer susceptibility candidate 2 (CASC2), an lncRNA located at chromosome 10q26, was originally identified as a downregulated gene in endometrial cancer (6). Previous studies suggested that CASC2 may act as a tumor suppressor gene, with epigenetic and genetic alterations contributing to gene inactivation (6-13). Downregulation of CASC2 may provide a growth advantage in EOC cells (7). Recent studies identified that CASC2 is associated with tumor development and prognosis in various cancer types, including renal cell carcinoma, osteosarcoma, bladder cancer, pancreatic cancer, endometrial cancer, hepatocellular carcinoma, glioma, non-small cell lung cancer and gastric cancer (8-13). However, little is known regarding the role of CASC2 in EOC.

The present study aimed to investigate the expression, clinical significance and functional role of CASC2 in EOC. The expression levels of CASC2 in EOC cells and tissues were measured, and the association between CASC2 expression and the clincopathological characteristics of patients with EOC were statistically analyzed. Furthermore, CASC2 was overexpressed and silenced in EOC cells, and the functional alterations in the EOC cells were evaluated.

Patients and methods

Patients and tissue samples. A total of 126 female patients (aged 56.9±13.2 years old, ranging between 35-82 years old) with EOC who underwent surgical resection between August 2010 and August 2011 at Shanghai Jiao Tong University Affiliated Sixth People's Hospital (Shanghai, China) were enrolled in the present study, and written informed consent was obtained from each patient. The collected EOC tissues and paired tumor adjacent tissues were frozen in liquid nitrogen immediately following surgical resection and were stored at -80˚C until use. The exclusion criteria were patients who had undergone presurgical anticancer therapy and were diagnosed with two or more malignances. The present study was approved by the Ethics Committee of the Shanghai Jiao Tong University Affiliated Sixth People's Hospital.

Cell lines and cell culture. The EOC cell lines (SKOV3, OV90, A2780, ES2 and IGROV-1) and an immortalized ovarian epithelial cell line (Moody) (14) were purchased from the Cell Bank of Type Culture Collection of the Chinese Academy of Science (Beijing, China). Cells were cultured in RPMI-1640 medium (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA; SKOV3) or Dulbecco's modified Eagle's medium (Invitrogen; Thermo Fisher Scientific, Inc.; OV90, A2780, ES2 and IGROV-1) supplemented with 10% fetal bovine serum (FBS; Life Technologies; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin and 100 mg/ml streptomycin (Life Technologies; Thermo Fisher Scientific, Inc.) in a humidified atmosphere containing 5% CO_2 at 37˚C.

Gene ectopic expression, interference and cell transfection. Ectopic expression of CASC2 was achieved by subcloning the CASC2 sequence into the pcDNA3.1 vector (Invitrogen; Thermo Fisher Scientific, Inc.; pcDNA3.1-CASC2; 1.0 μg/μl), with an empty pCDNA3.1 vector serving as an empty control. Small interfering (si)RNAs [siCASC2-1 and siCASC2-2 (sequences unavailable); 5 nM] and non-targeting siRNA [siRNA negative control (siNC)] were all purchased from Genewiz, Inc. (Suzhou, China). When EOC cells were 60-70% confluent, transfection was performed using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. After 5 h of incubation, the transfection medium was replaced with the appropriate culture medium without antibiotics, followed by incubation for 48 h. The cells were subsequently used in experiments. The expression levels of CASC2 following ectopic expression and interference were confirmed by reverse transcription-quantitative polymerase chain reaction (RT-qPCR).

RNA extraction and RT-qPCR. Total RNA was extracted from EOC tissues and cell lines with TRIzol® (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. cDNA was reverse transcribed from total RNA (2.5 μg) using the Superscript III kit (Life Technologies; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Reverse transcription was performed according to the following temperature protocol: Incubation for 5 min at 25˚C, followed by 60 min at 42˚C and then 70˚C for 5 min. The relative expression of selected genes were quantified and analyzed by real-time PCR using the iQ SYBRGreen PCR Supermix kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The qPCR thermocycling conditions used were as follows: Initial denaturation at 95˚C for 5 min; followed by 40 cycles of denaturation at 95˚C for 30 sec, annealing at 50˚C for 30 sec and extension at 72˚C for 30 sec. The primers were as follows: CASC2 sense, 5'-GCACATTGGACGGTGTTTCC-3'; CASC2 antisense, 5'-CCCAGTCCTTCACAGGTCAC-3'; and GAPDH sense, 5'-AGAAGGCTGGGGCTCATTTG-3'; GAPDH antisense, 5'-AGGGGCCATCCACAGTCTTC-3'. The expression levels of the target genes were calculated using the $2^{-\Delta\Delta Cq}$ method based on the cycle threshold values of the genes compared with those of GAPDH (15). All samples were typically analyzed in triplicate in at least three independent runs.

Transwell assay and Matrigel assay. For the Transwell assay and the Matrigel assay, cells ($1x10^5$) were suspended in 200 μl serum-free medium and were seeded into the upper chamber of Matrigel-coated (Matrigel assay) or uncoated (Transwell assay) Boyden chambers (8 μM pore size; BD Biosciences, Franklin Lakes, NJ, USA). Medium containing 20% FBS was added to the lower chamber. After 24 h incubation, cells remaining in the upper chamber were removed with cotton swabs, and cells that had invaded through the membrane were fixed with ethanol for 30 min at room temperature, and subsequently stained with Giemsa for another 30 min at room temperature. The cells that migrated or invaded were counted in five independent fields using an Olympus CKX53 inverted light microscope (magnification, x10; Olympus Corporation, Tokyo, Japan), and images were obtained (magnification, x4). The results presented are representative of three independent experiments with technical duplicates.

Cell Counting kit-8 (CCK-8) assay. To analyze the effect of CASC2 on cell proliferation, a CCK-8 assay was performed using a CCK-8 kit (Roche Applied Science, Penzberg, Germany). In total, 5,000 cells were seeded into each well of 96-well plates after 48 h of transfection. CCK-8 was used to quantify the absorbance measurements at 450 nm at the indicated time points (0, 24, 48, 72 and 96 h). The absorbance values were normalized to those of cells transfected with empty vector or siNC. The results represent the average of three replicates under the same conditions.

Colony formation assay. For the colony formation assay, cells were incubated in 6-well plates (1,000 cells/well) in triplicates, and subsequently cultured in a humidified incubator with 5% CO_2 for 10-14 days. The medium was changed every 3 days. Subsequent to incubation, the cell colonies were incubated in methanol for 10 min at room temperature, stained with 1% crystal violet for 10 min at room temperature. All colonies in the 6-well plate were manually counted using a

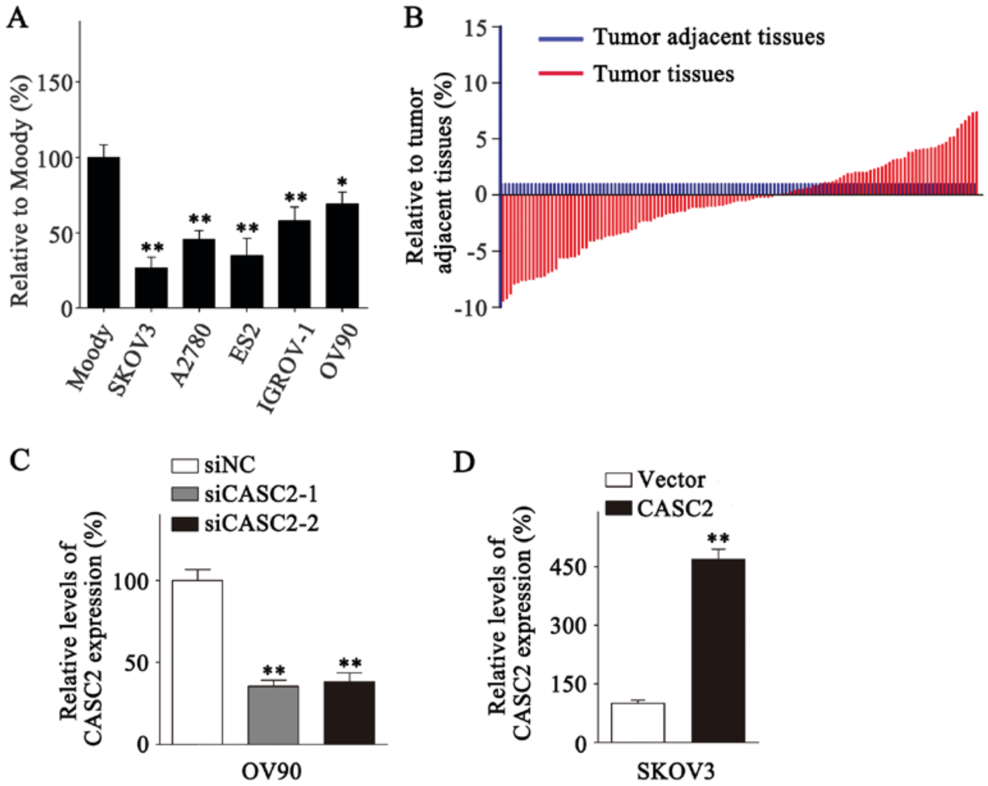

Figure 1. CASC2 expression is downregulated in EOC cells and tissues. (A) RT-qPCR assay was performed to detect the relative expression of CASC2 in EOC cells (SKOV3, A2780, ES2, IGROV-1 and OV90) compared with an immortalized ovarian epithelial cell line (Moody). *P<0.05, **P<0.01 vs. Moody based on one-way analysis of variance. (B) Expression of CASC2 was measured in 126 EOC tissues and paired tumor adjacent tissues by an RT-qPCR assay. (C) Expression levels of CASC2 were evaluated following siRNA-mediated interference in OV90 cells by an RT-qPCR assay. **P<0.01 vs. siNC based on one-way analysis of variance. (D) Expression levels of CASC2 were evaluated following ectopic overexpression in SKOV3 cells by RT-qPCR. **P<0.01 vs. vector based on Student's t-test. CASC2, cancer susceptibility candidate 2; EOC, epithelial ovarian cancer; RT-qPCR, reverse-transcription polymerase chain reaction; si, small interfering; NC, negative control.

light microscope (magnification, x10). The results presented are representative of three independent experiments.

Statistical analysis. The data were analyzed with SPSS 19.0 (IBM Corp., Armonk, NY, USA) and are presented as the mean ± standard deviation. To statistically evaluate the association between CASC2 expression and the clinical features of patients with EOC, the patients with EOC (n=126) were dichotomized with the mean level of CASC2 expression serving as the cutoff value. Associations between clinicopathological features and CASC2 expression levels were assessed using the χ^2 test. Student's t-test was used to analyze the difference between two groups. One-way analysis of variance and Dunnett's t-test was used for multiple comparisons. Survival analysis was conducted using the Kaplan-Meier method. The log-rank test was applied to compare the survival characteristics between groups. Univariate and multivariate Cox's proportional hazards regression models were applied to analyze the survival data. P<0.05 was considered to indicate a statistically significant difference.

Results

CASC2 is downregulated and correlates with tumor progression in EOC. The expression of CASC2 was evaluated in five EOC cell lines and one immortalized ovarian epithelial cell line (Moody) with an RT-qPCR assay. CASC2 exhibited a significantly higher expression level in Moody cells compared with the five EOC cell lines (Fig. 1A; P<0.05). Further detection of CASC2 expression levels in the 126 EOC tissues and paired tumor adjacent tissues revealed that CASC2 was downregulated in the EOC tissues compared with the tumor adjacent tissues [1 vs. (-0.5±3.92); (Fig. 1B)]. The results demonstrated that CASC2 downregulation was associated with histological subtype (P<0.001), lymph node metastasis (P=0.038), histological grade (P<0.001) and tumor size (P=0.001; Table I). Therefore, it may be postulated that CASC2 may be a tumor suppressor in EOC and is able to repress tumor progression.

CASC2 inhibits the migration, invasion and proliferation of EOC cells. Cancer metastasis and proliferation are two primary factors leading to tumor progression. Therefore, the functional role of CASC2 in EOC metastasis and cell proliferation was investigated in *in vitro* studies. To better elucidate the function of CASC2 in EOC cells, siRNAs were used to interfere with the expression of CASC2 in OV90 cells due to their relatively high CASC2 expression level among the six EOC cell lines (Fig. 1A and 1C), and ectopic overexpression was conducted in SKOV3 cells due to their relatively low CASC2 expression level among the six EOC cell lines (Fig. 1A and 1D). CASC2 expression was significantly downregulated and upregulated in OV90 and SKOV3 cells, respectively following transfection (Fig. 1C and D; P<0.01).

The effects of CASC2 interference and overexpression on the motility of EOC cells were determined with the Transwell assay and the Matrigel assay. The results of the migration assay demonstrated that OV90 cells with silenced CASC2 exhibited significantly increased migration ability in the Transwell assay (Fig. 2A; P<0.01); whereas, CASC2 upregulation significantly suppressed the migration of SKOV3 cells (Fig. 2B; P<0.01). Accordingly, CASC2 deficiency significantly promoted invasion in OV90 cells (Fig. 2C; P<0.01), and ectopic overexpression of CASC2 significantly decreased invasion in SKOV3 cells (Fig. 2D; P<0.01). Therefore, CASC2 is able to inhibit the metastasis of EOC cells.

The CCK-8 assay and the colony formation assay were used to investigate the role of CASC2 in EOC cell proliferation. The results identified that CASC2 silencing increased the colony numbers and the optical density values of OV90 cells in the colony formation assay and CCK-8 assay, respectively (Fig. 3A and B). However, CASC2 upregulation reduced the proliferative ability of SKOV3 cells (Fig. 3C and 3D). Overall, CASC2 suppressed the proliferation of EOC cells.

Low CASC2 expression is an independent risk factor for poor prognosis. To verify the prognostic role of CASC2 in EOC, statistical analysis was performed to evaluate the association between CASC2 expression level and EOC prognosis. Notably, patients with EOC with low CASC2 expression demonstrated a markedly poorer overall survival rate (Fig. 4A) and progression-free survival rate (Fig. 4B) compared with patients with high CASC2 expression. In addition, the subtype analysis identified that low CASC2 expression predicted shorter overall survival time in patients with EOC with Fédération Internationale de Gynécologie et d'Obstétrique (FIGO) (16) I/II (Fig. 4C) and FIGO III/IV (Fig. 4D).

Furthermore, the risk factors for overall survival in EOC were analyzed by univariate analysis and multivariate analysis. Lymph node metastasis (HR=1.754; 95% CI=1.075-2.860; P=0.024), advanced FIGO stage (HR=1.977; 95% CI=1.234-3.169; P=0.005) and low CASC2 expression (HR=0.403; 95% CI=0.245-0.663; P<0.001) were revealed to be risk factors for poor overall survival (Table II). The subsequent multivariate analysis identified advanced FIGO stage (HR=1.989; 95% CI=1.237-3.197; P=0.005) and low CASC2 expression (HR=0.417; 95% CI=0.251-0.693; P=0.001) as independent risk factors of EOC overall survival (Table II). In addition, the risk factors for progression-free survival were analyzed. As expected, lymph node metastasis (HR=2.021; 95% CI=1.238-3.300; P=0.005), advanced FIGO stage (HR=1.799; 95% CI=1.139-2.842; P=0.012) and low CASC2 expression (HR=0.405; 95% CI=0.249-0.659; P<0.001) were identified as risk factors for poor progression-free survival (Table III). The subsequent multivariate analysis identified the following three factors as independent risk factors for progression-free survival in EOC, lymph node metastasis (HR=1.680; 95% CI=1.019-2.768; P=0.042), advanced FIGO stage (HR=1.810; 95% CI=1.142-2.870; P=0.012), low CASC2 expression (HR=0.426; 95% CI=0.260-0.699; P=0.001; Table III). Taken together, these results suggest that CASC2 may be a promising biomarker for the prediction of prognosis in patients with EOC.

Table I. Correlation between long non-coding RNA CASC2 expression and epithelial ovarian cancer clinicopathological characteristics.

Parameters	Number of patients, n=126	CASC2 high/low, 50/76	P-value
Age			0.945
<60 years	60	24/36	
≥60 years	66	26/40	
Histology subtype			<0.001
Serous	52	46/6	
Others	74	30/44	
CA125			0.768
<35 U/ml	31	13/18	
≥35 U/ml	95	37/58	
Lymph node metastasis			0.038
Yes	77	25/52	
No	49	25/24	
Residual tumor size			0.218
<1 cm	80	35/45	
≥1 cm	46	15/31	
Histological grade			<0.001
G1+G2	68	37/31	
G3	58	13/45	
Tumor size			0.001
<2 cm	62	34/28	
≥2 cm	64	16/48	
FIGO stage			0.519
I+II	59	25/34	
III+IV	67	25/43	

CASC2, CASC2, cancer susceptibility candidate 2; FIGO, Fédération Internationale de Gynécologie et d'Obstétrique.

Discussion

Due to the various functional roles of lncRNAs in cancer biology, an increasing number of studies have focused on the role of lncRNAs in different types of cancer. Through analysis of the differential expression of lncRNAs in normal tissues and cancer tissues, lncRNAs have been observed to be involved in the initial of carcinogenesis (17,18). A larger number of lncRNAs have been discovered as a result of the extensive use of next-generation sequencing technologies, and additional functional roles of lncRNAs have been detected in carcinogenesis and cancer progression (17,18). Through analysis of the expression profiles of lncRNAs in EOC, specific differentially expressed lncRNAs have been identified (19). Further comprehensive analysis of the expression profiles of lncRNAs in EOC revealed that an eight-lncRNA signature may be a measure for predicting the chemotherapeutic response and identifying patients with platinum resistance, who may benefit from other more effective therapies (20). At present, a

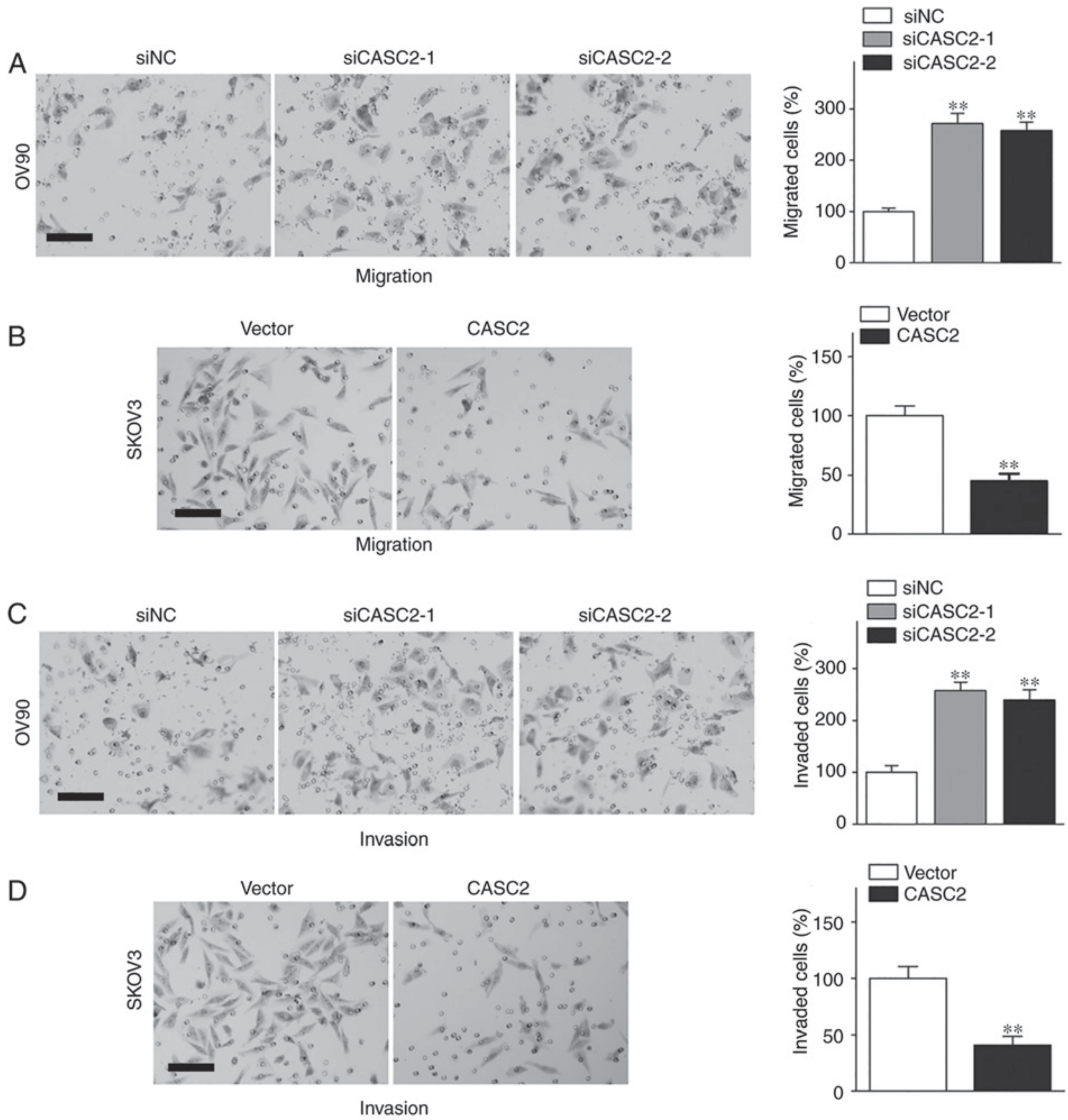

Figure 2. CASC2 suppresses the motility of epithelial ovarian cancer cells. (A) Interference with CASC2 in OV90 cells significantly promoted the migration of OV90 cells in the Transwell assay. **P<0.01 vs. siNC based on one-way analysis of variance. (B) Overexpression of CASC2 in SKOV3 cells significantly decreased the migration of SKOV3 cells in the Transwell assay. **P<0.01 vs. vector based on Student's t-test. (C) CASC2 silencing significantly increased the invasive ability of OV90 cells in the Matrigel assay. **P<0.01 vs. siNC based on one-way analysis of variance. (D) CASC2 overexpression significantly decreased the migration of SKOV3 cells. Scale bars, 50 μm. **P<0.01 vs. vector based on Student's t-test. CASC2, cancer susceptibility candidate 2; si, small interfering, NC, negative control.

number of lncRNAs have been suggested to be associated with EOC progression, chemoresistance and prognosis, including deleted in lymphocytic leukemia 1 (21), H19, imprinted maternally expressed transcript (22), HOX transcript antisense RNA (23-26), nuclear paraspeckle assembly transcript 1 (27,28) and metastasis associated lung adenocarcinoma transcript 1 (29). Mechanistically, lncRNAs have been identified to regulate EOC progression by mediating cancer cell proliferation, metastasis, inflammasome formation, and the epithelial to mesenchymal transition (23,30,31). Numerous lncRNAs are involved in regulating EOC biology through various ways. In addition, certain lncRNAs, including AB073614 (32), colon cancer associated transcript 2 (33) and neuroblastoma associated transcript 1 (34), have been demonstrated to be potential prognostic markers in patients with EOC. However, the significance of CASC2 in EOC has not been elucidated yet.

The lncRNA CASC2 was discovered in 2004 in patients with endometrial carcinoma as a potential tumor suppressor (6). CASC2 transcripts may be classified into three subgroups; CASC2a, CASC2b and CASC2c (5). Evidence suggested that the expression levels of CASC2b and CASC2c mRNA were similar in normal and neoplastic endometrial tissues; whereas, CASC2a was identified to be downregulated in neoplastic samples compared with the normal counterparts (5). Since

Table II. Univariate and multivariate analysis of clinicopathological features for overall survival of patients with epithelial ovarian cancer.

	Univariate analysis			Multivariate analysis		
Parameters	HR	95% CI	P-value	HR	95% CI	P-value
Age ≥60 years vs. <60 years	1.056	0.944-2.403	0.086			
Histology subtype serous vs. others	1.414	0.893-2.239	0.139			
CA125 <35 U/ml vs. ≥35 U/ml	1.768	0.987-3.168	0.055			
Lymph node metastasis yes vs. no	1.754	1.075-2.860	0.024	1.431	0.866-2.358	0.160
Residual tumor size <1 cm vs. ≥1 cm	1.113	0.698-1.775	0.653			
Histological grade G1+G2 vs. G3	1.052	0.665-1.664	0.827			
Tumor size ≥2 cm vs. <2 cm	1.061	0.672-1.674	0.799			
FIGO stage (III+IV) vs. (I +II)	1.977	1.234-3.169	0.005	1.989	1.237-3.197	0.005
CSCA2 low vs. high	0.403	0.245-0.663	<0.001	0.417	0.251-0.693	0.001

HR, hazard ratio; CI, confidence interval; CA125, cancer antigen 125; FIGO, Fédération Internationale de Gynécologie et d'Obstétrique; CASC2, CASC2, cancer susceptibility candidate 2.

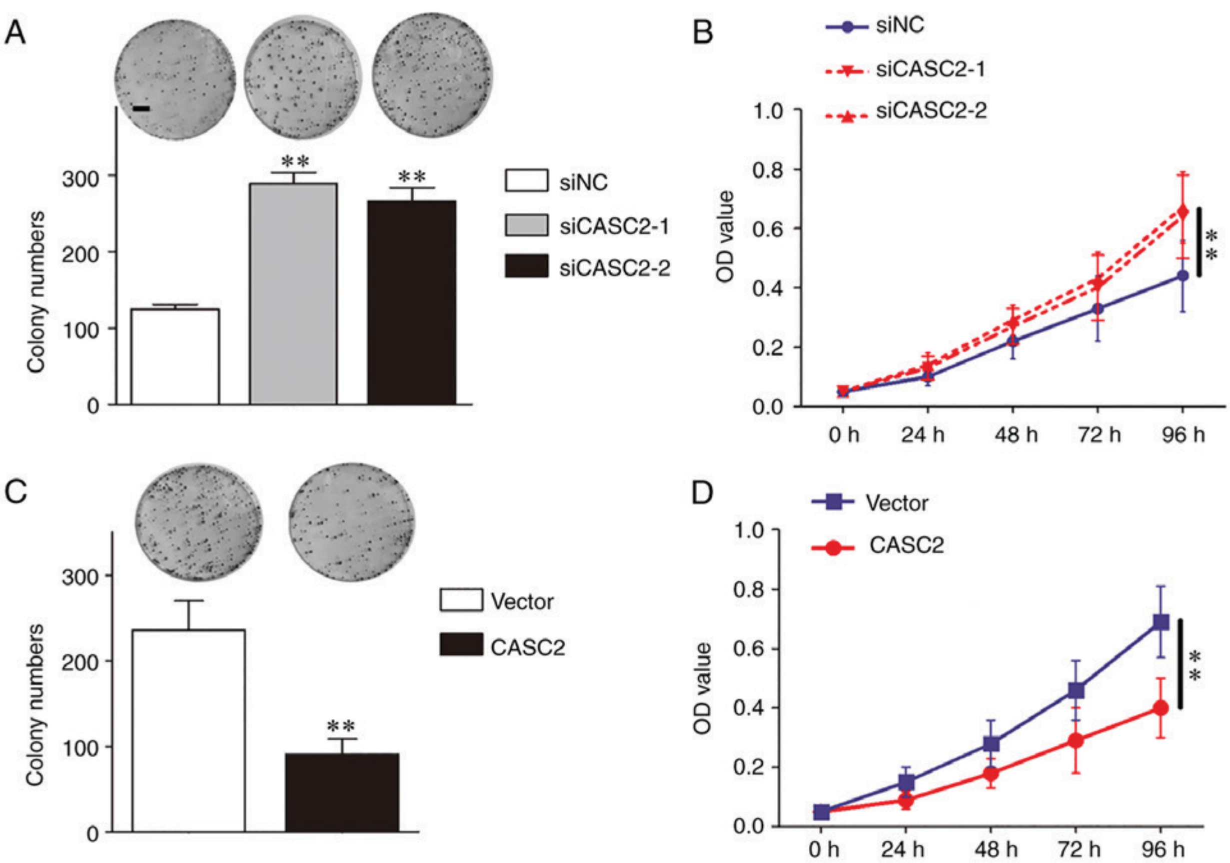

Figure 3. CASC2 represses the proliferation of epithelial ovarian cancer cells. (A) Colony formation assay demonstrated that CASC2 deficiency in OV90 cells increased the number of colonies. **P<0.01 vs. siNC based on one-way analysis of variance. (B) CCK-8 assay identified that CASC2 silencing markedly increased the OD value of OV90 cells. **P<0.01. (C) Colony formation assay demonstrated that CASC2 overexpression in SKOV3 cells markedly decreased the number of colonies. **P<0.01 vs. vector based on Student's t-test. (D) CCK-8 assay demonstrated that CASC2 upregulation markedly decreased the OD value of SKOV3 cells. Scale bar, 5 mm. **P<0.01. CASC2, cancer susceptibility candidate 2; CCK-8, Cell Counting kit-8; OD, optical density; si, small interfering; NC, negative control.

then, further studies in other types of neoplasia have been conducted, and the mechanisms and the interactions of CASC2 in cancer have been better elucidated (35); in addition, CASC2 has become synonymous with CASC2a. Aberrant expression of CASC2 was detected in renal cell carcinoma (12), osteosarcoma (36), pancreatic cancer (13), endometrial cancer (6), hepatocellular carcinoma (37), glioma (10,38,39), non-small cell lung cancer (8) and gastric cancer (11). Functionally,

Table III. Univariate and multivariate analysis of clinicopathological features for progression-free survival of patients with epithelial ovarian cancer.

	Univariate analysis			Multivariate analysis		
Parameters	HR	95% CI	P-value	HR	95% CI	P-value
Age ≥60 years vs. <60 years	1.460	0.925-2.303	0.104			
Histology subtype serous vs. others	1.485	0.947-2.328	0.085			
CA125 <35 U/ml vs. ≥35 U/ml	1.583	0.911-2.749	0.103			
Lymph node metastasis yes vs. no	2.021	1.238-3.300	0.005	1.680	1.019-2.768	0.042
Residual tumor size <1 cm vs. ≥1 cm	1.173	0.744-1.850	0.492			
Histological grade, G1+G2 vs. G3	1.155	0.734-1.818	0.533			
Tumor size ≥2 cm vs. <2 cm	1.127	0.720-1.764	0.600			
FIGO stage (III+IV) vs. (I +II)	1.799	1.139-2.842	0.012	1.810	1.142-2.870	0.012
CASC2 low vs. high	0.405	0.249-0.659	<0.001	0.426	0.260-0.699	0.001

HR, hazard ratio; CI, confidence interval; CA125, cancer antigen 125; FIGO, Fédération Internationale de Gynécologie et d'Obstétrique; CASC2, cancer susceptibility candidate 2.

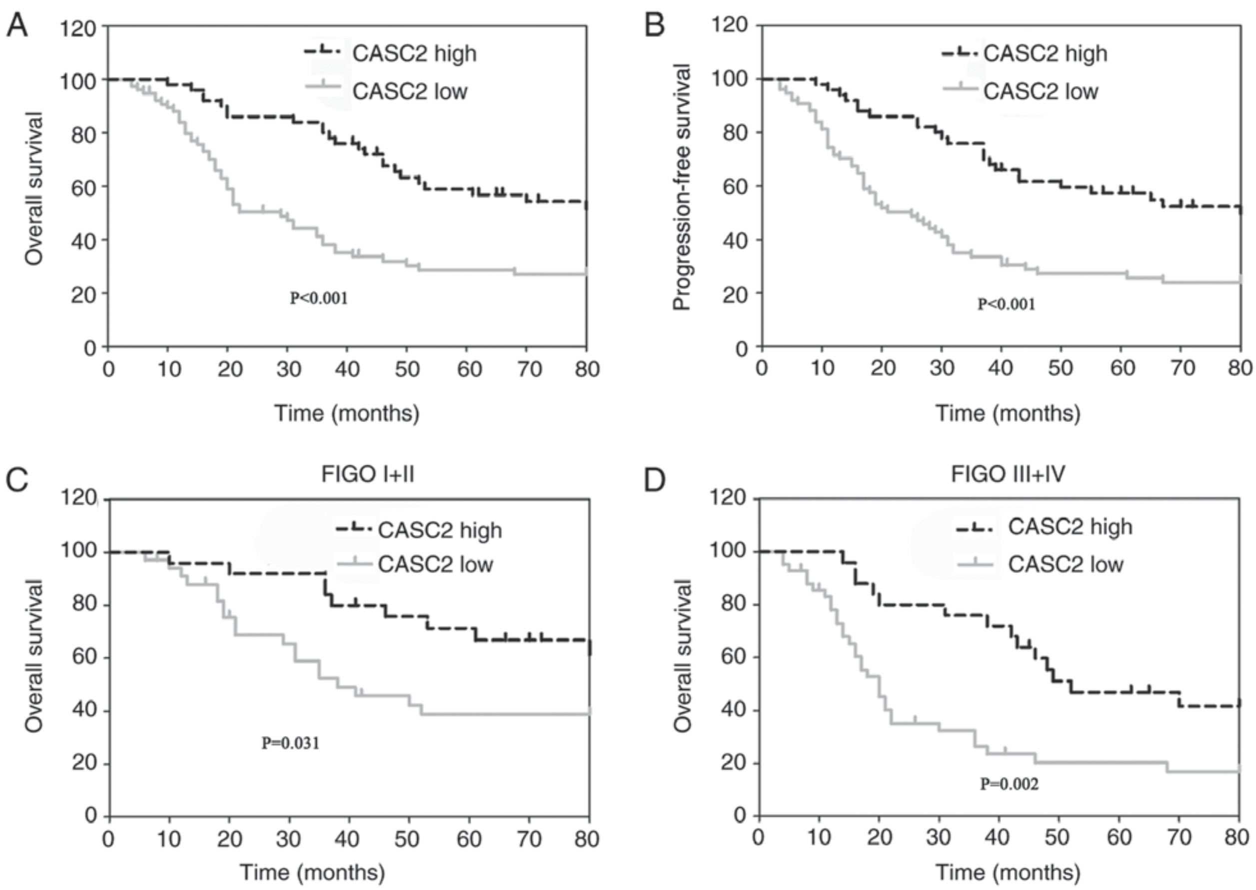

Figure 4. CASC2 expression level correlates with the prognosis of patients with EOC a total of 80 months post-treatment. (A) Overall survival rate of patients with EOC (80 months post-operation) with high CASC2 expression was compared with patients with low CASC2 expression. (B) Progression-free survival rate of patients with EOC with high CASC2 expression and those with low CASC2 was calculated. (C) Prognosis of patients categorized as FIGO stage (I+II) with low or high CASC2 expression levels was analyzed. (D) Prognosis of patients categorized as FIGO stage (III+IV) with low or high CASC2 expression levels was analyzed. CASC2, cancer susceptibility candidate 2; EOC, epithelial ovarian cancer; FIGO, Fédération Internationale de Gynécologie et d'Obstétrique.

different previous studies have verified that CASC2 may be involved in cancer tumorigenesis, autophagy, proliferation, invasion, metastasis and apoptosis (35). Mechanistically, CASC2 has been demonstrated to function by interacting with microRNAs (38,40-43), the phosphatase and tensin homology pathway (13,44) and the Wnt/β-catenin signaling pathway (45). Furthermore, survival analyses identified that low CASC2 expression predicts poor prognosis in thyroid cancer (46), glioma (10), astrocytoma (40) and non-small cell lung cancer (8). Taken together, these results suggest that CASC2 may act as a tumor suppressor in a wide range of cancer types through different signaling pathways.

The present study investigated the significance of CASC2 in EOC by determining its baseline expression level. In accordance with the results of previous studies (35), CASC2 demonstrated significantly decreased expression levels in EOC cells and tissues. In addition, the clinical significance analysis of the association CASC2 expression and the clinicopathological features of patients with EOC identified that low CASC2 expression was associated with the serous histological subtype, lymph node metastasis, poor histological grade and large tumor size, all of which contribute to EOC progression. Indeed, the prognostic evaluation revealed that patients with low CASC2 expression exhibited a markedly poorer overall survival rate and progression-free survival rate. In addition, further analysis revealed that patients with FIGO stage I/II or III/IV demonstrated a poorer overall survival rate if they additionally had low CASC2 expression. Furthermore, low CASC2 expression was confirmed to be an independent risk factor for poor prognosis in EOC. These results suggested that CASC2 has a primary role in inhibiting EOC progression in patients. To verify the function of CASC2 in EOC cells, CASC2 was overexpressed or silenced, and the results of the functional studies confirmed that CASC2 may inhibit the proliferation and metastasis of EOC cells. However, the clinical specimens used in the present study were collected in one medical center. Future studies investigating CASC2 should be performed in numerous different centers using a larger sample size. The underlying mechanism by which CASC2 exerts its function should be further explored.

In conclusion, CASC2 expression levels were decreased in EOC cells and tissues, and a low CASC2 expression level was associated with clinical progression in patients with EOC. The results of the functional studies identified that CASC2 may inhibit the proliferation and metastasis of EOC cells. Furthermore, patients with low CASC2 expression exhibited a poorer overall survival rate and a shorter progression-free survival period. Notably, low CASC2 expression was identified as an independent risk factor for overall survival and progression-free survival in patients with EOC. However, the detailed mechanisms of the influence of CASC2 on EOC progression were not further verified in the present study. The functional role of CASC2 as a tumor suppressor was demonstrated in EOC, and CASC2 may be considered a promising prognostic marker and therapeutic target in EOC.

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Availability of data and materials

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

Authors' contributions

ZX performed the statistical analysis of clinical data, performed *in vitro* assays and wrote the manuscript. XZ determined the expression levels of CASC2 in EOC cells and tissues, and performed statistical analysis. YT designed the study, performed the statistical analysis and wrote the manuscript. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the Shanghai Jiao Tong University Affiliated Sixth People's Hospital. Written informed consent was obtained from each patient.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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