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CKS2 promotes tumor progression and metastasis and is an independent predictor of poor prognosis in epithelial ovarian cancer

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Abstract. – OBJECTIVE: Accumulating evidence showed that dysregulation of cyclin-dependent kinases regulatory subunit 2 (CKS2) could contribute to tumor growth and metastasis of several tumors. However, its expression and function in epithelial ovarian cancer (EOC) have not been investigated. Here, we aimed to investigate the role of CKS2 in EOC.

PATIENTS AND METHODS: Real-time PCR and Western blotting were used to determine the mRNA and protein expression of CKS2 in EOC tissues and cell lines. Then, the associations of CKS2 expression with clinicopathological features and patient's overall survival were determined. Proliferation assay flow cytometric analysis and transwell assay were performed to detect the relation between CKS2 and malignant behaviors of EOC cells. We also evaluated the expression of related proteins of the Akt/mTOR pathway to determine the associated molecular mechanism.

RESULTS: We found that CKS2 expression was significantly up-regulated in both EOC tissues and cell lines. Clinically, high expression of CKS2 was associated with advanced FIGO stage, histological grade and shorter overall survival of EOC patients. We also found that knockdown of CKS2 suppressed proliferation, invasion, and migration of EOC cells *in vitro*, and CKS2 could promote EMT progress by modulating EMT-related molecules. Finally, Western blot demonstrated that down-regulation of CKS2 suppressed the expression of p-Akt and p-mTOR.

CONCLUSIONS: Our findings indicated that CKS2 might function as a tumor promoter by modulating Akt/mTOR pathway in EOC and could serve as a promising prognostic biomarker for EOC.

Key Words:
CKS2, Prognosis, Epithelial ovarian cancer, Metastasis, Akt/mTOR pathway.

Introduction

Ovarian cancer represents a leading cause of death in women, and one of the most lethal of gynecological malignancies, with an estimated 21,290 new cases reported in the USA in 2015[1,2]. Epithelial ovarian cancer (EOC) accounts for approximately 90% of ovarian cancer and is characterized by the frequent development of metastases during the asymptomatic stage of the disease[3]. The lack of early-stage biomarkers and non-typical symptoms of this malignancy makes approximately 70% of the patients with advanced stage of EOC at diagnosis when the tumor cells have dispersed beyond the ovaries to other organs, such as the pelvic and abdominal organs[4,5]. Despite recent advances in diagnostics and multimodal therapies, the 5-year survival rate for advanced EOC is poorly 30%[6,7]. The main reason for the poor prognosis in ovarian cancer is invasion and metastasis. Thus, a thorough understanding of the potential mechanism of EOC development and progression is very important for improving the treatment and prevention of this disease.

Cyclin-dependent kinase subunit 2 (CKS2), located in chromosome 9q22, is a cyclin-dependent kinase-interacting protein, a critical protein involved in cell cycle regulation[8]. CKS2 play an important role in the regulation of the development and formation of the human embryo and the process of somatic cell division[9]. It has been confirmed that CKS2 proteins are involved in the regulation of cells proliferation, metastasis, and apoptosis[10]. These findings suggest that CKS2 may play a functional role in the development and function of tumors[11,12]. Indeed, several studies have reported that CKS2 is abnormally expressed in several tumors and display a tumor-promotive role. For instance, Yu et al[13] reported that CKS2 expression was significantly up-regulated in colorectal cancer and associated with a shorter five-year overall survival rate by analyzing clinical data from 183 EOC patients. In addition, loss-of-function assays indicated that down-regulation of CKS2 suppressed cell proliferation and invasion and promoted cell

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apoptosis through modulating claudin1 expression. Wu et al[14] indicated that CKS2, a target of miR-26a, promoted the proliferation and metastasis, suggesting it as a potential therapeutic target for this disease. However, the expression levels, the roles and the potential mechanism of CKS2 in EOC remain largely unclear.

In this study, we explored whether CKS2 was abnormally expressed in EOC tissues by analyzing microarray data from TCGA datasets and Gene Expression Omnibus (GEO), and qRT-PCR was further performed to validate the above results. Then, we further conducted a follow-up of EOC patients over a 5-year period and a performed statistical analysis to explore the clinical significance of CKS2 expression in EOC patients. Loss-of-function assays indicated that the knockdown of CKS2 suppressed the proliferation and metastasis of EOC cells by suppression of Akt/mTOR signaling pathway. This study advances our understanding of the role of CKS2 as a tumor promoter in the progression of EOC.

Patients and Methods

Patients and Tissue Collection

Pathologically confirmed EOC tissue samples and matched non-cancerous normal specimens were obtained from 127 EOC patients underwent surgery with a written consent of each patient and ethics approval from Huai'an First People's Hospital, Nanjing Medical University. For all cases, the tissue samples were immediately snap frozen using liquid nitrogen after resection and preserved at -80°C. All patients' clinical features were shown in Table II.

Cell Lines and Cell Culture

All cell lines (IOSE80, OVCAR-3, SKOV3, and HEY) used in this study were purchased from BNBio Biological Co., Ltd. (Chaoyang, Beijing, China). RPMI 1640 medium (XF Biotech, Minhang, Shanghai, China) supplemented with 10% fetal bovine serum (FBS) was utilized for cell culture. The cells were all maintained in an incubator with 5% CO_2 at 37°C.

Cell Transfection

Briefly, SKOV3 or OVCAR-3 cells (1×10^5 cells/well) were placed in 6-well plates and continued to be cultured until the cell confluence reached about 70%. Then, the small interfering RNAs (siRNAs) targeting CKS2 (siRNA#1, siRNA#2) or negative control siRNAs (NC-siRNA) were transfected into these cells using Lipofectamine 3000 reagent (HaoJia Biotech, Wuhan, Hubei, China) according to relevant protocols. The siRNAs were synthesized by JRDUN Biotechnology Co., Ltd. (Baoshan, Shanghai, China).

Table I. Sequence of the primers used in this study.

Names	Sequences (5'-3')
CKS2: Forward	TTCGACGAACACTACGAGTACC
CKS2: Reverse	GGACACCAAGTCTCCTCCAC
GAPDH: Forward	GGAGCGAGATCCCTCCAAAAT
GAPDH: Reverse	GGCTGTTGTCATACTTCTCATGG

Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR)

Total RNA from EOC tissues or cells isolated by TRIzol reagent (Huamaike, Fangshan, Beijing, China) was first reversely transcribed to cDNA using a First Strand cDNA Synthesis kit (HengFei Biotech, Minhang, Shanghai, China). Subsequently, the program of PCR amplification was conducted by an SYBR Green qPCR Mix kit (PuKaiRui, Haidian, Beijing, China) on a SLAN-96P real-time PCR apparatus (HongShi, Jiading, Shanghai, China). The relative expression of CKS2 was normalized to GAPDH. The primers were displayed in Table I. The relative expression was calculated using $2^{-\Delta\Delta Ct}$ method.

Western Blot Analysis

Cell lysis buffer (QianChen, Pudong, Shanghai, China) was utilized to lyse the collected SKOV3 or OVCAR-3 cells. Then, the proteins were separated by SDS-PAGE on 8-12% gels and sequentially electrotransferred onto polyvinylidene difluoride membranes. Before being incubated with corresponding primary antibodies, the membranes were blocked with Bull Serum Albumin (BSA) solution (5%). After being washed with TBST for three times, the membranes were incubated with matched secondary antibodies, and an ECL assay kit (DingGuo, Haidian, Beijing, China) was finally applied to visualize the protein bands. The primary antibodies targeting caspase 3, vimentin, p-AKT and p-mTOR were purchased from YBio Biotechnology Co., Ltd. (Minhang, Shanghai, China). The primary antibodies against caspase 9, N-cadherin, GAPDH and β-actin were obtained from BOSTOR Biotechnology Co., Ltd. (Wuhan, Hubei, China).

Table II. Association of CKS2 expression with clinicopathological features of EOC.

Variables	Cases (N)	CKS2 expression		*p*-value
		High	Low	
Age (years)				0.249
<50	73	33	40	
≥50	54	30	24	
FIGO stage				0.006
I-II	89	37	52	
III-IV	38	26	12	
Histological grade				0.022
G1-G2	81	34	47	
G3	46	29	17	
Lymph node metastasis				0.056
Absent	96	43	53	
Present	31	20	11	
CA125 level (U/ml)				0.427
<600	58	31	27	
≥600	69	32	37	
Ascites				0.424
<100	63	29	34	
≥100	64	34	30	

Cell Viability Assays

Cell viability assays were conducted using the CCK-8 assay kit (XinRun, Wuxi, Jiangsu, China). In short, SKOV3 or OVCAR-3 cells after treatment were harvested and plated into 96-well plates in triplicate (2000 cells/well). After the cells attached the plates, CCK-8 reagent (10 µl/well) was added into the cells, and the plates were incubated at 37°C for 1-2 h. Then, a DNM-9602 microplate reader apparatus (Perlang Medical, Shunyi, Beijing, China) was utilized to determine the OD 450nm absorbance.

Colony Formation Assay

The CKS2 siRNAs-transfected SKOV3 or OVCAR-3 cells were first trypsinized into single-cell suspensions. Then, about 500 cells were plated into 6-well plates, and the cells were continued to be maintained at 37˚C with 5% CO_2 for about 2 weeks. The cell colonies were stained with crystal violet (0.1%) and imaged by a microscope (XHC-BV1; HuaCe, Chaoyang, Beijing, China).

Flow Cytometry Analysis

The cell cycle and cell apoptosis were determined by flow cytometry analysis. In brief, SKOV3 or OVCAR-3 cells after treatment were trypsinized into single-cell suspensions. For cell cycle analysis, the cell suspensions were added with propidium iodide (PI; 20 µg/ml) and RNase (10 µl; Beyotime, Haimen, Jiangsu, China); for cell apoptosis analysis, the cell suspensions were added with Annexin V-FITC/PI solution (KeyGEN, Nanjing, Jiangsu, China). After the treated cell suspensions were kept in the dark for 20 min, the cells were subjected to flow cytometry analysis using a BD Biosciences Accuri C6 flow cytometer (Rantai Biotech, Minhang, Shanghai, China).

Wound Healing Assays

The cell migratory capacity of SKOV3 and OVCAR-3 cells after transfection of CKS2 siRNAs were determined by wound healing assays. Briefly, cells were plated in 12-well plates until 70-80% conflux. Then, CKS2 siRNAs or negative control siRNAs were separately transfected into the cells. When the cell confluence reached 90-100%, a 200 µl plastic pipette tip was utilized to create the wound. Finally, a microscope (XHC-BV1; HuaCe, Chaoyang, Beijing, China) was used to take pictures of the wounded areas immediately after the wound made and 48 h later.

Transwell Invasion Assays

Cell invasion was determined by using the Corning 24-well transwell chambers (pore size: 8 µm; Unique, Chaoyang, Beijing, China). In brief, the CKS2 siRNAs or negative control siRNAs-transfected SKOV3 or OVCAR-3 cells were digested into single-cell suspensions (without serum; at a density of 2×10^5 cells per ml), and 100 µl of the cell suspension was added into the upper side of each chamber (pre-coated with Matrigel). Then, complete medium (containing 15% FBS) was added into the lower chamber. Twenty-four hours later, the cells that passed through the transwell membranes (on the lower surfaces) were fixed (with 4% paraformaldehyde) and stained using crystal violet (0.1%). After rinsing with phosphate buffer saline, the invaded cells were photographed by a microscope (XHC-BV1; HuaCe, Chaoyang, Beijing, China).

Statistical Analysis

Statistical analysis was carried out using SPSS 20.0 statistics software (SPSS, Inc., Chicago, IL, USA). The Student's *t*-test was used to evaluate the differences between data from two groups, and one-way ANOVA was applied to assess the differences among multiple groups. The paired comparison was performed by the Student-Newman-Keuls (SNK) approach. The association between CKS2 and clinicopathological parameters was analyzed by the Chi-square test. The Ka-

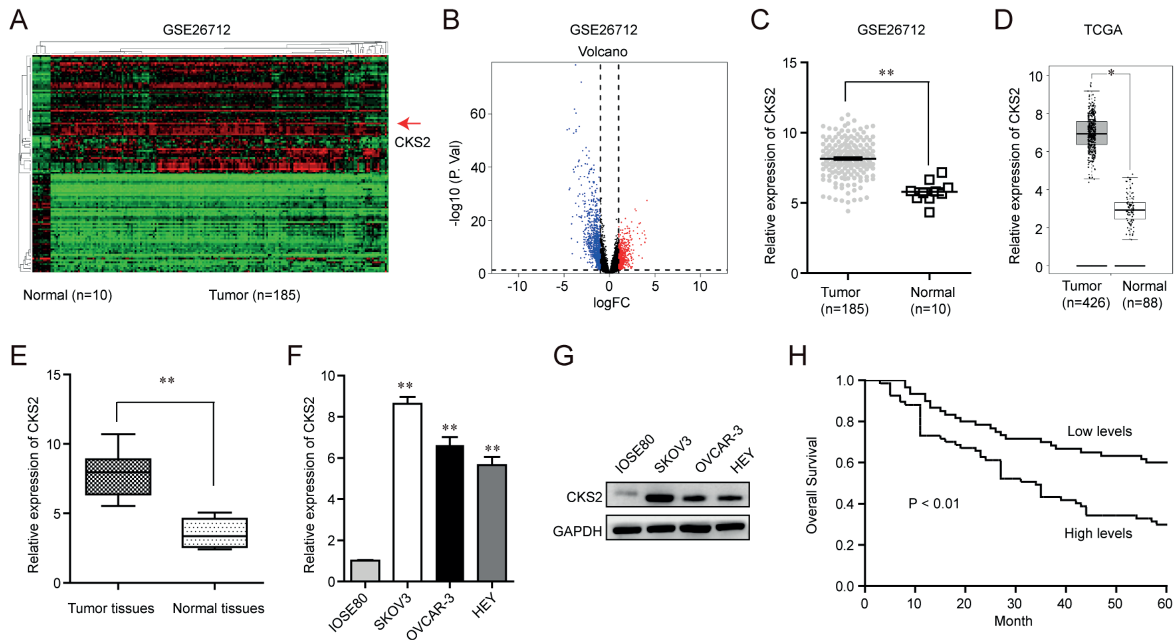

Figure 1. Expression levels of CKS2 in EOC tissues and cells. ***A,*** Hierarchical clustering analysis of the differentially expressed mRNAs in GSE26712 (|fold change| > 1.5, $p < 0.05$). ***B,*** Volcano plot of the aberrantly expressed miRNAs between EOC and adjacent normal tissues in GSE26712 datasets. ***C,*** Data mining of CKS2 expression levels in the EOC tissue samples from gene profiling (GSE26712). ***D,*** CKS2 expression levels in 127 pair-matched tumor tissues and adjacent normal tissues from the TCGA database. ***E,*** Expression levels of CKS2 were significantly increased in EOC tissues compared with matched adjacent non-tumor tissues. ***F,*** The relative level of CKS2 mRNA in EOC cell lines and IOSE80 cell line by RT-PCR. ***G,*** The relative level of CKS2 proteins in EOC cell lines and IOSE80 cell line by Western blot. ***H,*** Kaplan-Meier overall survival curves by high and low CKS2 expression in 127 EOC patient cases. *$p<0.05$, **$p<0.01$.

plan-Meier method was used to calculate survival curves which were compared using the log-rank test. Cox proportional hazards models were generated to assess the survival data. A $p<0.05$ was regarded to be statistically significant.

Results

CKS2 was Up-Regulated in EOC and Correlated with Pathogenic Condition and Prognosis

To investigate aberrant mRNAs in EOC, we first analyzed the microarray data from GEO datasets (GSE26712) and found that CKS23 was the highest up-regulated mRNA in GSE26712 dataset (Figure 1A). Then, the volcano plots showed the variation of mRNAs expression between the EOC cancerous tissues and the adjacent normal tissues (Figure 1B). In addition, Figure 3A showed that CKS2 mRNA was significantly up-regulated in EOC tissues compared with adjacent non-tumor tissues in both GSE26712 and TCGA datasets ($p<0.01$). To further co-validate the online results, we detected the expression of CKS2 mRNA in 127 paired EOC tissues and matched normal tissues by RT-PCR, finding that CKS2 mRNA was significantly up-regulated in EOC tissues compared to matched normal tissues ($p<0.01$). We also confirmed that the expression of CKS2 mRNA and proteins were significantly up-regulated in three EOC cell lines (Figure F and G). These results indicated that CKS2 was highly expressed in EOC and may act as a tumor promoter. Then, we further explored the clinical significance of CKS2 in EOC patients, and the results of the Chi-square test showed that high CKS2 expression was significantly correlated with FIGO stage ($p = 0.006$) and Histological grade ($p = 0.022$) (Table II). Moreover, Kaplan-Meier survival analysis and the log-rank test for overall survival curves were used to reveal the relationship between CKS2 expression and patients' survival; we found that patients with decreased CKS2 expression had better overall survival than those with elevated expression of CKS2 ($p<0.01$). More importantly, univariate and multivariate analysis showed that lower CKS2 expression was an independent prognostic risk fac-

tor of overall survival for EOC patients ($p<0.05$, Table III). Overall, our results provided evidence that CKS2 was highly expressed in EOC and associated with poor prognosis of EOC patients.

CKS2 Regulated the Proliferation and Apoptosis of SKOV3 and OVCAR-3 Cells

To investigate the relevant influence of CKS2 on EOC cell proliferation and apoptosis, siRNAs targeting CKS2 (siRNA#1 and siRNA#2) was transfected into SKOV3 and OVCAR-3 cells. The results of qRT-PCR assays revealed that transfection of CKS2 siRNAs remarkably reduced the intracellular CKS2 expression (Figure 2A). Subsequently, the cellular viability was determined by CCK-8 assays. We found that repressing expression of CKS2 led to a notable decrease in the proliferation in both SKOV3 and OVCAR-3 cells (Figure 2B). Additionally, cell colony forming was conducted to evaluate the roles of CKS2 in EOC cell colony formation. According to the data, the silence of CKS2 resulted in a marked decline of colony formation in SKOV3 and OVCAR-3 cells (Figure 2C). Moreover, cell cycle analysis was also carried out to ascertain whether the effects of CKS2 on cellular proliferation of EOC cells were due to cell cycle arrest. It was shown that the percentages of CKS2 siRNAs-transfected SKOV3 and OVCAR-3 cells at G0/G1 phase were significantly increased when comparing to the controls, while the percentages of CKS2 siRNAs-transfected SKOV3 and OVCAR-3 cells at S phase were notably decreased (Figure 2D). These data indicated that the knockdown of CKS2 induced G0/G1 phase arrest of ovarian cell cycle. Besides, we also determined the cell apoptosis using flow cytometry. The data suggested that much more apoptotic SKOV3 and OVCAR-3 cells were observed in the CKS2 deficiency groups compared with the controls (Figure 2E). Accordingly, the molecular mechanism research revealed that the protein expression of apoptotic relevant genes was dramatically elevated in SKOV3 and OVCAR-3 cells when they were transfected with CKS2 siRNAs (Figure 2F). Taken together, these data showed that silence of CKS2 suppressed the cell proliferation and promoted apoptosis of EOC cells.

The Migration and Invasion of SKOV3 and OVCAR-3 Cells Were Inhibited by CKS2 Depletion

To further explore the biological significance of CKS2 in EOC, we next evaluated the influence of CKS2 on the metastatic potentials of SKOV3 and OVCAR-3 cells. The results from the examination of transwell invasion assays showed that transfection of CKS2 siRNAs resulted in a marked decrease in invasive potentials of SKOV3 and OVCAR-3 cells (Figure 3A). In addition, the wound healing assays demonstrated that the mobility of SKOV3 and OVCAR-3 cells was significantly reduced when the cells were transfected with CKS2 siRNAs (Figure 3B). Considering that the cellular migration and invasion were the key phenotypes of epithelial-mesenchymal transition (EMT), we next employed Western blot assays to evaluate the expressing changes of EMT related proteins in EOC cells after transfection of CKS2 siRNAs. The data indicated that knockdown of CKS2 caused remarkably decreased expression of vimentin and N-cadherin in SKOV3 and OVCAR-3 cells (Figure 3C and D). Therefore, these data validated that down-regulation of CKS2 impeded the migration and invasion of EOC cells by inhibiting EMT.

Table III. Univariate and multivariate Cox regression analysis of overall survival in EOC patients.

	Univariate analysis		Multivariate analysis	
Prognostic variables	**HR (95% CI)**	***p***	**HR (95% CI)**	***p***
Age	1.426 (0.782-2.321)	0.342	-	-
FIGO stage	2.665 (1.437-4.995)	0.006	2.426 (1.218-4.557)	0.014
Histological grade	3.128 (1.539-5.237)	0.003	2.557 (1.258-4.461)	0.009
Lymph node metastasis	2.462 (1.231-3.759)	0.036	2.288 (0.852-3.055)	0.073
CA125 level	1.587 (0.563-2.471)	0.218	-	-
Ascites	1.322 (0.859-2.154)	0.429	-	-
CKS2 expression	2.895 (1.388-4.785)	0.006	2.578 (1.123-4.237)	0.017

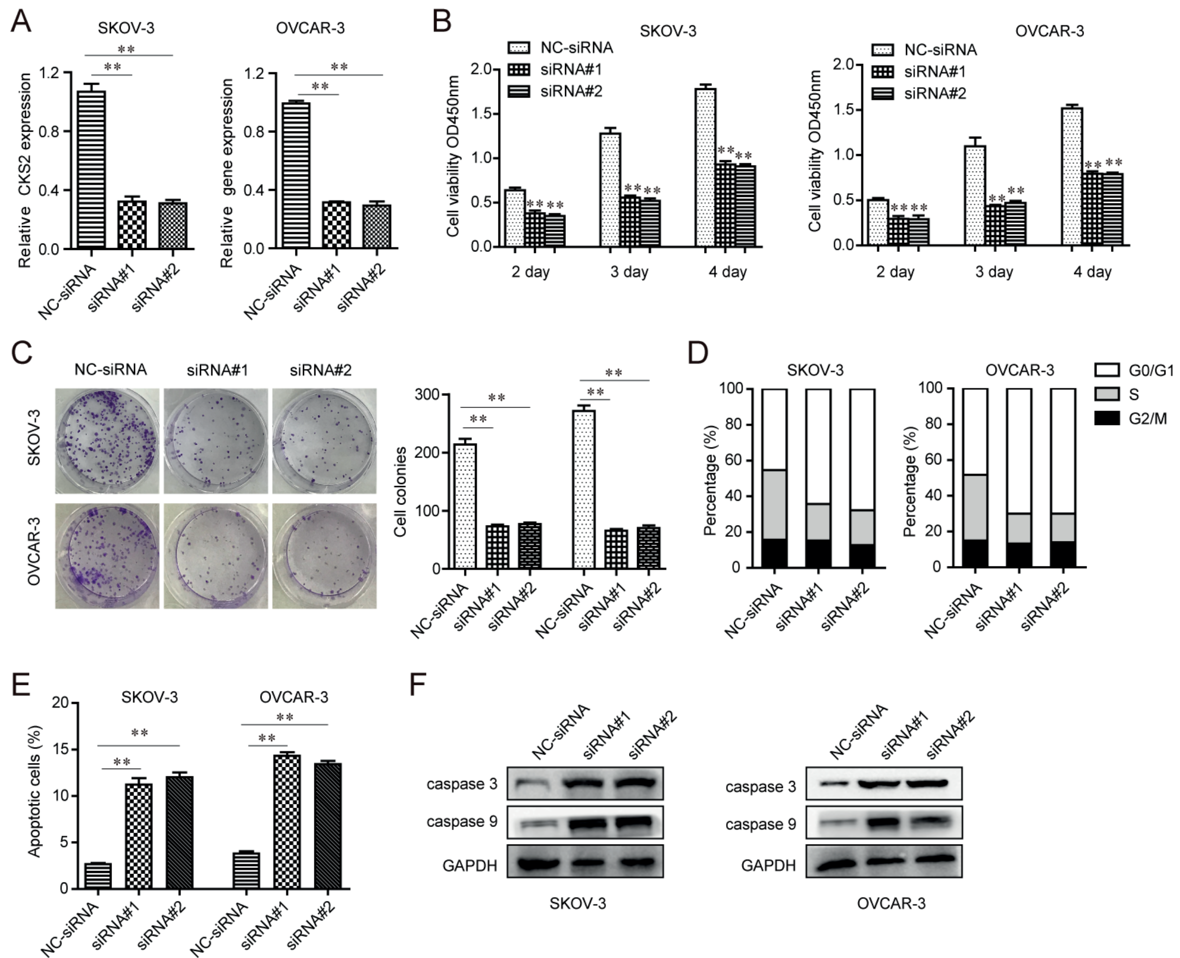

Figure 2. CKS2 promoted the malignant progression of EOC cells. ***A,*** QRT-PCR analysis of CKS2 expression in SKOV3 and OVCAR-3 cells transfected with CKS2 siRNAs (siRNA#1 and siRNA#2) and negative control siRNAs (NC-siRNA). ***B,*** Cell viability of SKOV3 and OVCAR-3 cells after treatment was determined by the CCK-8 assays at 2, 3, and 4 days. ***(C)*** Representative images of the colony formation assays and statistical analysis of the treated SKOV3 and OVCAR-3 cell colony number (magnification: 10×). ***D,*** The effects of CKS2 depletion on the cell-cycle progression of SKOV3 and OVCAR-3 cells. ***E,*** The effects of CKS2 knockdown on promoting apoptosis of SKOV3 and OVCAR-3 cells. ***F,*** Western blot assays detected the protein levels of caspase 3 and caspase 9. *p<0.05, **p<0.01.

Silence of CKS2 Suppressed AKT/mTOR Signaling Pathway in SKOV3 and OVCAR-3 Cells

For a further determination of the detailed molecular mechanism underlying the inhibitory effects of CKS2 deficiency on EOC cells, we next aimed to carry out Western blot assays to evaluate the changes of relevant tumorigenesis signaling pathway in SKOV3 and OVCAR-3 cells after transfection of CKS2 siRNAs. As AKT/mTOR signaling pathway played critical roles in various aspects of cancer development and progression, we next measured the protein levels of phosphorylated-AKT (p-AKT) and phosphorylated-mTOR (p-mTOR) because the activation of AKT and mTOR was dependent on protein phosphorylation. The results from Western blot assays demonstrated that knockdown of CKS2 dramatically reduced the protein levels of p-AKT and p-mTOR in SKOV3 and OVCAR-3 cells, which indicated that CKS2 was capable to affect AKT/mTOR signaling pathway (Figure 4A). Besides, we next wondered whether activating the AKT signaling was able to abrogate the inhibitory effects of CKS2 depletion on EOC cell phenotypes. Hence, we added an activator of AKT: IGF-1 (10 ng/ml; Sigma-Aldrich, Pudong, Shanghai, China), into the CKS2 siRNAs-transfected SKOV3 and OVCAR-3 cells, and sequentially performed CCK-8 assays to evaluate the cellular viability changes.

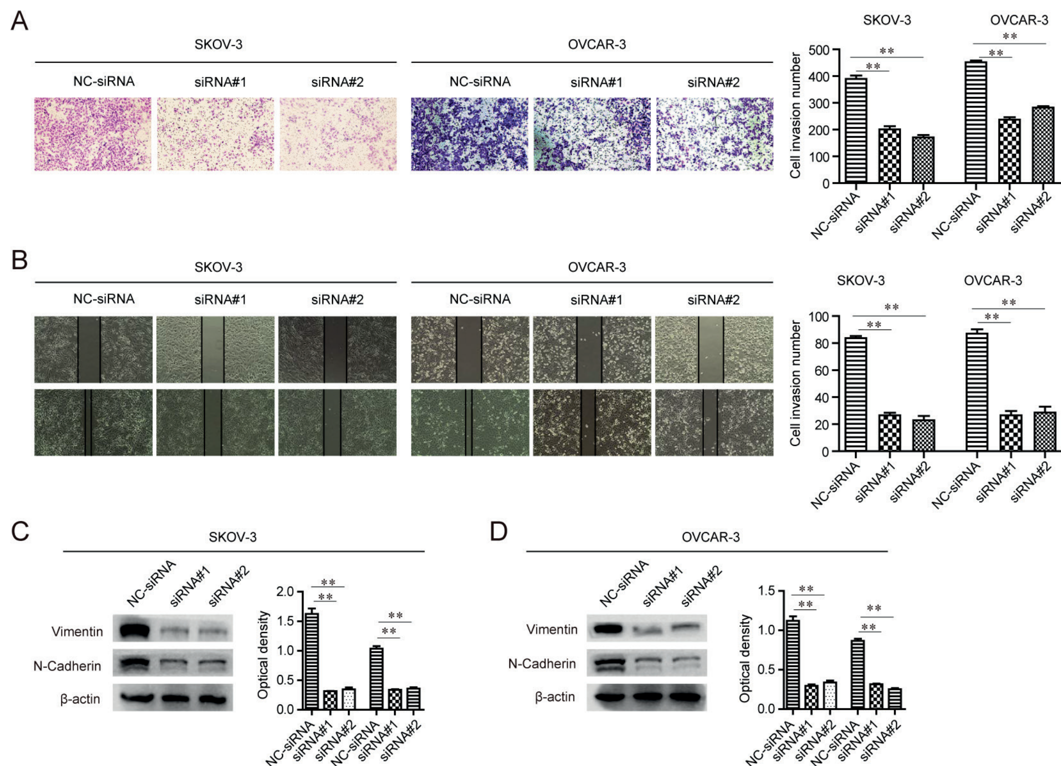

Figure 3. CKS2 knockdown resulted in decreased cell migration and invasion. ***A,*** Transwell invasion assays evaluated the potentials of invasion in SKOV3 and OVCAR-3 cells transfected with CKS2 siRNAs (siRNA#1 and siRNA#2) and negative control siRNAs (NC-siRNA) (magnification: 40×). ***B,*** Wound healing assays were utilized to determine the migratory ability of SKOV3 and OVCAR-3 cells with downregulated CKS2 (magnification: 10×). ***C,*** and ***D,*** The protein levels of vimentin and N-cadherin were detected by Western blot assays. *p<0.05, **p<0.01.

The data confirmed that transfection of CKS2 siRNAs (siRNA#1) significantly reduced the cell proliferation, while the introduction of IGF-1 remarkably restored the cellular viability of SKOV3 and OVCAR-3 cells (Figure 4B). Therefore, our data suggested that the depression of CKS2 resulted in suppression of EOC progression via AKT/mTOR signaling pathway inhibition.

Discussion

EOC, one of the most prevalent gynecologic malignancies, will represent a serious threat to women's life in China in the following years[15]. Up to date, the treatment of EOC patients usually involves tumor resection and chemotherapy, and sometimes radiotherapy, regardless of the subtype

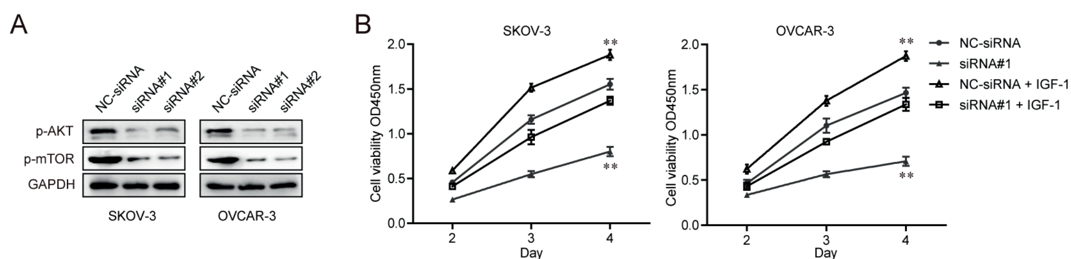

Figure 4. AKT/mTOR signaling pathway was inactivated in EOC cells after knockdown of CKS2. ***A,*** Western blot assays detected the protein levels of p-AKT and p-mTOR in SKOV3 and OVCAR-3 cells. ***B,*** Transfection of CKS2 siRNAs (siRNA#1) suppressed the proliferation of SKOV3 and OVCAR-3 cells, while the inhibitory effects were rescued by introduction of IGF-1. *p<0.05, **p<0.01.

of EOC[16,17]. However, tumor resection alone is correlated with high recurrence rates, and the treatment effect of chemotherapy and radiotherapy are controversial[18]. On the other hand, many patients relapse after surgery or develop resistance to chemotherapy drugs[19]. Despite improvements in the therapeutic tool, the overall survival of EOC with advanced stage remains low. As a multifactorial and multistep disease, EOC is involved in abnormal activation of oncogenes and inactivation of anti-oncogenes[20,21]. Because of poor understanding of nosogenesis of EOC, it was hard to develop a great method for the early diagnosis and prediction of prognosis. With the development of biological technologies, more and more functional genes are reported to be significantly abnormally expressed in various tumors and shown possess diagnostic and prognostic value[22,23]. Thus, the identification of novel diagnostic markers and therapeutic targets in EOC are urgently needed.

In this study, we firstly reported that CKS2 expression was significantly up-regulated in both EOC tissues and cells by analyzing TCGA datasets and GEO datasets and using RT-PCR. Then, clinical data showed that higher expression of CKS2 was associated with advanced FIGO stage and histological grade, suggesting that CKS2 may contribute to malignant progression of EOC. Moreover, survival assays indicated that patients with high CKS2 expression levels tended to have worse overall survival than those with low levels of CKS2 expression, which revealed CKS2 as a potential biomarker for EOC. More importantly, univariate and multivariate analyses revealed that increased expression of CKS2 might be a potential unfavorable prognostic factor for patients with EOC. Recently, previous studies also reported the prognostic value of CKS2 on other tumors. It was reported that CKS2 was associated with five-year overall survival of patients of colorectal cancer, prostatic cancer, and breast cancer. Our results were in line with previous findings indicating that higher CKS2 expression has the potential to be a novel diagnostic and prognostic biomarker.

Functionally, CKS2 has been found to be involved in the progression of cells growth and cell cycle, suggesting that CKS2 may act as a cancer gene. Recently, the oncogenic roles of CKS2 have been reported in several tumors. For instance, Ji et al[24] detected that CKS2 expression was significantly up-regulated in hepatocellular carcinoma and predicted poor prognosis of patients with hepatocellular carcinoma. Functional researches showed that the suppression of CKS2 significantly suppressed hepatocellular carcinoma cells proliferation by modulating PTEN and P53 pathway. In recent years, the study of miRNAs had attracted increasing attention. The regulation network of miRNA/mRNA developed our understanding of the pathogenesis of tumors. Recently, CKS2 was also reported to be targeted by several miRNAs in different tumors, such as miRNA-7 targeting CKS2 in thyroid papillary cancer and miRNA-26a targeting CKS2 in laryngeal squamous cell carcinoma[14,25]. However, whether or not CKS2 also played a similar oncogenic role in EOC remains unknown. In this study, we found that CKS2 silencing in SKOV-3 and OVCAR-3 cells significantly suppressed cells proliferation and induced cell apoptosis, along with increased protein expression of caspase-3 and caspase-9. Moreover, we performed wound healing assays and transwell invasion assays to explore whether CKS2 could influence the ability of metastasis of EOC cells, finding that the knockdown of CKS2 in SKOV-3 and OVCAR-3 cells significantly suppressed cells migration and invasion, along with decreased protein expression of Vimentin and N-cadherin. Our results suggested that CKS2 may act as a positive regulator in the progress of EMT which is an essential step in invasion and metastasis of human cancers[26]. Overall, we first provided evidence that the up-regulation of CKS2 may be related to the fast growth, invasiveness, and metastatic potential of EOC.

The PI3K/AKT/mTOR pathway is a well-known biological signal pathway involved in the regulation of diverse cellular processes related to cell proliferation, survival, differentiation, motility, and invasion[27,28]. The dysregulation of the PI3K/Akt signaling pathway has been observed in various cancers and has been shown to play a critical role in cancer cells proliferation and metastasis[29,30]. In addition, it was reported that PI3K/Akt signaling pathway plays an important role in EMT, which is the main cause of tumor metastasis[31,32]. Downstream factors of AKT and mTOR are involved in survival, growth and metabolic-related pathways and has been observed in high-grade and late-stage serous EOC[33]. To further clarify the molecular mechanism involved in CKS2 induced EOC cell growth and metastasis, we analyzed the expression levels of p-AKT and p-mTOR, finding that overexpression of CKS2 decreased the level of p-Akt and p-mTOR. The suppression of the phosphorylation of AKT and mTOR, suggests that CKS2 may display its oncogenic role

through suppressing the AKT/mTOR signaling pathway.

Conclusions

We provided important evidence that the overexpression of the oncogenic CKS2 is relevant in the progression of human EOC. These findings shed new light on the prospect for manipulating CKS2 expression as a potential novel therapeutic targeting to anti-cancer therapy of EOC as well as a potential diagnostic and prognostic biomarker.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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