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LINC01116 promotes the progression of epithelial ovarian cancer via regulating cell apoptosis

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Abstract. – OBJECTIVE: To explore the biological function of LINC01116 in epithelial ovarian cancer (EOC) and its underlying mechanism.

PATIENTS AND METHODS: The expression level of LINC01116 in 60 EOC tissues, 30 normal ovarian tissues and EOC cells were detected by qRT-PCR (quantitative real-time polymerase chain reaction). Disease-free survival (DFS) and overall survival (OS) of enrolled EOC patients were recorded. The correlation between LINC01116 expression, DFS and OS of EOC patients was analyzed using ROC curve. Influencing factors for DFS and OS were analyzed by univariable and multivariable Cox regression model. For *in vitro* experiments, the effect of LINC01116 knockdown on proliferation, invasion and apoptosis of EOC cells were detected by CCK-8 (cell counting kit-8), transwell assay and flow cytometry, respectively. Protein expressions of apoptosis-related genes in EOC cells transfected with pc-DNA-LINC01116 or si-LINC01116 were detected by Western blot.

RESULTS: LINC01116 was overexpressed in EOC tissues than that of paracancerous tissues. DFS and OS in EOC patients with higher expression of LINC01116 were remarkably shorter than those with lower expression. FIGO (International Federation of Gynecology and Obstetrics) clinical stage and LINC01116 expression were the independent factors that affected DFS and OS of EOC patients. Besides, LINC01116 expression was positively correlated to the diagnostic sensitivity of EOC patients. *In vitro* experiments found that LINC01116 overexpression promoted proliferation and invasion of EOC cells. Overexpressed LINC01116 resulted in upregulated Bcl-2, and downregulated cleaved Caspase-3 and cleaved Caspase-9 in EOC cells.

CONCLUSIONS: Overexpressed LINC01116 promotes EOC progression via increasing proliferation and migration, and inhibiting cell apoptosis.

Key Words:
LINC01116, Apoptosis, Disease-free survival, Overall survival.

Introduction

The mortality of EOC ranks the first in gynecologic malignancies, with the 5-year survival rate of only 30%[1]. It is of great significance to investigate the potential mechanism of EOC. Currently, relative researches mainly focus on finding novel biomarkers and targets of EOC. Identifying high-risk population and establishment of effective management help to improve clinical outcomes of EOC patients[2].

LncRNA (long non-coding RNA) is a non-coding RNA with more than 200 nt in length, which is normally transcribed in eukaryotes. Functionally, lncRNA regulates gene expression at pre-transcriptional, transcriptional and post-transcriptional level. However, it could not encode proteins[3]. LncRNA was initially considered as by-product of RNA polymerase II transcription without any cellular function[4]. In recent years, studies have demonstrated that lncRNA is involved in X chromosome silence, genetic imprinting, chromatin modification, transcription, and nuclear transportation[5].

A large number of studies[6-8] have indicated that differentially expressed lncRNAs are closely related to various tumors. The specific role of lncRNA in EOC, however, has not been fully elucidated. This research aims to clarify the role of LINC01116 in EOC and its underlying mechanism. Our results provide new ideas for predicting, diagnosing and treating for EOC, so as to improve clinical outcomes of affected population.

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Patients and Methods

Sample Collection

EOC patients treated in The First People's Hospital of Jining from July 2012 to July 2017 were enrolled. All included patients were pathologically diagnosed as EOC without other major complications. Patients did not receive preoperative therapies. Clinical data of these patients were retrospectively analyzed. 60 EOC tissues and 30 normal ovarian tissues were surgically resected and preserved in -80°C for the following experiments. This study was approved by The First People's Hospital of Jining Ethic Committee and patients were all informed consent.

Cell Culture and Transfection

Normal ovarian cell line (IOSE-386 cells) and EOC cell lines (HO8910, HEY and A2780 cells) were obtained from the Institute of Biochemistry and Cell Biology, Beijing, China. Cells were cultured in F12 or RPMI-1640 (Roswell Park Memorial Institute-1640) containing 10% FBS (fetal bovine serum), 100 U/mL penicillin and 100 μg/mL streptomycin (HyClone, South Logan, UT, USA). Cells were incubated at 37°C in a 5% CO_2 incubator.

Cells were seeded in the 6-well plates and 96-well plates at a density of 1×10^5/ml. Cell transfection was performed when the cell confluence was up to 60-80% according to the instructions of Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

RNA Extraction and qRT-PCR (Quantitative Real-Time Polymerase Chain Reaction)

Total RNA in treated cells was extracted using TRIzol method for reverse transcription according to the instructions of PrimeScript RT reagent Kit (TaKaRa, Otsu, Shiga, Japan). RNA concentration was detected using spectrometer and those samples with A260/A280 ratio of 1.8-2.0 were selected for the following qRT-PCR reaction. QRT-PCR was then performed based on the instructions of SYBR Premix Ex Taq TM (TaKaRa, Otsu, Shiga, Japan), with 3 replicates in each group. QRT-PCR reaction parameters were as follows: denaturalization at 95°C for 60 s, extension at 95°C for 30 s and annealing at 60°C for 40 s, for a total of 40 cycles.

CCK-8 (Cell Counting Kit-8) Assay

EOC cells were seeded into 96-well plates at a density of 2×10^3/μL. 10 μL of CCK-8 solution (Cell Counting Kit-8, Dojindo, Kumamoto, Japan) were added in each well after cell culture for 0, 6, 24, 48, 72 and 96 h, respectively. The absorbance at 450 nm of each sample was measure by a microplate reader (Bio-Rad, Hercules, CA, USA). Each group had 5 replicates.

Colony Formation Assay

EOC cells in logarithmic growth phase were washed with PBS, digested with trypsin and centrifuged at 100 rpm/min for 3 min. After cell density was adjusted to 1×10^4/L, cells were seeded in the 6-well plates with 2000 cells per well. Cells were fixed with 4% methanol for 30 min and stained with 0.1% crystal violet for another 30 min (Sigma-Aldrich, St. Louis, MO, USA), followed by the detection of colony formation.

Cell Apoptosis Detection

EOC cells were digested with EDTA-free trypsin and centrifuged at 1000 rpm/min for 5 min. Cells were resuspended in 100 μL of 1 × Annexin at a density of 3×10^5/ mL. Subsequently, 1 μL of propidium iodide (PI) and 5 μL of Annexin V-FITC (fluorescein isothiocyanate) were added in 100 μL of cell suspension, and incubated at room temperature in dark for 15 min. Finally, cell apoptosis was analyzed by flow cytometry (Partec AG, Arlesheim, Switzerland).

Western Blot

Total protein was extracted from treated cells by RIPA (radioimmunoprecipitation assay) solution (Yeasen, Shanghai, China). Protein sample was separated by electrophoresis on 10% SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis). Samples were then transferred to PVDF (polyvinylidene difluoride) membrane (Roche, Basel, Switzerland). After membranes were blocked with skimmed milk, the membranes were incubated with primary antibodies (Cell Signaling Technology, Danvers, MA, USA) overnight at 4°C. The membranes were then washed with TBST (Tris-buffered Saline with Tween 20) and followed by the incubation of secondary antibody at room temperature for 1 h. The protein blot on the membrane was exposed by enhanced chemiluminescence.

Statistical Analysis

SPSS22.0 (Statistical Product and Service Solutions) statistical software (IBM, Armonk, NY, USA) was used for data analysis. Graph-

Pad Prism 5.0 (La Jolla, CA, USA) was introduced for editing figures. Measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$) and compared using the *t*-test. Cox regression analysis was performed to evaluate patient prognosis and ROC (receiver operating characteristics) curve was introduced for calculating the diagnostic sensitivity. Classification data were compared with x^2-test. $p < 0.05$ considered the difference was statistically significant.

Results

LINC01116 was Overexpressed in EOC Tissues

LINC01116 was overexpressed in EOC tissues than that of normal ovarian tissues ($p < 0.05$, Figure 1A). Follow-up data of enrolled patients were collected. Survival analysis indicated that DFS (HR = 2.067, $p = 0.0264$) and OS (HR = 2.081, $p = 0.0244$) in EOC patients with higher expression of LINC01116 were remarkably shorter than those with lower expression (Figure 1B and 1C). ROC curve showed that LINC01116 expression is highly sensitive to the diagnosis of EOC ($p < 0.05$, Figure 1D). The above results demonstrated that LINC01116 expression is negatively correlated to prognosis of EOC patients.

Independent Risk Factors for EOC

Univariable and multivariable COX regression analyses confirmed that FIGO clinical stage ($p = 0.035$) and LINC01116 expression ($p = 0.028$) were the independent risk factors for DFS of EOC patients (Figure 2A and 2B). FIGO clinical stage ($p = 0.033$) and LINC01116 ($p = 0.013$) were also the independent risk factors for OS of EOC patients (Figure 2C and 2D).

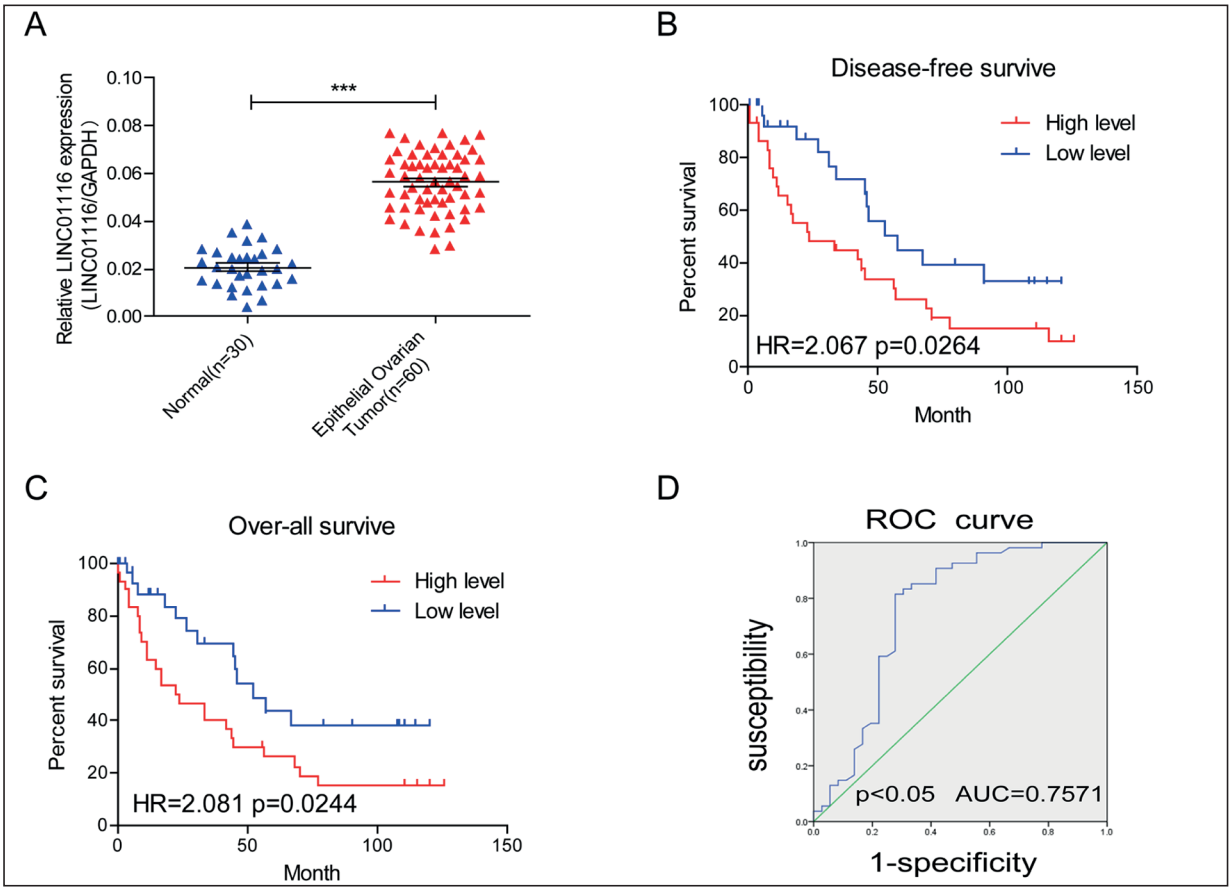

Figure 1. LINC01116 was overexpressed in epithelial ovarian cancer tissues. ***A,*** LINC01116 was overexpressed in epithelial ovarian cancer tissues than that of paracancerous tissues. ***B,*** DFS in epithelial ovarian cancer patients with higher expression of LINC01116 was remarkably shorter than those with lower expression. ***C,*** OS in epithelial ovarian cancer patients with higher expression of LINC01116 was remarkably shorter than those with lower expression. ***D,*** LINC01116 expression was highly sensitive to diagnosis of epithelial ovarian cancer.

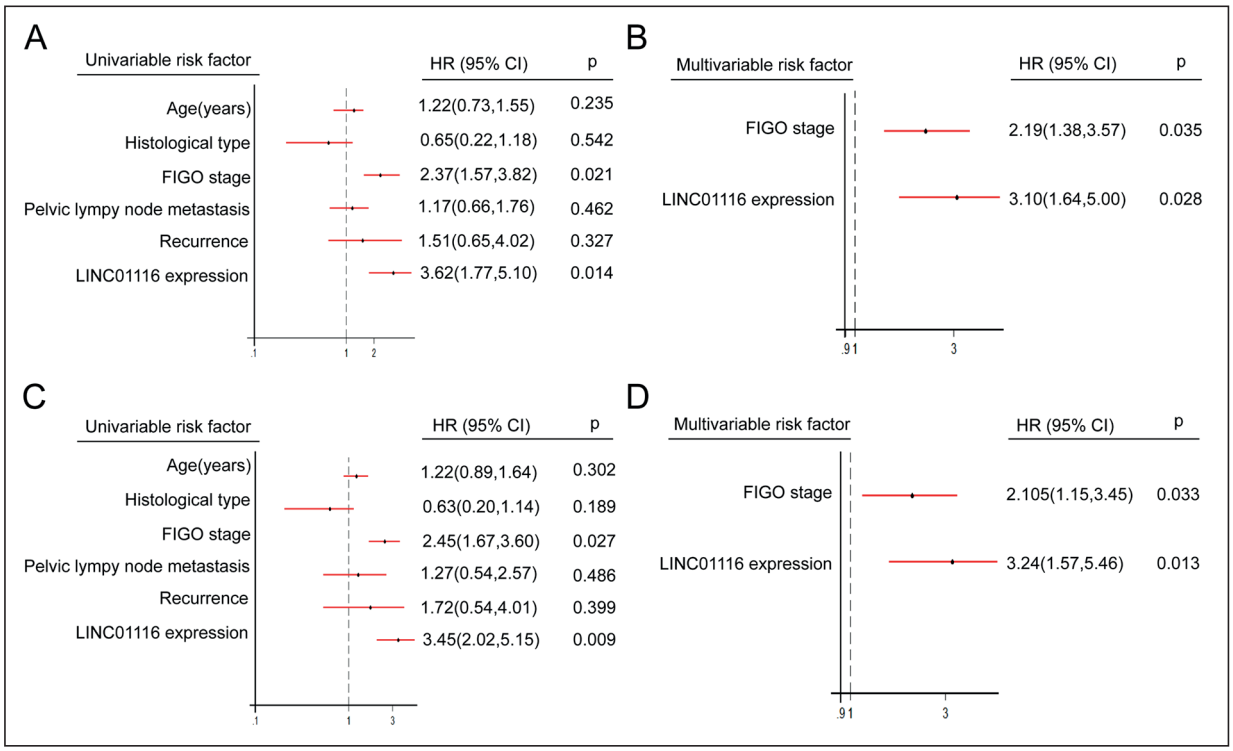

Figure 2. Independent risk factors for epithelial ovarian cancer. ***A, B,*** FIGO clinical stage (***A***) and LINC01116 expression (***B***) were the independent risk factors for DFS of epithelial ovarian cancer patients. ***C, D,*** FIGO clinical stage (***C***) and LINC01116 expression (***D***) were the independent risk factors for OS of epithelial ovarian cancer patients.

Overexpressed LINC01116 Promoted Proliferation and Migration of EOC cells

LINC01116 was overexpressed in EOC cell lines (HO8910, HEY and A2780) compared with that of normal ovarian cell line IOSE-386 ($p < 0.05$, Figure 3A). We selected HO8910 cells for the following *in vitro* experiments. Subsequently, we constructed pc-DNA-LINC01116 and si-LINC01116. The transfection efficacy of corresponding plasmids was verified by qRT-PCR (Figure 3B and 3C). LINC01116 knockdown led to decreased viability and migration of EOC cells (Figure 3D and 3E). LINC01116 overexpression in HO8910 cells obtained the opposite findings (Figure 3F and 3G).

Overexpressed LINC01116 Inhibited Apoptosis of EOC Cells

Flow cytometric results demonstrated that LINC01116 knockdown remarkably stimulated apoptosis of HO8910 cells ($p < 0.05$, Figure 4A). The transfection of pc-DNA-LINC01116 in HO8910 cells obtained the opposite result (Figure 4B). Protein expressions of apoptosis-related genes in HO8910 cells were detected by Western blot, including Bcl-2, cleaved Caspase-3 and cleaved Caspase-9. Overexpressed LINC01116 upregulated Bcl-2, whereas downregulated cleaved Caspase-3 and Caspase-9 (Figure 4C and 4D). The above data elucidated that LINC01116 promotes EOC progression *via* inhibiting cell apoptosis.

Discussion

EOC is one of the most common genital tumors in women. The incidence rate of EOC ranks the second only to cervical cancer[9-11]. More seriously, insidious onset, high metastatic rate, and lack of effective early diagnosis all trigger the rapid progression of EOC. The 5-year survival rate of EOC is only 20-30%[12,13]. Although many oncogenes, tumor suppressor genes, and tumor-associated signaling pathways have been recently confirmed to be involved in EOC, their pathogenesis remains unclear. LncRNA is a non-coding RNA with more than 200 nt in length[14-16]. With the rapid development of science and technology, lncRNAs

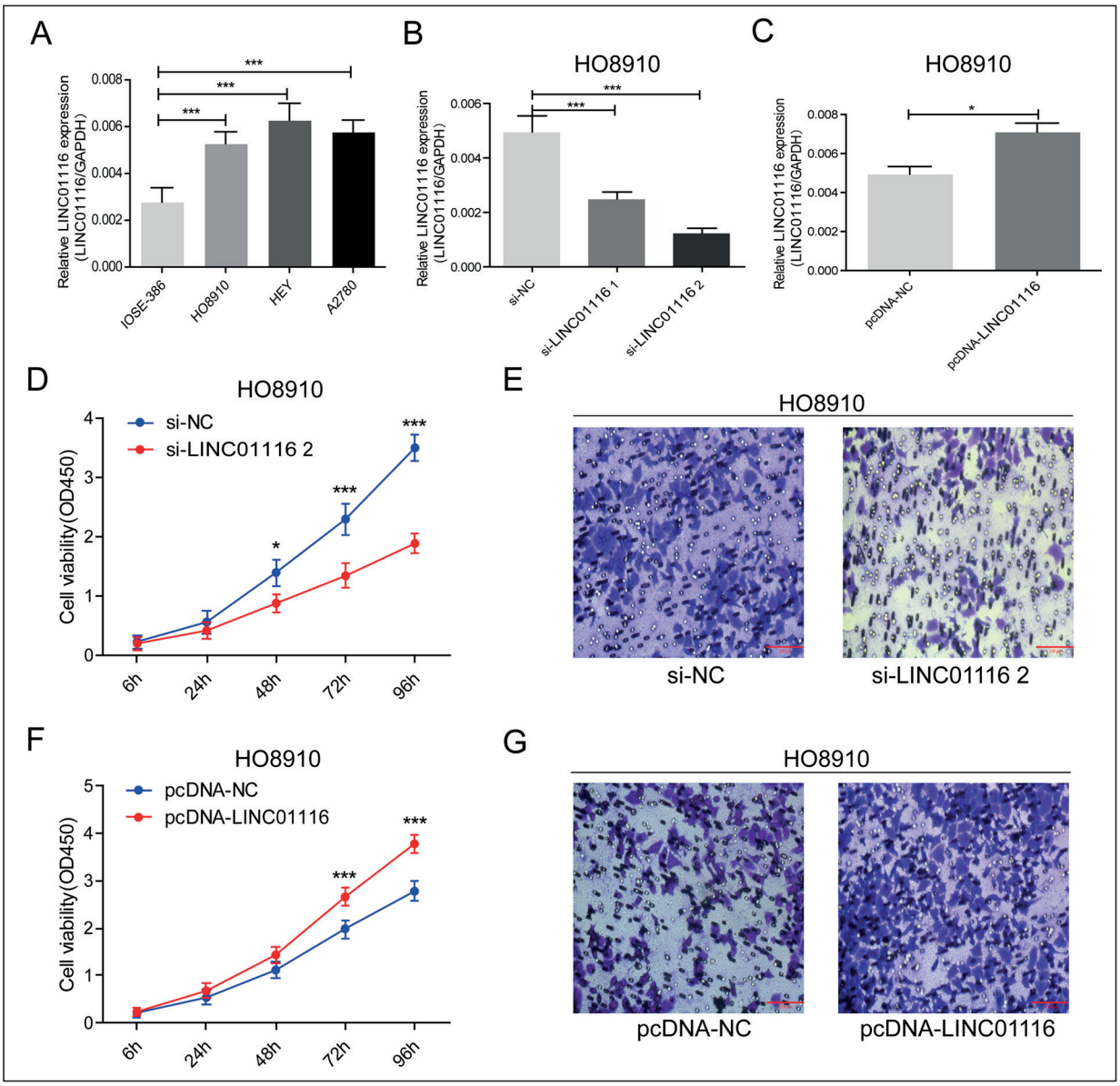

Figure 3. Overexpressed LINC01116 promoted proliferation and migration of epithelial ovarian cancer cells. ***A,*** LINC01116 was overexpressed in epithelial ovarian cancer cell lines (HO8910, HEY and A2780) compared with that of normal ovarian cell line IOSE-386. ***B, C,*** The transfection efficacy of pc-DNA-LINC01116 (***B***) and si-LINC01116 (***C***) was verified by qRT-PCR. ***D, E,*** LINC01116 knockdown led to decreased viability (***D***) and migration (***E***) of epithelial ovarian cancer cells. ***F, G,*** LINC01116 overexpression in HO8910 cells led to increased viability (***F***) and migration (***G***) of epithelial ovarian cancer cells.

have been confirmed to participate in occurrence and progression of many diseases, especially in the complex pathogenesis of tumors[17,18]. It is reported that that some certain lncRNAs are capable of regulating proliferation, apoptosis, invasion, metastasis, and chemotherapy resistance of EOC[19]. For example, overexpressed lncRNA H19 is served as a predictive factor for serous ovarian cancer[20]. Overexpression of lncRNA HOTAIR can promote metastasis of EOC[21]. HOST2, acts as an endogenous competitive inhibitor, promotes proliferation and invasion of ovarian tumor cell *via* targeting let-7b[22]. In the present study, we first found that LINC01116 was overexpressed in EOC tissues and cells. LINC01116 knockdown inhibited proliferation and induced apoptosis of EOC cells. In the present study, DFS and OS in EOC patients with higher expression of LINC01116

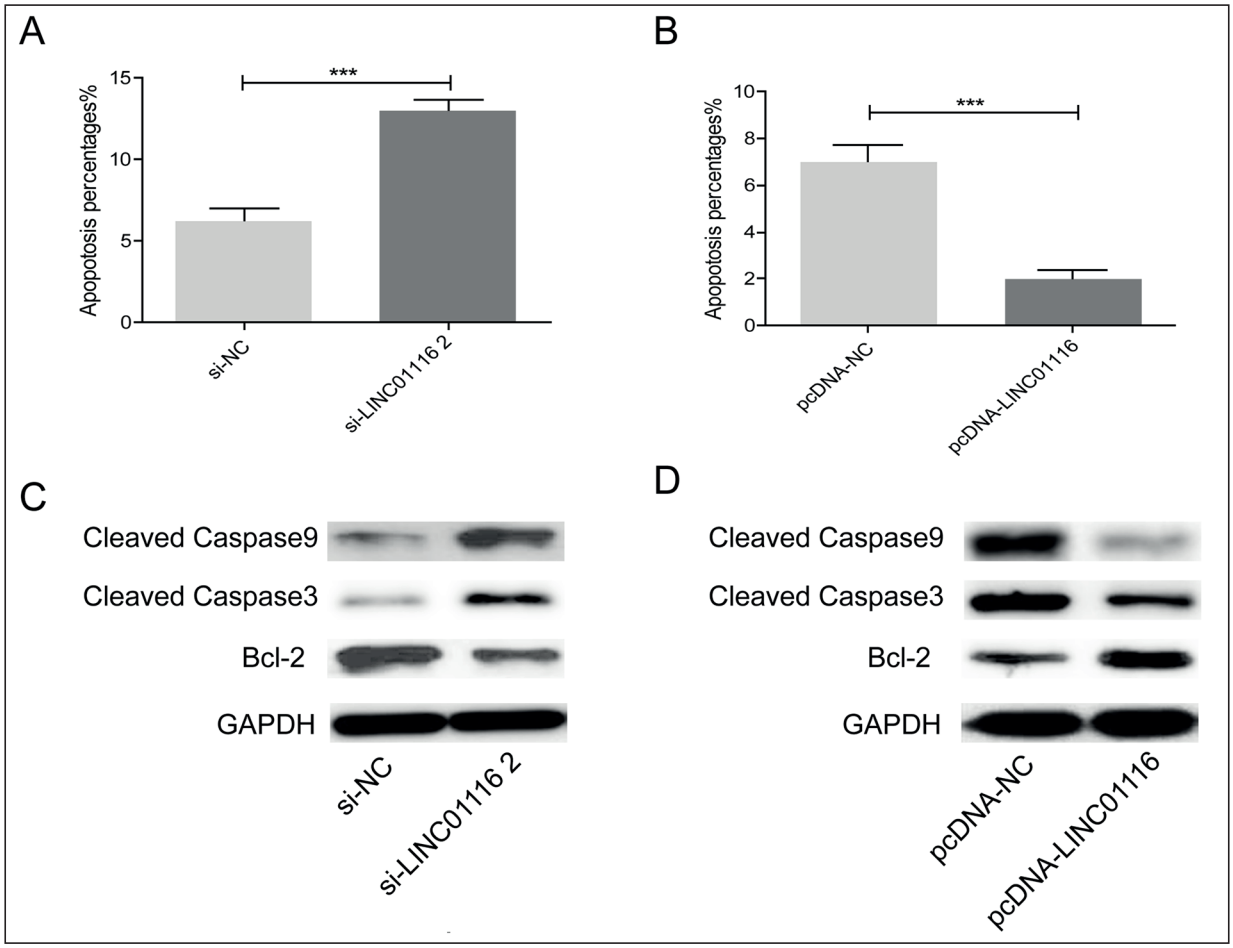

Figure 4. Overexpressed LINC01116 inhibited apoptosis of epithelial ovarian cancer cells. ***A,*** LINC01116 knockdown remarkably stimulated apoptosis of HO8910 cells. ***B,*** LINC01116 overexpression remarkably inhibited apoptosis of HO8910 cells. ***C,*** Overexpressed LINC01116 upregulated Bcl-2, whereas downregulated cleaved Caspase-3 and cleaved Caspase-9 in HO8910 cells. ***D,*** LINC01116 knockdown downregulated Bcl-2, whereas upregulated cleaved Caspase-3 and cleaved Caspase-9 in HO8910 cells.

were remarkably shorter than those with lower expression. FIGO clinical stage and LINC01116 expression were the independent factors that affected DFS and OS of EOC patients, indicating the significant role of LINC01116 in the occurrence, progression and prognosis of EOC. Our *in vitro* results showed that LINC01116 overexpression promotes proliferation and invasion of EOC cells. Overexpressed LINC01116 downregulated protein expressions of cleaved Caspase-3 and Caspase-9, further suggesting that LINC01116 inhibits apoptosis of EOC cells.

To sum up, LINC01116 overexpression promoted the occurrence, progression of EOC. LINC01116 knockdown could partially reverse the disease condition of EOC, which provided a new basis for treating EOC.

Conclusions

We found that overexpressed LINC01116 promotes EOC progression *via* increasing proliferation and migration, and inhibiting cell apoptosis.

Acknowledgements

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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