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Research paper

# Up-regulation of long intergenic noncoding RNA 01296 in ovarian cancer impacts invasion, apoptosis and cell cycle distribution via regulating EMT

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ABSTRACT

*Background:* Recently, long intergenic non-coding RNA 01296 (LINC01296) has been demonstrated to regulate the initiation and progression of several cancers, but the functions of LINC01296 in ovarian cancer still remain unclear. The objective of our study was to determine the expression, biological roles, and clinical significance of LINC01296 in ovarian cancer.

*Methods:* LINC01296 expression was measured in ovarian cancer tissues or cell lines. Next, the relationships between LINC01296 levels and the clinical factors of ovarian cancer, such as progression-free survival and overall survival were analyzed. Additionally, cell proliferation, migration and invasion capacities, apoptosis, cell cycle distribution were investigated after silencing of LINC01296. To confirm whether LINC01296 mediates EMT initiation in ovarian cancer cells, the effect of LINC01296 silence on E-cadherin, N-cadherin and vimentin was assessed in SKOV3 and OVCAR3 cells.

*Results:* We found that LINC01296 was over-expressed in ovarian cancer tissues and cell lines, when comparing with adjacent normal tissue samples and normal cells. Higher LINC01296 expression was significantly correlated with shorter progression-free survival and overall survival. For the functional experiments, knockdown of LINC01296 suppressed cell proliferation, inhibited colony formation ability, abrogated cell migration and invasion potential, and enhanced cell apoptosis. Cell cycle analysis suggested that LINC01296 positively regulated cell cycle progression in ovarian cancer cells. Moreover, western blotting analysis displayed that knockdown of LINC01296 significantly increased E-cadherin, but reduced N-cadherin and vimentin expressions in SKOV3 and OVCAR3 cells, compared with no-transfection cells.

*Conclusions:* LINC01296 plays an important role in promoting the progression of ovarian cancer. Over-expression of LINC01296 might function as an indicator for diagnosis and prognosis of ovarian cancer patients.

## 1. Background

Ovarian cancer is a main type of gynecological malignant tumors, which has high mortality rate, high incidence, high invasiveness as well as metastatic features [1,2]. Because of the advanced stages at diagnosis and chemotherapy-resistance, the prognosis is very bad with the 5-year survival rate of only 30% [3–6]. Moreover, insufficiency exploration of the mechanisms of the progression of ovarian cancer remains a challenge in improving clinical outcomes [7]. Lacking effective biomarkers for early diagnosis of this cancer is the major reason of the low survival rate. Therefore, revealing the biomarkers of ovarian cancer progression is important for improving the survival of ovarian cancer patients.

Recent investigations have implicated that long noncoding RNAs (lncRNAs), a kind of noncoding RNA of > 200 nucleotides in length, functionally act as tumor suppressors or oncogenes, and participate in multiple biological processed in cancer, including cell proliferation, migration and metastasis [8]. Several lncRNAs have been demonstrated to be potential signatures for diagnosis and prognosis prediction in many cancers [9,10]. Recently, lncRNA GHET1 knockdown has been reported to inhibit cell proliferation of gastric cancer [11]. Additionally, lncRNA ABHD11-AS1 has been suggested to promote cell proliferation, invasion and metastasis, and suppress cell apoptosis in ovarian cancer [12]. Moreover, down-regulation of lncRNA GAS5 has been demonstrated to be connected with poor overall survival (OS) rate, and the over-expression of GAS5 suppresses ovarian cancer growth via mediating the expression of apoptosis protease activating factor 1,

*Abbreviations:* LINC01296, long intergenic non-coding RNA 01296; lncRNAs, long non-coding RNAs; OS, overall survival; GEO, Gene Expression Omnibus; PFS, progression-free survival; HRP, horseradish peroxidase; SD, standard deviation; siRNAs, small interfering RNAs

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p21, and cyclin D1 [13]. Thus, identification of critical lncRNAs has resulted in the discovery of novel biomarkers that are of value in the early diagnosis of cancer and treatment of ovarian cancer.

One newly identified lncRNA that has attracted more attention is LINC01296, located at chromosome 14q11.2. LINC01296 has been demonstrated to be differentially expressed in many cancers. For instance, LINC01296 expression has been reported to be over-expressed in gastric cancer [14], esophageal squamous cell carcinoma tissues [15], prostate cancer [16], and cholangiocarcinoma [17]. On the contrary, this lncRNA was found to act as a tumor suppressor in colorectal cancer [18,19]. Furthermore, over-expressed LINC01296 has been demonstrated to be a predictor of poor prognosis for prostate cancer patients and facilitates cell proliferation, and invasion [16]. LINC01296 has also been reported to be connected with unfavorable prognosis in esophageal squamous cell carcinoma tissues and aggravates cell growth, migration as well as invasion [20]. However, to date, no study has reported the roles as well as clinical significance of LINC001296 in ovarian cancer.

Given its important roles in the pathogenesis of the cancers described above, we planned to determine the expression level, roles, as well as clinical significance of LINC01296 in ovarian cancer. In the current study, we observed that LINC01296 was up-regulated in ovarian cancer tissues and cells. Moreover, silence of LINC01296 decreased cell proliferation, inhibited migration/invasion, facilitated apoptosis, and influenced cell cycle distribution. Hence, LINC01296 might be a novel biomarker involved in ovarian cancer development and progression, and might act as a potential prognostic signature and treatment target.

## 2. Materials and methods

### 2.1. Ovarian cancer data from GEO database and survival analysis

Gene expression profiles and clinical data of patients with ovarian cancer were downloaded from Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/). Two independent datasets were obtained in our study, under the accessing number of GSE14407, and GSE54388. GSE14407 consisted of 12 laser capture microdissected serous ovarian cancer epithelia cases and 12 healthy ovarian surface epithelia samples. GSE54388 included 16 serous ovarian cancer samples and 6 healthy normal samples.

A total of 450 cases were enrolled to perform the progression-free survival (PFS) and OS analysis, including 285 patients from GSE9891, 107 patients from GSE26193, and 58 patients from GSE30161. Kaplan-Meier was employed to construct curves according to lncRNA expression status, and log-rank test was used to compare the survival differences.

### 2.2. Cell culture

Four ovarian cancer cell lines (SKOV3, Caov-3, HO-8910, and OVCAR3) were obtained from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China) and ATCC. and a human ovarian surface epithelial cell line (IOSE80) were purchased from Shanghai Sixin Biotechnology Co. Ltd. Cells were incubated in RPMI 1640 medium with 10% fetal bovine serum, 100 U/ml penicillin, and 0.1 mg/ml streptomycin at 37 °C with 5% $CO_2$.

### 2.3. Cell transfection

Small interfering RNAs (siRNAs) targeting LINC01296 (si-LINC01296) and one negative control (si-control) without definite target were synthesized by Shanghai GenePharma Co. Ltd. (Shanghai, China). Lipofectamine 2000 (Invitrogen, Grand Island, NY, USA) was utilized to carry out the transfection based on the manufacturer's protocols. Quantitative real-time polymerase chain reaction (qRT-PCR) and western blotting were used to determine the transfection efficiency 24 h after transfection, and silencing efficacy of > 90% was selected for further experiments.

Sequences of siRNA for LINC01296 were:

5′-CUGAAACAUAUUCCGUGGUTT-3′ (sense) for siRNA LINC01296 1# and 5′-GGCUGGAGAAUAUUUC CUATTTT-3′ (sense) for siRNA LINC01296 2#.

The sequence for negative control was: 5′-AATTCTCCGAACGTGTC ACGT-3′ (sence).

### 2.4. RNA extraction and qRT-PCR

TRIzol (Life Technologies, Carlsbad, CA, USA) was applied to extract total RNA of ovarian cancer cell lines. Then, reverse transcription reaction system was employed to synthesize cDNA. Subsequently, RT-PCR was carried out using SYBR Green supermix.

at ABI 7500 detection system. GAPDH was an internal reference.

We listed the primers below: LINC01296:

F:5′- AAGTGGCACCAGCCTCACT -3′,

R: 5′- CGGCCAAGT TCTTTACCATC -3′,

GAPDH: F: 5'-GGAGCGA GATCCCTCCAAAAT -3′.

R: 5'–GGCTGTTGTCATACTTCTCATGG-3'.

Relative expression level of LINC01296 was calculated relying on the $2^{-\Delta\Delta Ct}$ method.

### 2.5. Western blotting analysis

Total protein was extracted with RIPA buffer based on the protocols. The extracted protein concentration was measured using a BCA protein assay kit (Thermo, Waltham, MA, USA). Proteins were electrophoretically separated using SDS-PAGE gels and.

transferred to PVDF membranes. The membranes were blocked with 5% non-fat milk for 1 h at room temperature and then incubated with primary antibodies at 4 °C overnight. Primary antibodies used in our study were included as follows:

anti-Cyclin A (1:1000, Santa Cruz), anti-*E*-cad (1:1000, Santa Cruz), anti-N-cad (1:1000, Santa Cruz), anti-Vimentin (1:1000, Santa Cruz), anti-Bcl2, anti-Bax, anti-Caspase-3, anti-GAPDH (1:1000, Santa Cruz). After three washes using TBST, horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG was used to incubate the blots for 2 h at room temperature. Finally, the bands were visualized by enhanced chemiluminescence.

### 2.6. CCK-8 and colony formation experiment

Cell proliferation was evaluated by CCK-8 and colony formation experiment. For the CCK-8 experiment, ovarian cancer cells were plated into 96-well plates. Next, 10 μl of CCK-8 was added to each well at the predefined time-points and incubated for 2 h, and then the density was recorded at 450 nm by means of the microplate reader. With regard to colony formation assay, cells were seeded in 6-well plates. After 2 weeks incubation, the colonies were fixed using 4% paraformaldehyde for 30 min and stained using 0.1% crystal violet for 30 min. The experiments were repeatedly carried out in triplicate.

### 2.7. Wound-healing analysis

Cells were plated in 6-well plate. The monolayer was scratched after a monolayer was produced. Then, the wound images were photographed at 0 and 24 h. The percentage of wound was determined according to the wound width at 0 h [21].

### 2.8. Cell migration and invasion assay

Transwells were used to assess the migration and invasion capacity of ovarian cancer cells. Inserts were either non-coated for migration or

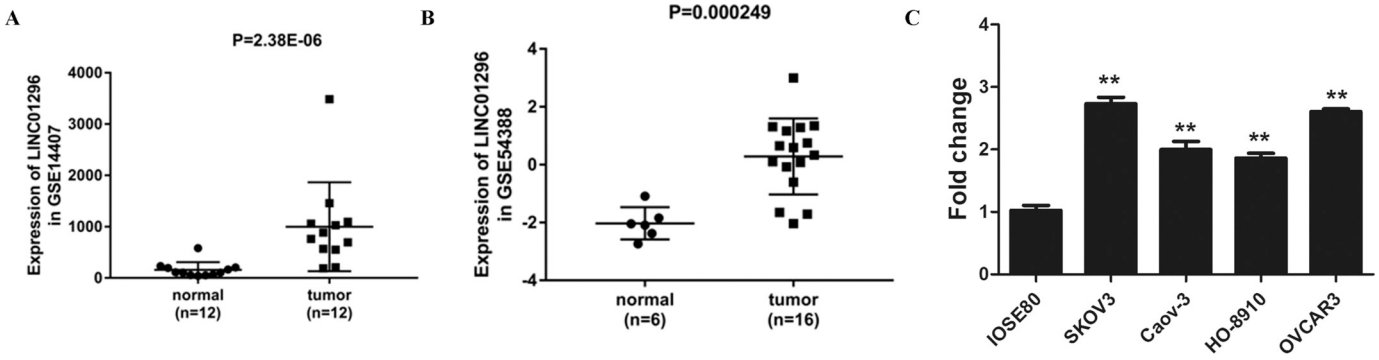


**Fig. 1.** Expression of LINC01296 in ovarian cancer. A and B. Box plots exhibited elevated levels of LINC01296 in ovarian cancer compared to normal samples obtained from two microarray datasets ($P$ = 2.38E-06 for GSE14407 and $P$ = .000249 for GSE54388). C. qRT-PCR analyzed the expression of LINC01296 in a human ovarian surface epithelial cell line (IOSE80) and four ovarian cancer cells (SKOV3, Caov-3, HO-8910, and OVCAR3). $^{**}P < .01$ compared to the human ovarian surface epithelial cell line IOSE80 which was used as normal control.

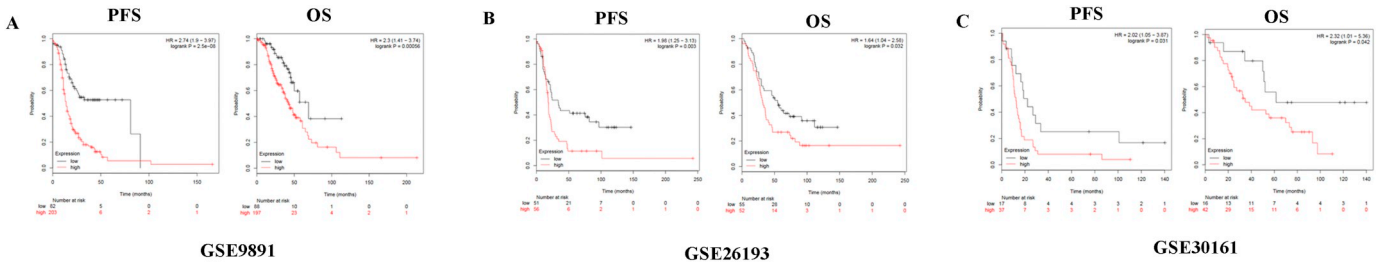


**Fig. 2.** Kaplan-Meier survival curves for PFS and OS difference of ovarian cancer patients from three microarray datasets (A. for GSE9891, B. for GSE26193, and C. for GSE30161) based on the expression level of LINC01296.

covered with Matrigel for invasion experiment. For each assay, cells transfected with siRNA LINC01296 or siRNA NC were putted to the upper chamber of each insert in serum-free medium, and serum medium was used in the lower chamber as the chemoattractant. After 24 h of incubation at 37 °C, the invaded cells were fixed using 4% paraformaldehyde for 30 min, stained with 0.1% crystal violet for 20 min, and then counted. Each experiment was implemented in triplicate.

### 2.9. Flow cytometric analysis of apoptosis assay

Apoptosis was measured through flow cytometric analyses using Annexin V/FITC Apoptosis Detection Kits (BD Biosciences, USA). In detail, cells infected with siLINC01296 and siRNA NC were putted into the 6-well plates, respectively, and cultured for 48 h. Subsequently, PBS was used to wash and resuspend the cells. Afterwards, cells were stained using Annexin V-FITC and PI in the dark at room temperature for 20 min. The cell populations were examined with a flow cytometer.

### 2.10. Cell cycle analysis

Cell cycle distribution was investigated by staining ethanol-fixed cells with PI through flow cytometry described previously [22]. After the cells were seeded in fresh medium containing 10% FBS to 90% confluency, these cells were harvested in 5 ml PBS. Next, a volume of 2 ml 70% cold ethanol was added for immobilization overnight at 4 °C. Then, the cells were incubated in the dark for 20 min after stained with Annexin V-FITC and PI. The ratio of cells in different phases was determined via flow cytometry, and analyzed with the ModFit LT software (Verity Software House Inc., USA).

### 2.11. Statistical analysis

Results were analyzed based on SPSS 22.0 (SPSS Inc., Chicago, IL, USA). All data were shown as means ± standard deviation (SD). Student's *t*-test as well as one-way ANOVA were applied to determine the significance of differences between two/multiple groups. A $P$ value < .05 was regarded to be statistically significant.

## 3. Results

### 3.1. LINC01296 is up-regulated in ovarian cancer tissues and cell lines

After analyzing the published expression profiles of ovarian cancer and normal tissues collected from the two datasets GSE14407, and GSE54388, it was observed that LINC01296 was significantly over-expressed in ovarian cancer compared with the corresponding normal tissue (Fig. 1A and B). Consistently, qRT-PCR analysis exhibited that LINC01296 was remarkably up-regulated in four ovarian cancer cell lines (SKOV3, Caov-3, HO-8910 and OVCAR3) compared with normal cells IOSE80 (Fig. 1C). The results suggested that LINC01296 functioned as an oncogene in the progression of ovarian cancer. Significantly, among the four ovarian cancer cell lines, SKOV3 and OVCAR3 expressed relatively higher LINC01296 level and therefore, these two cell lines were selected for further study.

*Over-expression of LINC01296 is associated with poor prognosis in ovarian cancer.*

Subsequently, we used the Kaplan-Meier and log-rank test to investigate the relationship between LINC01296 expression level and patients'PFS and.

OS. As displayed in Fig. 2, those patients with lower LINC01296 had longer PFS and OS according to the GEO datasets (A. for GSE9891, B. for GSE26193, and C. for GSE30161).

### 3.2. Knockdown of LINC01296 inhibits cell proliferation and colony formation in SKOV3 and OVCAR3 cells

The biological functions of LINC01296 in ovarian cancer remain poor. Hence, we utilized loss-of-function model to investigate the roles of LINC01296 in SKOV3.

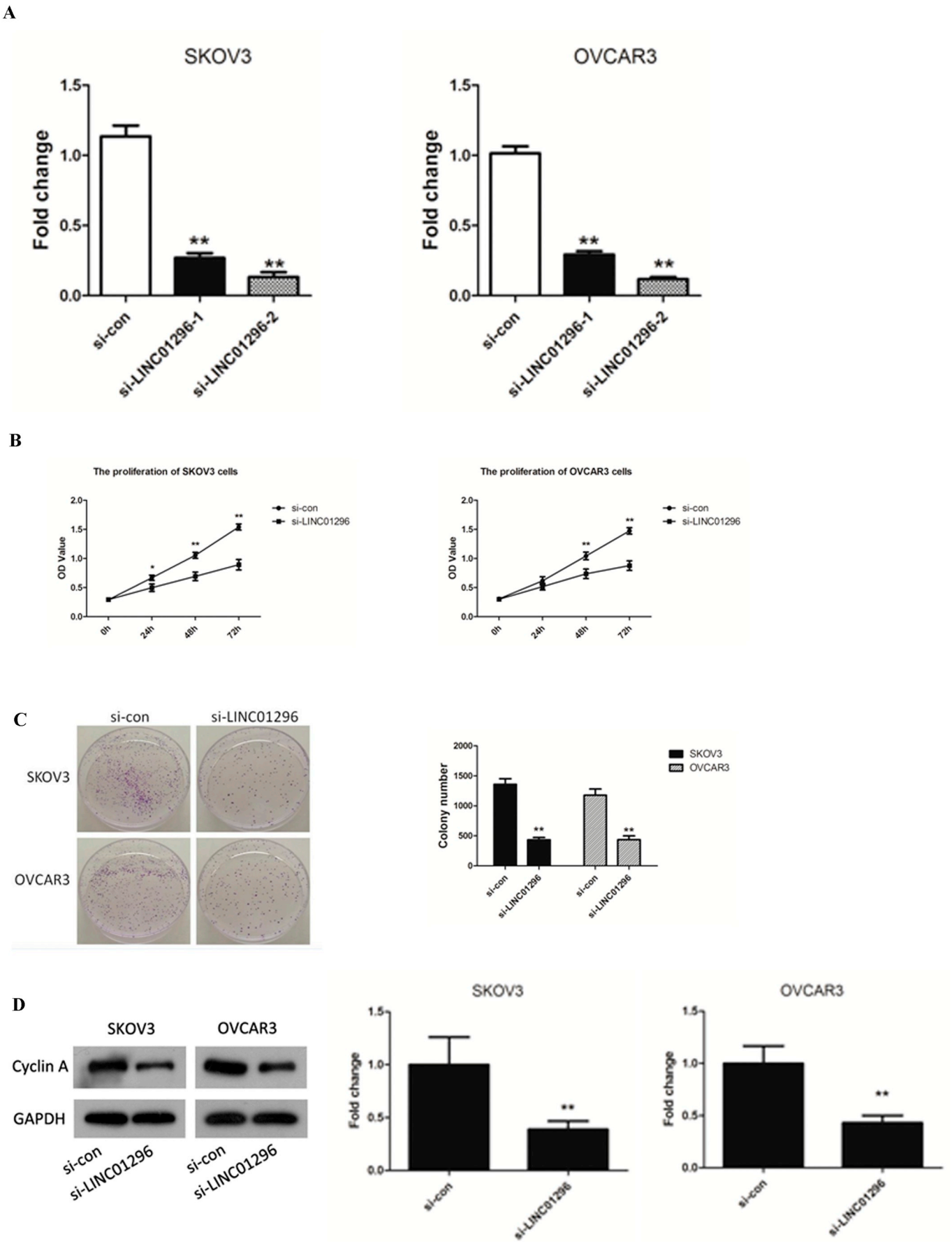


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**Fig. 3.** LINC01296 inhibited ells proliferation. (A) LINC01296 expression in ovarian cancer cells (SKOV3 and OVCAR3) transfected with two interfering oligonucleotides. (B) CCK-8 assay exhibited the proliferation capacity of ovarian cancer cells was inhibited after LINC01296 transfection. (C) Colony formation assay displayed that the clone formation number was reduced after LINC01296 silence. (D) Western blotting analysis revealed that the expression level of Cyclin A was reduced after LINC01296 knockdown. Data are presented as the mean ± SD. *$P < .05$, **P < .01 compared to si-control group.

and OVCAR3 cells. Firstly, the LINC01296 expression level was measured in a loss-of-function model (Fig. 3A). After SKOV3 and OVCAR3 cells were transfected using two interfering oligonucleotides, we observed that LINC01296 was remarkably decreased compared with negative control group (Fig. 3A). Significantly, we found that the transfection efficiency of siRNA LINC01296 2# was higher than siRNA LINC01296 1#. Thus, in the subsequent assays, siRNA LINC01296 2# was used in our study. CCK-8 assay exhibited that LINC01296 knockdown inhibited the proliferation ability of ovarian cancer cells (Fig. 3B). Colony formation assay revealed that clone number was reduced in the LINC01296 transfection group (Fig. 3C). As shown in Fig. 3D, LINC01296 knockdown inhibited the expression level of Cyclin A based on the western blotting analysis. In a nutshell, these findings implicated that LINC01296 knockdown inhibited ovarian cancer cells proliferation.

### 3.3. Knockdown of LINC01296 suppresses migration and invasion properties, facilitates the apoptosis in SKOV3 and OVCAR3 cells

To explore the roles of LINC01296, several functional experiments were carried out. Firstly, the migration ability of SKOV3 and OVCAR3 cells was investigated based on wound healing assay. The results exhibited that the would closure areas was smaller in the LINC01296 transfection group compared with control group, implicating LINC01296 silence suppressed the migration ability of SKOV3 and OVCAR3 cells (Fig. 4A). After that, transwell assays were conducted and obtained a similar result. LINC01296 knockdown inhibited the invasion of SKOV3 (Fig. 4B) and OVCAR3 (Fig. 4C) cells. These findings above implied that LINC01296 silence could remarkably attenuate the migration and invasion properties of ovarian cancer cells.

Next, we also evaluated whether silence of LINC01296 contributes

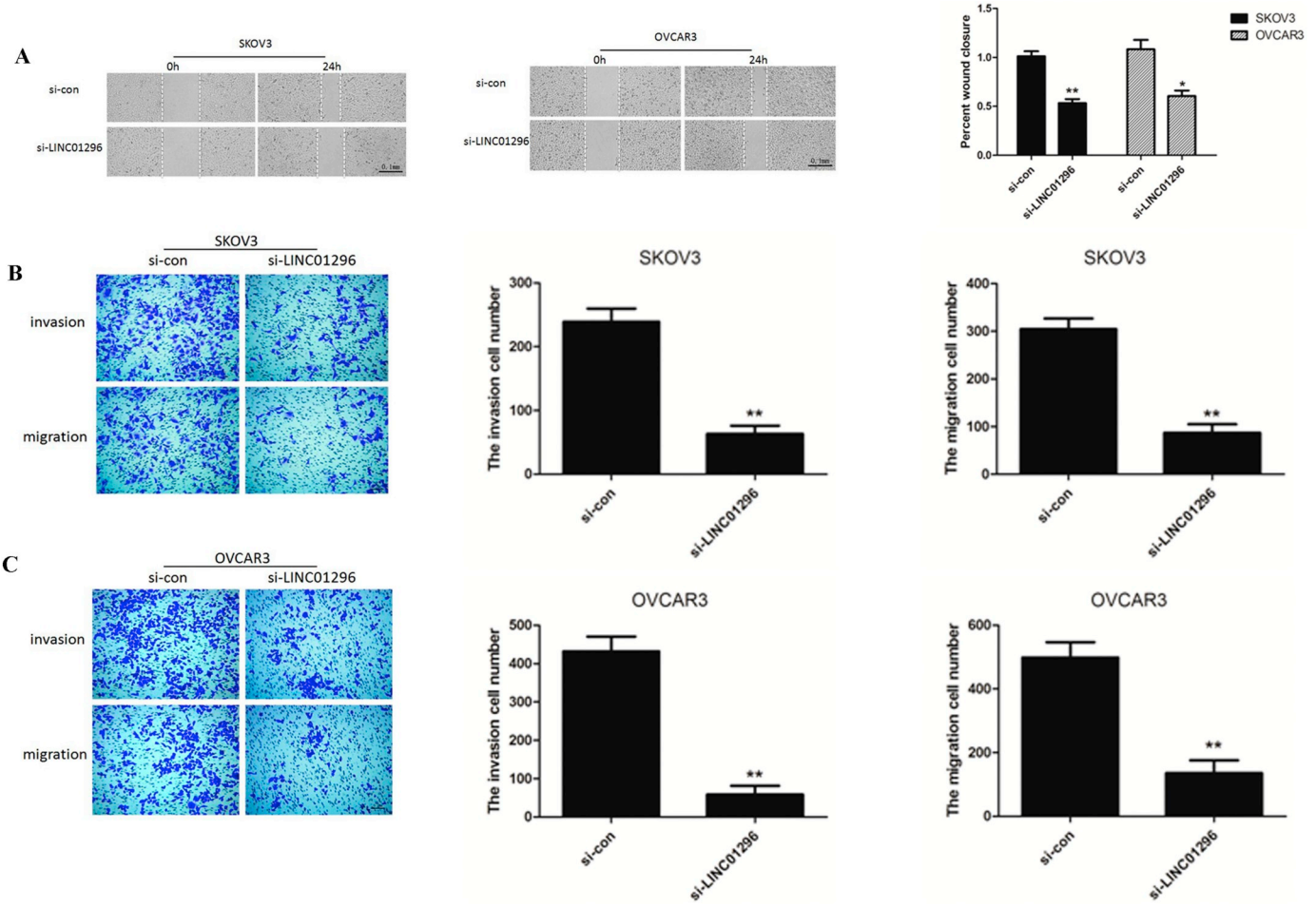


**Fig. 4.** LINC01296 knockdown inhibited ovarian cancer cells migration and invasion. (A) Wound healing assay revealed the migration capacity of ovarian cancer cells transfected with si-LINC01296 or si-NC. (B and C) Transwell assay showed the invasion of ovarian cancer cells transfected with si-LINC01296 or si-NC. Bar = 100 μm. **P < .01 compared to si-control group.

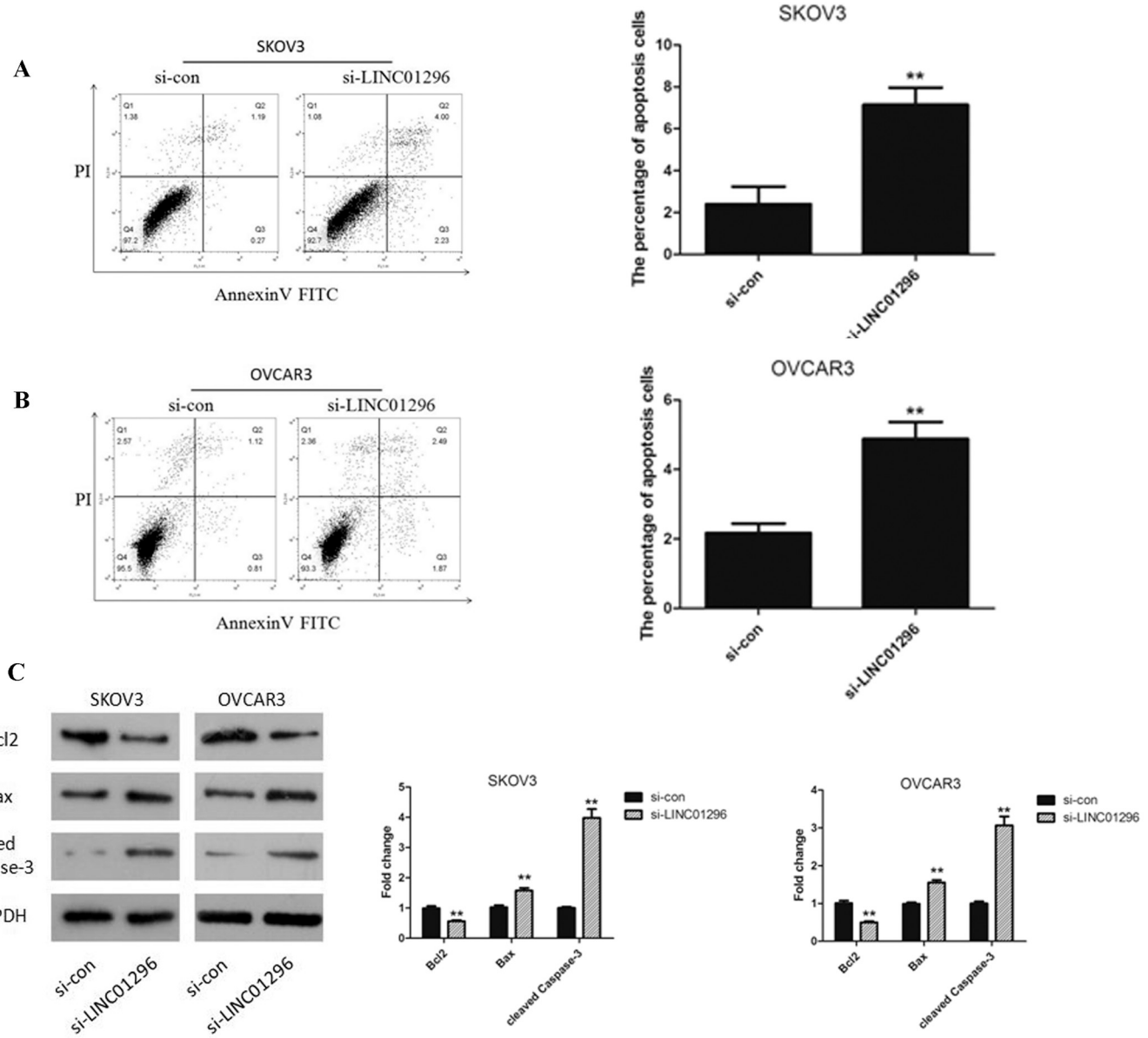


**Fig. 5.** (A and B) Apoptosis cells were analyzed using flow cytometry after cells were stained with Annexin V/PI. The cells were analyzed for early apoptotic cells (bottom right quadrant) and late apoptotic or dead cells (top right quadrant). The percentages of cells in each quadrant are exhibited. (A) LINC01296 knockdown induced SKOV3 cell apoptosis. (B) Knockdown of LINC01296 significantly induced apoptosis in OVCAR3 cells. (C) Western blotting used to measure the expression level of apoptosis-related markers (Bcl-2, Bax, and Cleaved Caspase-3). **P < .01 compared to si-control group.

to cell apoptosis. In detail, cells were stained using Annexin V/PI and analyzed using flow cytometry. We observed that the proportion of apoptotic cells in SKOV3 (Fig. 5A) and OVCAR3 (Fig. 5B) cells after LINC01296 knockdown was significantly increased, relative to that in the si-NC group.

Caspase-3, Bcl-2, and Bax were considered as markers of apoptosis. Thus, we used western blotting to measure the protein expression of Caspase-3, Bcl-2, and Bax. We found that LINC01296 knockdown induced a significant increase of cleaved Caspase-3 and Bax expression, but LINC01296 silence markedly decreased the expression of BCL-2 in these two cancer cell lines SKOV3 and OVCAR3 (Fig. 5C). These results implicate that LINC01296 is negatively connected with the ovarian cancer cell apoptosis.

### 3.4. Cell cycle analysis after knockdown of LINC01296

For OVCAR3, the G1 phase cells were remarkably enhanced (from 24.3% to 42.6%; $p < .001$), whereas the cells in both S phase (from 18.8% to 9.67%; $p < .001$) and G2 phase (from 36% to 29.8%; $p < .05$) were reduced (Fig. 6).

Similar results were found in SKOV3 cells. LINC01296 transfected SKOV3 cells exhibited a significantly increased proportion in the G1 phase (from.

36.4% to 53.5%; $p < .01$), while S phase cells (from 9.06% to 4.95%; $p < .05$) and G2 phase cells (from 40.5% to 28.7%; $p < .001$) were decreased (Fig. 6). This implies that LINC01296 might positively regulate cell cycle progression in ovarian cancer cells.

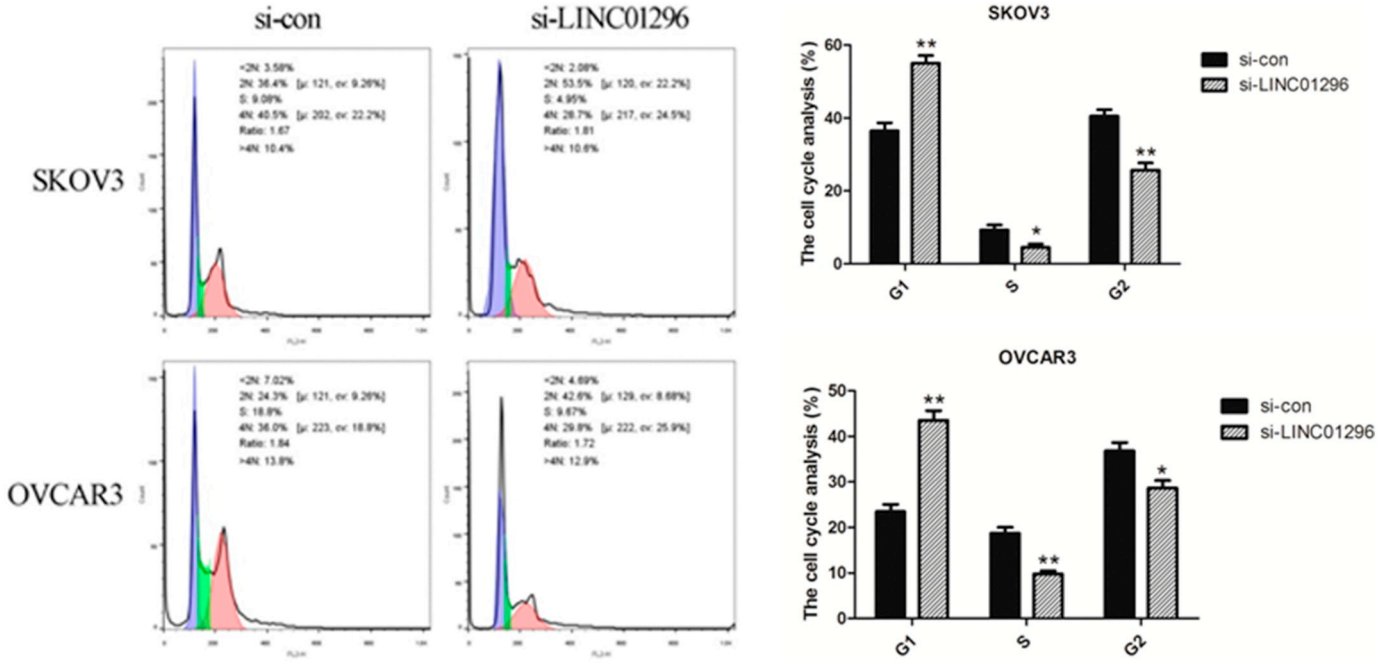


**Fig. 6.** Cell cycle analyses of SKOV3 and OVCAR3 cells, determined by flow cytometry. After LINC01296 transfection, cancer cells contained less cells in S and G2 phases, and more cells in G1-phase in SKOV3 cells, and OVCAR3 cells.*P < .05, **P < .01 compared to si-control group.

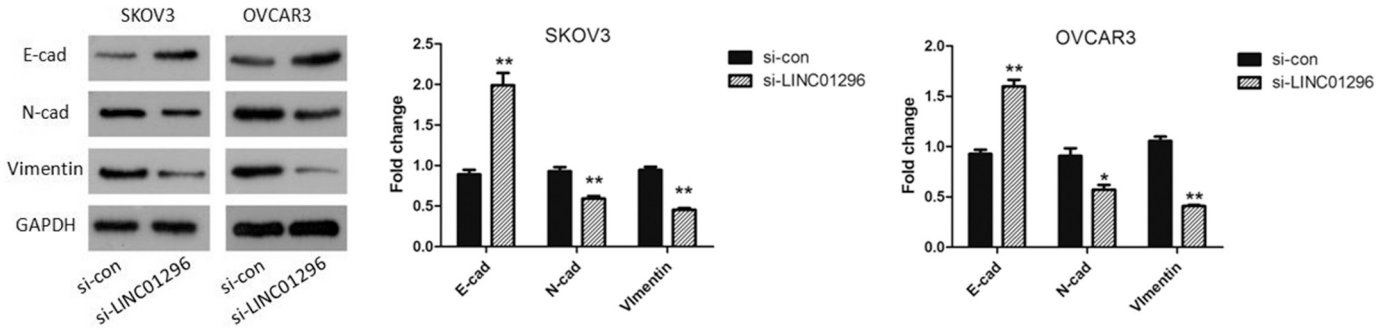


**Fig. 7.** The expressions of E-cadherin, N-cadherin, and Vimentin in ovarian cancer cell lines (SKOV3 and OVCAR3) after LINC01296 transfection analyzed using western blotting. Bars present means ± SD. The proteins were normalized with the expression of GAPDH. * *P < .01, and * P < .05 compared with the respective non-transfected cells.

### *3.5. Effect of LINC01296 knockdown on E-cadherin, N-cadherin, and Vimentin expressions in SKOV3 and OVCAR3 cells*

To confirm whether LINC01296 mediates EMT initiation in ovarian cancer cells, the effect of LINC01296 silence was assessed in SKOV3 and OVCAR3 cells. Western blotting analysis displayed that knockdown of LINC01296 significantly increased E-cadherin, but reduced N-cadherin and vimentin expressions in SKOV3 and OVCAR3 cells, compared with no-transfection cells (Fig. 7). These indicate that LINC01296 may mediate EMT initiation and progression in ovarian cancer cells.

## 4. Discussion

Relapse, metastasis, and chemotherapy resistance are the main challenges.

in the treatment of ovarian cancer clinically [23]. Hence, identification of molecular drivers is pivotal for developing novel therapeutic target in the treatment of ovarian cancer [24]. LINC01296, located at chromosome 14q11.2., has been demonstrated to be up-regulated in many types of cancer and serve as an oncogene, for example, esophageal squamous cell carcinoma tissues [15], gastric cancer [14], and non-small cell lung cancer [25]. However, there is no report about the alteration and prognostic significance of LINC01296 in ovarian cancer. In line with the previous studies, in our study, over-expression of LINC01296 was observed in ovarian cancer samples and was linked to poor prognosis of patients with ovarian cancer. Consistently, the evidence of highly expressed LINC01296 was found in ovarian cancer cells as determined by RT-PCR. These findings demonstrate that LINC01296 might also act as an oncogene in ovarian cancer progression.

Uncontrolled cell proliferation is one of the basis features of cancer. In a former in vitro analysis, LINC01296 silencing has been documented to decrease cell proliferation in bladder cancer cells [26]. In line with our expectation, proliferation rate and colony formation capacity of ovarian cancer cells were remarkably decreased in the siLINC01296 transfected cells in our study. Significantly, imbalance between cell proliferation and apoptosis can result in the development and progression of cancer [27]. Thus, we assessed the cell apoptosis when LINC01296 was silenced, and we found that cell apoptosis ratio is boosted in the cells transfected with LINC01296 based on flow cytometric analysis. Caspase-3 and Bcl-2 family (Bcl-2 and Bax) are apoptosis-related factors [28]. In the current work, a significant increase of Caspase-3 and Bax expression were found after silencing of LINC01296,

whereas Bcl-2 was decreased in the LINC01296 transfected group. Thus, the blockade of ovarian cancer progression was induced by regulating the Bcl-2/Caspase-3, thereby further inhibiting cell proliferation.

Up-regulation of LINC01296 has been reported to facilitate cell migration and invasion [16]. Growing evidence has implied that cell migration is associated with the formation of metastases [29,30]. Metastasis, as a primary biological feature of malignant cancer, is a lethal factor for cancer patients. So far, the molecular mechanisms of lncRNAs involved in tumor migration and metastasis have not been fully defined, a challenge was raised as to whether LINC01296 increases ovarian cancer cell metastasis via mediating gene expression that codes metastasis-related proteins. EMT is believed as one of the possible mechanisms, which is characterized by decreased cell-cell junction and promoted cell motility. These features of EMT have been demonstrated to change invasion properties, induce cell migration, and inhibit apoptosis [31]. Low level of E-cadherin expression is a crucial initiating event in EMT in cancer development and progression, and repressors that down-regulate E-cadherin have been considered as the inducers of EMT [31]. Down-regulation of E-cadherin is connected with a poor prognosis for many cancers [32]. Cells with high expression of vimentin and N-cadherin exhibited migratory and invasive properties [33]. In our study, low-expression of LINC01296 inhibited migration and invasion of ovarian cancer cells in vitro, and further suggested that up-regulation of LINC01296 was connected to metastasis in ovarian cancer. Moreover, we found that gene associated with EMT E-cadherin was significantly over-expressed and the other EMT genes N-cadherin and vimentin were low-expressed in SKOV3 and OVCAR3 cells after LINC01296 transfection, demonstrating LINC01296-mediated regulation of EMT mechanistically. Similarly, Yin et al. [34] have reported that knockdown of lncRNA HOCA11 is related to the expression of genes associated with EMT (MMP-9, Twist, E-cadherin, B-catenin, Snail, and vimentin) in ovarian cancer. Moreover, lncRNA MALAT1 has been implicated to function as an inducer of EMT in breast cancer [35]. Herein, we infer that differential expression of EMT-related genes might participate in LINC01296-related cell migration and invasion of ovarian cancer.

## 5. Conclusion

In summary, our study first implicated that the LINC01296 expression level was increased in ovarian cancer tissue samples and cells, and this up-regulation was closely associated with unfavorable prognosis of ovarian cancer patients. Moreover, knockdown of LINC01296 abrogated cell growth, and inhibited migration and invasion properties in SKOV3 and OVCAR3 cells. Significantly, cell apoptotic proportion was increased following knockdown of LINC01296 partly through Bcl-2/caspase-3 pathway. These imply that LINC01296 might be a potential anti-cancer target and a prognostic signature for ovarian cancer. However, in vitro experiments were conducted using only stable cell lines in our study. We did not repeat the experiments using primary ovarian cancer cells, at least obtained from HGSOCs, because it is difficulty to collect ennough ovarian cancer samples in a short time, and the primary ovarian cancer cells in the market are not available for purchase. Thus, in the future, we will try our best to collect clinical samples and explore the stable condition of separation of primary ovarian cancer cells for the later experiment to further investigate more complicated regulation mechanisms of LINC01296 in ovarian cancer.

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Availability of data and materials

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

## Competing interests

The authors declare that they have no competing interests.

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## Author contributions

Conceptualization, H.X. and P.S.L; Formal Analysis, J.F.Z. and C.Z.H; Data Curation, Y. L. and C.Z.H.; Writing – Original Draft Preparation, H.X. and P.S.L.; Writing – Review & Editing, P.S.L.

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Not applicable.

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