Original Article



# H3K27ac-activated lncRNA KTN1-AS1 aggravates tumor progression by miR-505-3p/ZNF326 axis in ovarian cancer

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**Background:** Numerous studies indicate that long noncoding RNA (lncRNA) is aberrantly expressed in ovarian cancer (OC). Our research investigated the regulatory role of lncRNA KTN1 antisense RNA1 (KTN1-AS1) in the progression of OC through the miR-505-3p/ZNF326 axis.

**Methods:** Expression of *KTN1-AS1*, microRNA-505-3p (*miR-505-3p*), and zinc-finger protein-326 (*ZNF326*) in OC was evaluated by using RT-qPCR analysis. The biological function of *KTN1-AS1* was inspected using the loss-of-function assay. Luciferase reporter assay and RIP assay were performed to determine the competitive endogenous RNA (ceRNA) network of *KTN1-AS1/miR-505-3p/ZNF326*.

**Results:** The data showed that *KTN1-AS1* and *ZNF326* had a high expression in OC than in the normal tissue, and *miR-505-3p* exhibited a low expression in OC than in the normal tissue. The knockdown of *KTN1-AS1* caused an inhibition in OC cell proliferation, migration, and invasion, and promoted cell apoptosis. In terms of mechanical exploration, *KTN1-AS1* was transcriptionally activated by histone H3 on lysine 27 acetylation (H3K27ac) at the promoter region, and *KTN1-AS1* increased *ZNF326* expression by competitively adsorbing *miR-505-3p*.

**Conclusions:** This study indicated that H3K27ac-induced lncRNA *KTN1-AS1* expression, and facilitated proliferation, migration, and invasion of OC cells by the *KTN1-AS1/miR-505-3p/ZNF326* axis.

**Keywords:** H3K27ac; *KTN1-AS1*; *miR-505-3p*; *ZNF326*; ovarian cancer (OC)

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## Introduction

Ovarian cancer (OC) is a common gynecologic cancer, and its occurrence rate is the seventh highest of all malignancies in females (1). Importantly, OC is also the fifth leading reason for tumor-relevant deaths in females (2). Reformative techniques in diagnosis and therapy have decreased the incidence and mortality rate of patients with OC. Nevertheless, the rate of 5-year survival rates remains unsatisfying in advanced OC patients because of extensive metastases, drug resistance, and relapse of OC (3). Therefore, it is essential to perform further researches for the regulatory mechanism of OC progression and development.

Long noncoding RNA (lncRNA) is a cluster of transcripts

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(≥200 nucleotides) with little or without capacity in coding proteins (4). Previously, these transcripts were considered as transcriptional *noise*; however, emerging research found that some of these transcripts exhibited essential regulatory properties in various physiological or pathological processes (5,6). Based on some studies, lncRNAs were widely reported to modulate genomic imprinting (7), X chromosome inactivation (8), stem cell differentiation (9), and cancer metastasis (10-12). The modulatory roles of lncRNAs have been reported in colon cancer (13), gastric cancer (14), and cervical cancer (15). Recent research also found that a series of lncRNAs with an abnormal expression exerted important functions in OC, such as *PTAR* (16), *PVT1* (17), *MALAT1* (18), and *HOST2* (19). Nonetheless, effects of most lncRNAs remain largely unclear in OC, and there is need to study these effects further.

KTN1 antisense RNA 1 (*KTN1-AS1*) is a newly recognized tumor-associated lncRNA that is known to play a regulatory role in diverse cell types. In hepatocellular carcinoma (HCC), *KTN1-AS1* sponges *miR-23c* to affect *ERBB2IP* expression, thereby promoting the growth of HCC (20). *KTN1-AS1* also expresses at a high level and is associated with unsatisfying clinical outcomes in lung cancer patients (21). In addition, *KTN1-AS1* has shown an upregulated expression level and facilitates the migration and invasion of bladder cancer cells (22). However, the expression and biological function of *KTN1-AS1* were not inspected in relation to OC.

H3K27ac is associated with gene activation and is mainly concentrated in the enhancer and promoter regions. When the promoter or enhancer region is modified with H3K27ac, the promoter or enhancer will be activated to promote gene expression.

In our study, we explored the function and molecular mechanism of *KTN1-AS1* in OC cells and found the *KTN1-AS1/miR-505-3p/ZNF326* axis. This finding offers a promising insight into the treatment of OC. We present the following article in accordance with the MDAR reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-443/rc).

## Methods

### *Clinical specimens*

Sixty-four OC and ten normal ovarian tissues were collected from patients at Guangzhou Women and Children's Medical Center, Guangzhou Medical University. An informed consent form was signed by each patient, and none of the patients accepted anti-cancer therapy before surgery. All collected tissues were preserved at –80 ℃ in liquid nitrogen. The study was approved by the Ethics Committee of Guangzhou Women and Children's Medical Center, Guangzhou Medical University (approval No. 2020-KY-021-03). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

### *Cell lines and reagent*

IOSE-80 (the human ovarian cell line) and A2780, OVCAR-3, SKOV3 and TOV112D (OC cell lines) were provided by ATCC (Manassas, VA, USA). Dulbecco's modified Eagle's medium (DMEM; HyClone, Logan, UT, USA), which contains 10% fetal bovine serum (FBS; HyClone, Logan, UT, USA), was utilized to culture these cell lines at 37 ℃ with 5% $CO_2$. The acetylation inhibitor (C646) was commercially obtained from Sigma Chemical (St. Louis, MO, USA).

### *Cell transfection*

The short hairpin RNA (shRNA) against *KTN1-AS1 (sh-KTN1-AS1#1/2)* or *CBP (sh-CBP#1/2)*, *miR-505-3p* mimics, and their relative reference (sh-NC and NC mimics) were synthesized by GenePharma (Shanghai, China). To overexpress *ZNF326*, *pcDNA3.1/ZNF326 (*GenePharma, Shanghai, China) was synthesized, and the empty *pcDNA3.1* vector was taken as an internal reference. Cell transfection was performed through Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Forty-eight hours later, transfection efficiency was proved by RT-qPCR.

### *RT-qPCR*

TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was used to isolate the total RNA from OC tissues and cells. Then, PrimeScript RT reagent Kit (Takara, Tokyo, Japan) was employed to reverse-transcribe the isolated RNA into complementary DNA (cDNA). On an ABI7300 real-time PCR machine (Applied Biosystems, Carlsbad, CA, USA), SYBR Green PCR Master Mix (Roche, Basel, Switzerland) was used for RT-qPCR to determine relative RNA expression. The appointed genes were normalized to Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*)

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**Table 1** Sequences of the primers and target shRNA

| Name | Sequences of the primers |
|---|---|
| *KTN1-AS1-F* | GCGAAGCCGTTAGTCCCTTA |
| *KTN1-AS1-R* | TTGGGTGAAAGTGGACCTGG |
| *ZNF326-F* | AATGAACCCGAACAAAGCCG |
| *ZNF326-R* | ACCTCCGAAAGAGTCAAGGC |
| *hsa-miR-505-3p-F* | CGTCAACACTTGCTGGTTTCCT |
| *CYTH1-F* | CACCATGGAGGAGGACGAC |
| *CYTH1-R* | CCGTCGGATGTTCTCCAGTT |
| *DNAJB9-F* | AGAGCGCCAAATCAAGAAGG |
| *DNAJB9-R* | TTCAGCATCCGGGCTCTTATT |
| *U6-F* | ATGGACTATCATATGCTTACCGTA |
| *NCK2-F* | CACGGCGAGATTTCATGTGTT |
| *NCK2-R* | GCAGGGATTCTCCAAATCCTCA |
| *KTN1-AS1 ShRNA* | GACTGTGGATAGAGATAGATAGATTCAAGA GAGACTGTGGATAGAGATAGATTTTTT |

(control for lncRNA and mRNA) or *U6* (miRNA control) using the $2^{-\Delta\Delta Ct}$ method. The primer sequences used in this study are listed in *Table 1*.

### *Colony formation assay*

Transfected A2780 and OVCAR-3 cells were planted in 6-well plates including a complete medium with 10% fetal bovine serum (FBS). Two weeks later, phosphate-buffered saline (PBS; Thermo Fisher Scientific, Carlsbad, CA, USA) was used to wash the colonies twice. Then, the colonies were fixed by 4% polyoxymethylene and colored with 1% crystal violet. Colonies (exceeding 50 cells) were manually counted.

### *Flow cytometry analysis*

In 6-well plates, transfected A2780 and OVCAR-3 cells were rinsed in PBS. After trypsinization, the cells were re-suspended in a binding buffer (100 µL). FITC-conjugated with Annexin V and PI (1 µL; Invitrogen, Carlsbad, CA, USA) was added into the binding buffer. Fifteen minutes later, the apoptotic OC cells were analyzed using flow cytometry (BD Biosciences, NY, USA).

### *Wound healing assay*

The transfected A2780 and OVCAR-3 cells were seeded in 6-well plates ($5\times10^5$ cells/well). At 80% confluence, a 200 µL pipette tip was used in the monolayer of cells to generate wounds. Then, cells were washed with PBS, and the function of genes on cell migration was assessed after adding the medium containing mitomycin C (20 µg/mL) without FBS. Photographs were taken to evaluate healing 0 and 24 hours after wounding.

### *Transwell assay*

Invasion of transfected A2780 and OVCAR-3 cells was assessed by applying a Transwell chamber with Matrigel (Corning, NY, USA). Add a serum-free medium containing $1\times10^5$ cells to the upper chamber, and add a medium containing 10% FBS to the lower chamber., and the medium containing 10% FBS was placed into the lower chamber. This was incubated for 48 hours, then invaded cells were fixed, stained with 1% crystal violet staining solution, then rinsed with PBS and counted with five fields in random.

### *ChIP assay*

The ChIP Assay Kit (Thermo Fisher Scientific, Carlsbad, CA, USA) was used to examine the ChIP assay in A2780 and OVCAR-3 cells. DNA-protein cross-links were formed after the incubation of A2780 and OVCAR-3 cells with formaldehyde for 10 minutes. Next, cross-linked chromatin DNAs were broken into segments sized 200–1,000 bp using an ultrasound machine. Then, the lysate was immunoprecipitated with anti-H3K27ac (Abcam, Boston, MA, USA), anti-*CBP* (Abcam, Boston, MA, USA), or IgG antibody (control, Abcam, Boston, MA, USA), and precipitates were recovered for RT-qPCR analysis.

### *RNA pull down assay*

The protein was extracted from A2780 and OVCAR-3 cells and later treated with purified biotinylated RNA (*KTN1-AS1* biotin probe) and biotinylated NC (*KTN1-AS1* no-biotin probe). The purified biotinylated transcripts were commercially obtained from Sangon Biotech (Shanghai, China) and were used to incubate with cell lysates at 25 ℃ for 1 hour. Later, streptavidin agarose beads (Invitrogen,

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Carlsbad, CA, USA) were applied to isolate biotin-coupled RNA complexes, and RT-qPCR was utilized to analyze miRNAs enrichment in the pull-down products.

### *Luciferase reporter assay*

*KTN1-AS1* full-length and *ZNF326* 3'-UTR of the wild-type (WT) and mutant-type (Mut) *miR-505-3p* binding sites were generated in Genechem Co., Ltd. (Shanghai, China). *KTN1-AS1*-WT/Mut or *ZNF326*-WT/Mut was transfected into A2780 and OVCAR-3 cells with *miR-505-3p* mimics/NC mimics. Luciferase activity was evaluated using Dual-Luciferase Reporter Assay (Promega, Madison, USA) after 48 hours of transfection.

### *RIP assay*

A2780 and OVCAR-3 cells were lysed in the lysis buffer, and the supernatant was collected after the lysate was centrifuged. Later, cell lysate was adopted to incubate with an anti-Ago2 or anti-IgG antibody (Millipore, MO, USA), and then magnetic beads were added for the immunoprecipitate RNA. After purification, RNA was detected by RT-qPCR.

### *Statistical analysis*

Statistical analyses were performed with GraphPad Prism Software (San Diego, CA, USA), and results were shown as mean ± standard deviation (SD) based on no less than three repeats. A Student's *t*-test or one-way Analysis of Variance (ANOVA) was used to estimate group difference, and statistical significance was set as $P<0.05$.

## Results

### *KTN1-AS1 was upregulated and enhanced cell proliferation and invasion in OC*

To determine the relationship between *KTN1-AS1* and the biological processes of OC, we first detected *KTN1-AS1* expression using RT-qPCR analysis. The results confirmed the increased *KTN1-AS1* expression in OC tissues compared to the expression in adjacent normal tissues (*Figure 1A*). Consistent with this, we compared these results with the IOSE-80 cell line; *KTN1-AS1* was present in a high level in OC cell lines (*Figure 1B*). To examine whether upregulated *KTN1-AS1* impacted OC cell growth, we performed a loss-of-function assay by constructing shRNA-mediated silencing of *KTN1-AS1* in A2780 and OVCAR-3 cells. The results showed that sh-*KTN1-AS1*#1/2 transfection caused a decrease in *KTN1-AS1* expression (*Figure 1C*). The function of silenced *KTN1-AS1* in cell proliferation was estimated using a colony formation assay, and data showed that KTN-AS1 deficiency slowed the proliferation of A2780 and OVCAR-3 cells (*Figure 1D*). Flow cytometry analysis showed that the apoptosis increased in *KTN1-AS1*-silenced A2780 and OVCAR-3 cells (*Figure 1E*). Subsequently, the wound healing assay demonstrated inhibitive migration in the sh-*KTN1-AS1*#1/2 group (*Figure 1F*). Furthermore, OC cell invasion was also suppressed by the deficiency of *KTN1-AS1* in the Transwell assay (*Figure 1G*). Taken together, *KTN1-AS1* was highly expressed and played a facilitative role in OC cell proliferation and invasion.

### *KTN1-AS1 was transcriptionally upregulated by CBP-mediated H3K27ac*

Then, we probed the mechanism of *KTN1-AS1* upregulation in OC. Previous research has shown that histone H3 lysine 27 acetylation (*H3K27ac*) could activate lncRNAs at the transcriptional level (23,24). By using UCSC (http://genome.ucsc.edu/), we also found that the *KTN1-AS1* promoter region presented high H3K27ac enrichment (*Figure 2A*). Therefore, we hypothesized that high expression of *KTN1-AS1* was triggered by H3K27ac at its promoter region. To verify this, ChIP assay was carried out to evaluate the H3K27ac level at the *KTN1-AS1* promoter region in OC cells (A2780 and OVCAR-3) and in the IOSE-80 cell line. Results showed that the H3K27ac level in the *KTN1-AS1* promoter region was higher in A2780 and OVCAR-3 cells compared to the IOSE-80 cell line (*Figure 2B*). Then, the histone acetyltransferase (HAT) inhibitor (C646) was used, and we found that *KTN1-AS1* expression was reduced by C646 treatment compared to dimethyl sulfoxide (DMSO) treatment (*Figure 2C*). These results indicated that *KTN1-AS1* was induced by H3K27ac. Considering that *CBP* acts as a key modulator in gene transcription and histone acetylation (25), we examined whether *CBP* was in charge of H3K27ac in the *KTN1-AS1* promoter. Using ChIP analysis, the enrichment of the *KTN1-AS1* promoter in *CBP* precipitates was validated (*Figure 2D*), highlighting the binding of *CBP* to the *KTN1-AS1* promoter. Later, *CBP* expression was knocked down in A2780 and OVCAR-3 cells (*Figure 2E*). We further found that *KTN1-AS1* expression decreased upon *CBP* knockdown

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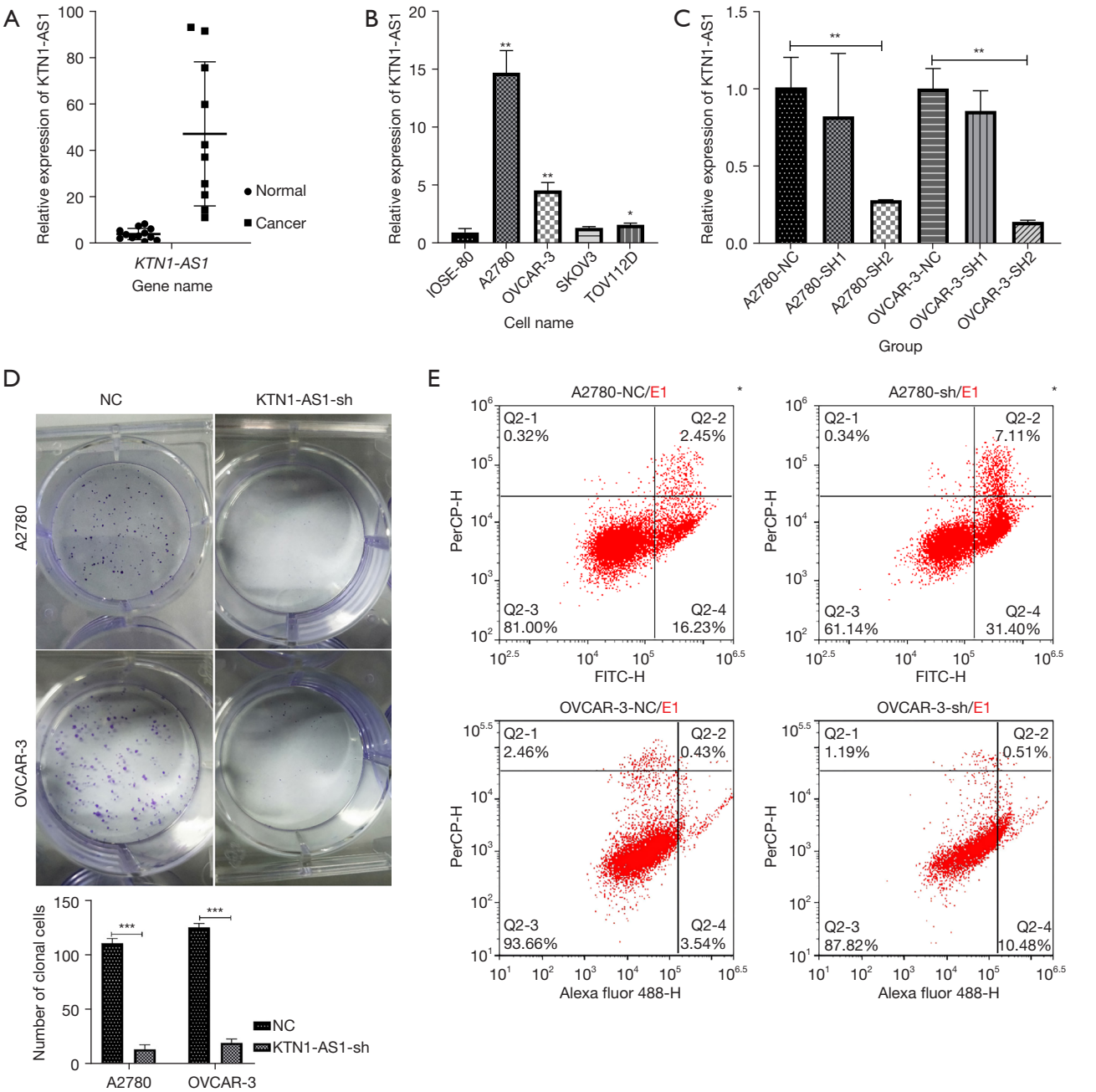


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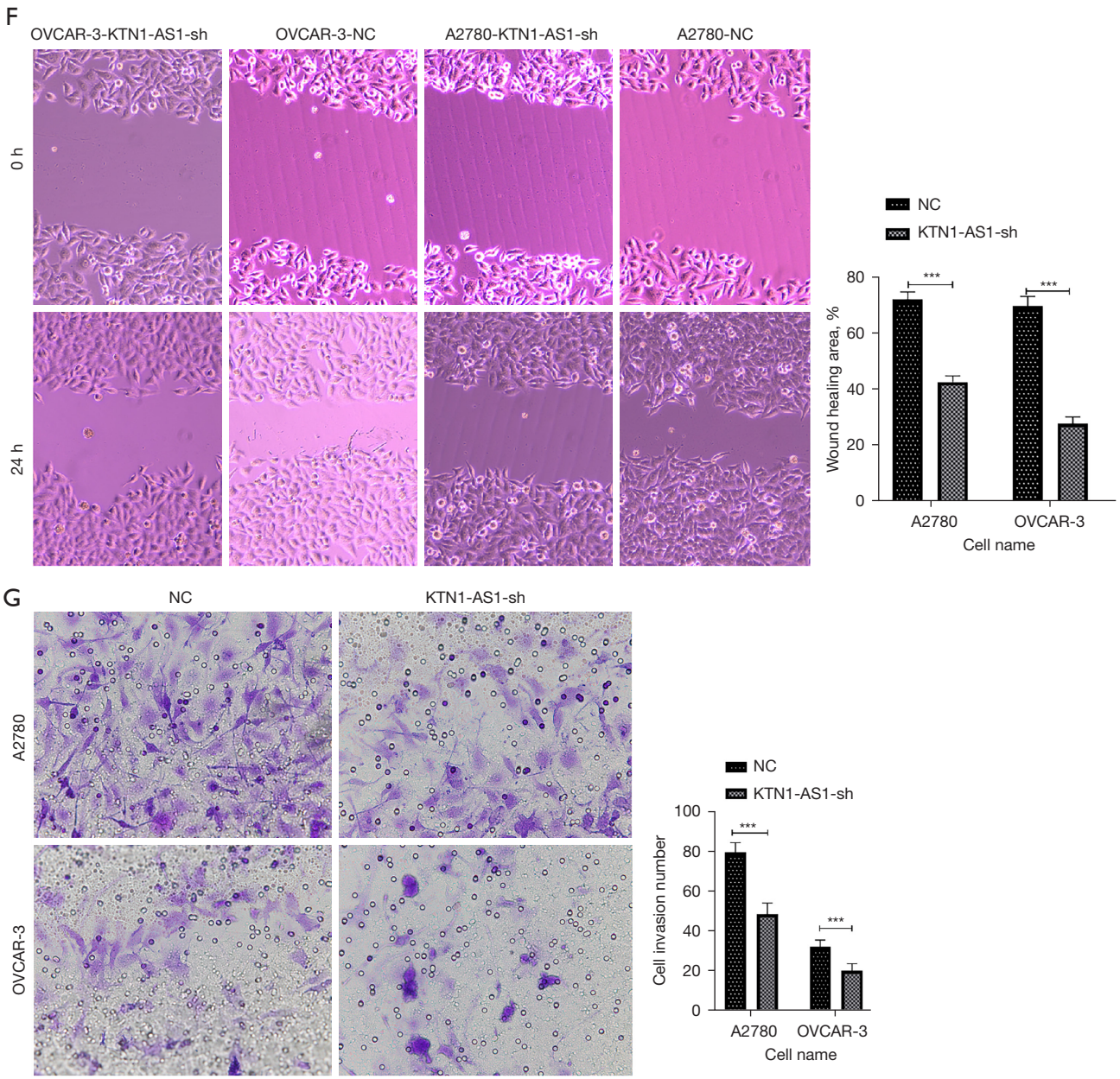


**Figure 1** KTN1-AS1 exhibited high level and oncogenic property in OC cells. (A) RT-qPCR analysis of *KTN1-AS1* expression in 64 pairs of OC and adjacent normal tissues. (B) RT-qPCR analysis of *KTN1-AS1* expression in OC cell lines (A2780, OVCAR-3, SKOV3, and TOV112D) and normal human ovarian cell line (IOSE-80). (C) *KTN1-AS1* expression was detected in A2780 and OVCAR-3 cells transfected with *KTN1-AS1* shRNAs by qRT-PCR. (D) Colony formation assay in *KTN1-AS1-SH*#1/2 transfected A2780 and OVCAR-3 cells. (E) Flow cytometry analysis used to evaluate the effect of *KTN1-AS1* silencing on cell apoptosis. (F) Wound healing assay used to estimate the migration ability upon *KTN1-AS1* knockdown. Only use 1% crystal violet staining solution for staining. The magnification is 100×. (G) Transwell assay used to assess the function of *KTN1-AS1* deficiency in cell invasion. Only use 1% crystal violet staining solution for staining. The magnification is 400×. *, P<0.05; **, P<0.01; ***, P<0.001. NC, negative control; OC, ovarian cancer; RT-qPCR, real-time quantitative polymerase chain reaction.

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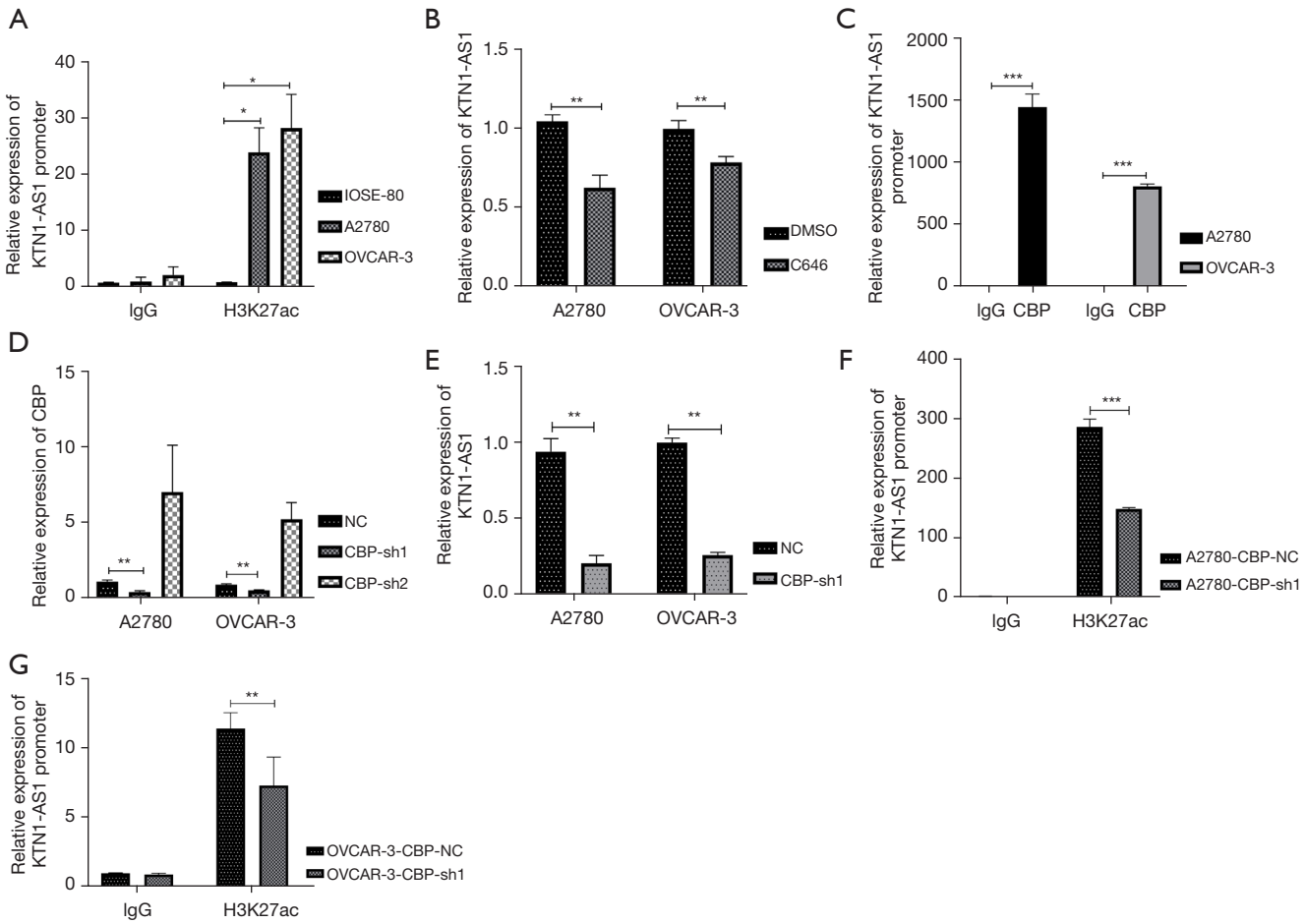


**Figure 2** Transcriptional activation of KTN1-AS1 by *CBP* (*CREB binding protein*)-mediated H3K27ac (histone H3 on lysine 27 acetylation). (A) UCSC (University of California, Santa Cruz) was used to predict H3K27ac enrichment at *KTN1-AS1* promoter. (B) H3K27ac level on *KTN1-AS1* promoter was determined by ChIP assay in A2780, OVCAR-3 and IOSE-80 cells. A2780 cell, human ovarian cancer epithelial cell line, OVCAR-3 cells, human ovarian adenocarcinoma epithelial cell line, IOSE-80 cells, human normal ovarian epithelial cell line. (C) RT-qPCR result of *KTN1-AS1* expression in C646- or DMSO-treated A2780 and OVCAR-3 cells. C646, a histone acetyltransferase inhibitor. (D) The binding of *CBP* to *KTN1-AS1* promoter was confirmed by ChIP assay. (E) RT-qPCR validated the silence of *CBP* in OC cells. (F) *KTN1-AS1* expression in OC cells upon *CBP* deficiency was detected by RT-qPCR. (G) The function of silenced *CBP* in H3K27ac level on *KTN1-AS1* promoter was determined by ChIP assay. *, P<0.05; **, P<0.01; ***, P<0.001. NC, negative control; OC, ovarian cancer; RT-qPCR, real-time quantitative polymerase chain reaction.

in OC cells (*Figure 2F*). Importantly, we confirmed that *CBP* silencing decreased the H3K27ac level in the *KTN1-AS1* promoter region (*Figure 2G*). Conclusively, *KTN1-AS1* was induced by *CBP*-mediated H3K27ac in OC.

### *KTN1-AS1 served as competitive endogenous RNA (ceRNA) for miR-505-3p*

Later, we investigated the downstream regulatory pattern of *KTN1-AS1* in OC. Previous studies showed that *KTN1-AS1* was involved in the ceRNA network in some cancers (20,21). Thus, we used starBase (http://starbase.sysu.edu.cn/) to seek microRNAs (miRNAs) that could theoretically bind to *KTN1-AS1*, and five potential miRNAs were found (*Figure 3A*). The RNA pull down assay with biotin-labeled *KTN1-AS1* implied that *miR-505-3p* was pulled down by the *KTN1-AS1* biotin probe (*Figure 3B*). Thus, we selected *miR-505-3p* to conduct subsequent research. *miR-505-3p* expression was examined in OC tissues and cells, and RT-qPCR demonstrated that *miR-505-3p* expressed at a low

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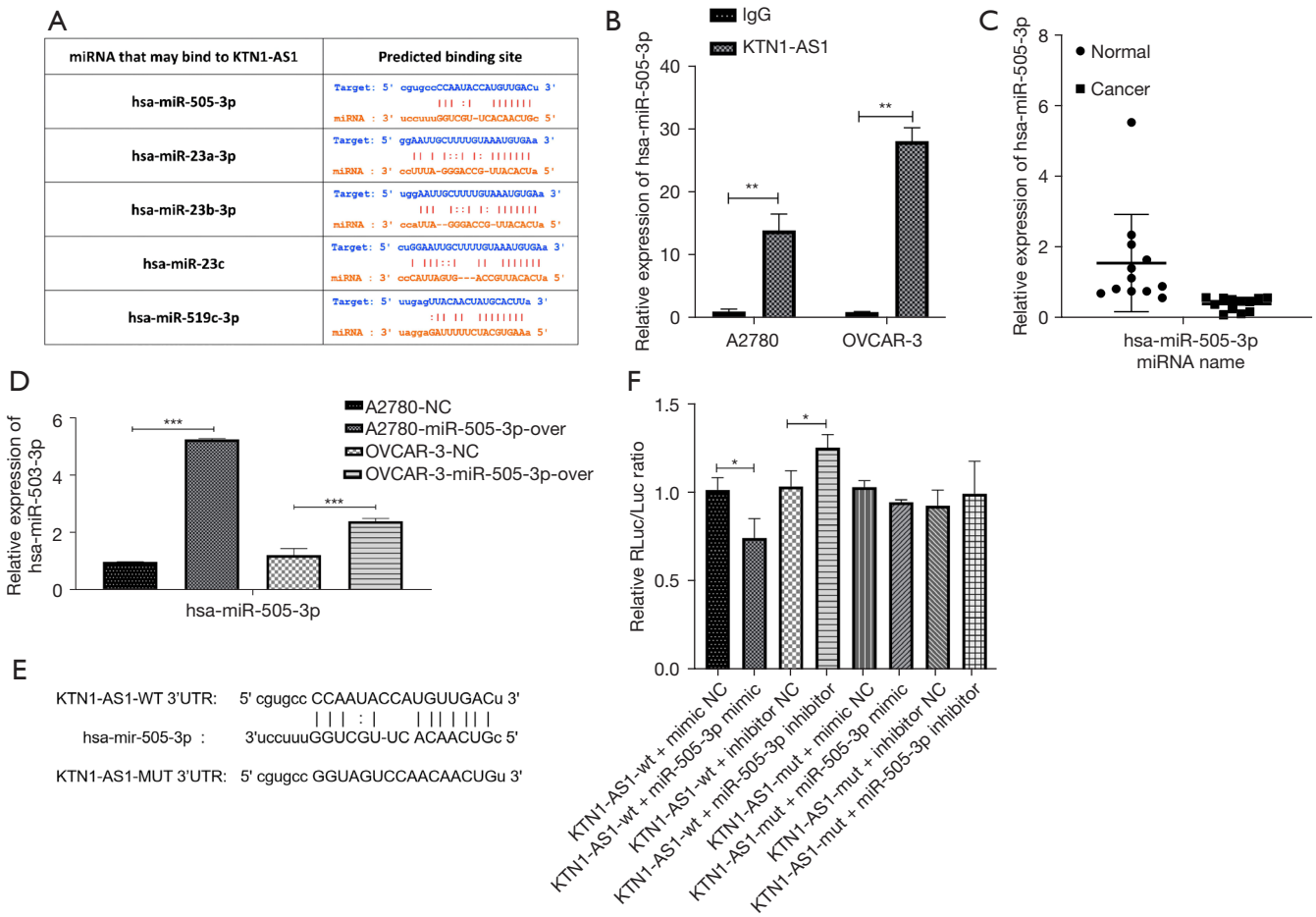


**Figure 3** KTN1-AS1 sponged miR-505-3p in OC. (A) The potential miRNAs for *KTN1-AS1* in starBase. (B) The relative enrichment of predicted miRNAs interacting with *KTN1-AS1* was quantified by RT-qPCR after biotinylated-*KTN1-AS1* pull-down experiment in OC cells. (C) *miR-505-3p* expression in OC tissues and cells was examined by RT-qPCR. (D) Overexpression of *miR-505-3p* in A2780 and OVCAR-3 cells was verified by RT-qPCR. (E) *miR-505-3p* binding site and mutated site on *KTN1-AS1*. (F) The luciferase activity of *KTN1-AS1*-WT/Mut in OC cells with *miR-505-3p* mimics was validated by luciferase reporter assay. *, P<0.05; **, P<0.01; ***, P<0.001. NC, negative control; OC, ovarian cancer; RT-qPCR, real-time quantitative polymerase chain reaction.

level (*Figure 3C*). Furthermore, we elevated *miR-505-3p* expression by transfecting *miR-505-3p* mimics (*Figure 3D*). The binding sequences of *miR-505-3p* in *KTN1-AS1*-WT and *KTN1-AS1*-Mut are represented in *Figure 3E*. In addition, we observed that the luciferase activity of the *KTN1-AS1*-WT reporter was reduced, and that of the *KTN1-AS1*-Mut reporter was not affected under the transfection of *miR-505-3p* mimics (*Figure 3F*). All data showed that *KTN1-AS1* interacted with *miR-505-3p* in OC.

### *ZNF326 was targeted by miR-505-3p*

Subsequently, the target gene of *miR-505-3p* was inspected to further support the ceRNA hypothesis. By using starBase (CLIP Data ≥5, Degradome Data ≥3), we identified five candidate targets for *miR-505-3p* (*Figure 4A*). To further identify, expression of these targets was estimated in *miR-505-3p*-overexpressed A2780 and OVCAR-3 cells. Compared to other genes, we found that *ZNF326* expression was significantly lowered upon *miR-505-3p* upregulation (*Figure 4B*). Moreover, RT-qPCR results showed that OC tissues and cells expressed a high level of *ZNF326* (*Figure 4C*). Furthermore, the complementary base pairs between the *miR-505-3p* seed region and *ZNF326* 3'-UTR were found (*Figure 4D*). To examine whether *miR-505-3p* could interact with *ZNF326*, we performed a

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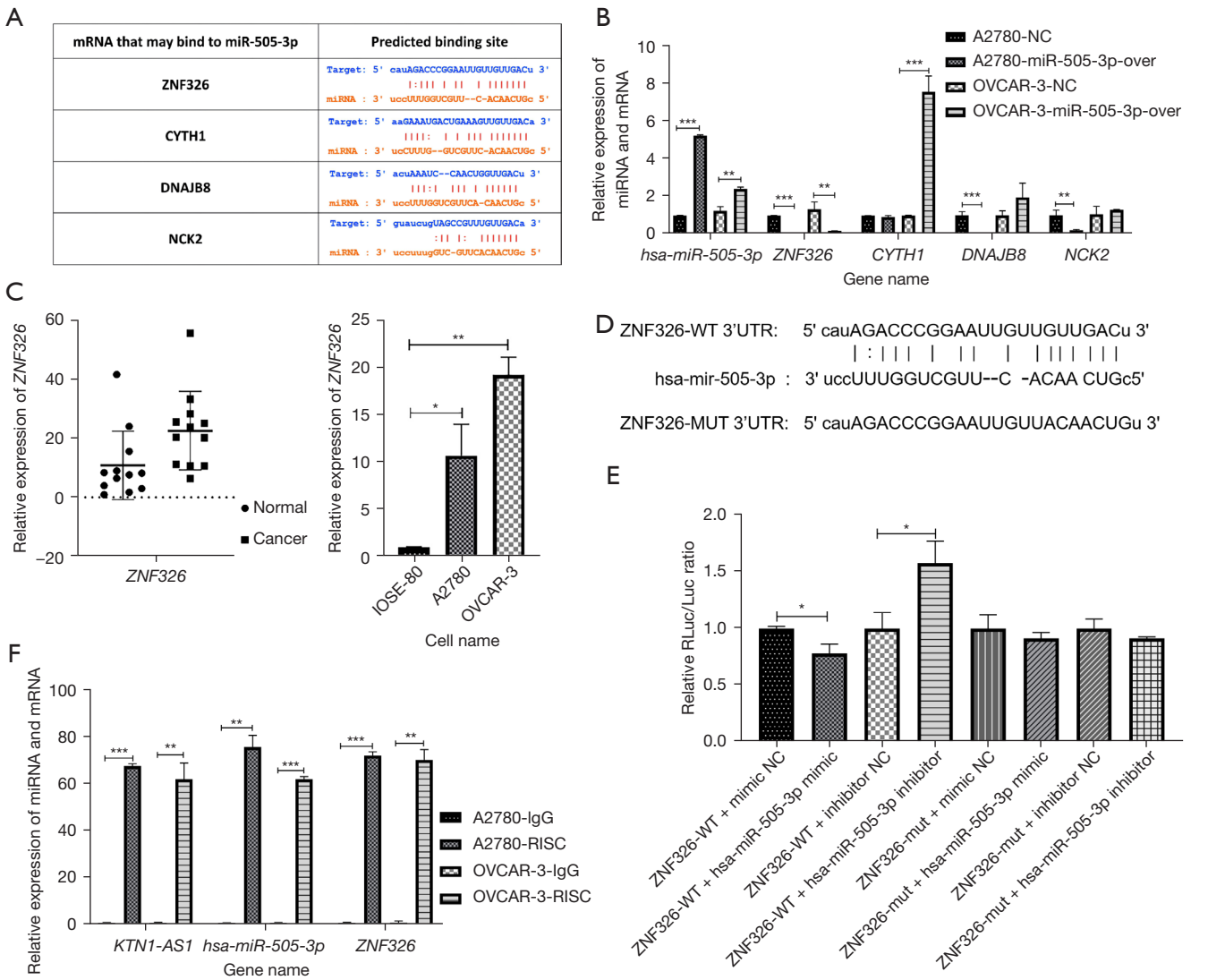


**Figure 4** MiR-505-3p targeted to ZNF326. (A) The potential mRNAs that could bind to *miR-505-3p* was predicted using starBase under the indicated selection condition. (B) Effect of *miR-505-3p* overexpression on the expression of predicted genes. (C) *ZNF326* expression in OC tissues and cells was validated by RT-qPCR. (D) The wide type and mutant type binding sequences between *miR-505-3p* and *ZNF326*. (E) Luciferase reporter assay was utilized to estimate the binding of *miR-505-3p* to *ZNF326*. (F) The enrichment of *KTN1-AS1*, *miR-505-3p* and *ZNF326* in Ago2-conjugated beads was measured by RIP assay. *, P<0.05; **, P<0.01; ***, P<0.001. NC, negative control; OC, ovarian cancer; RT-qPCR, real-time quantitative polymerase chain reaction.

luciferase reporter assay. Results found that the luciferase activity of the *ZNF326*-WT reporter but not the *ZNF326*-Mut reporter decreased in *miR-505-3p* mimics-transfected OC cells (*Figure 4E*). RIP assay displayed that *KTN1-AS1*, *miR-505-3p*, and *ZNF326* were markedly enriched in the AgO2-containing miRNA ribonucleoprotein complex relative to those in IgG (*Figure 4F*). In conclusion, *ZNF326* was the target gene of *miR-505-3p*.

### *KTN1-AS1 promoted cell proliferation and invasion by upregulating ZNF326 in OC*

Finally, we determined whether *KTN1-AS1* enhanced OC cell growth and invasion by depending on *ZNF326*. At first, pcDNA3.1/*ZNF326* was transfected into OC cells for upregulating the *ZNF326* level (*Figure 5A*). The colony formation assay demonstrated that overexpressing

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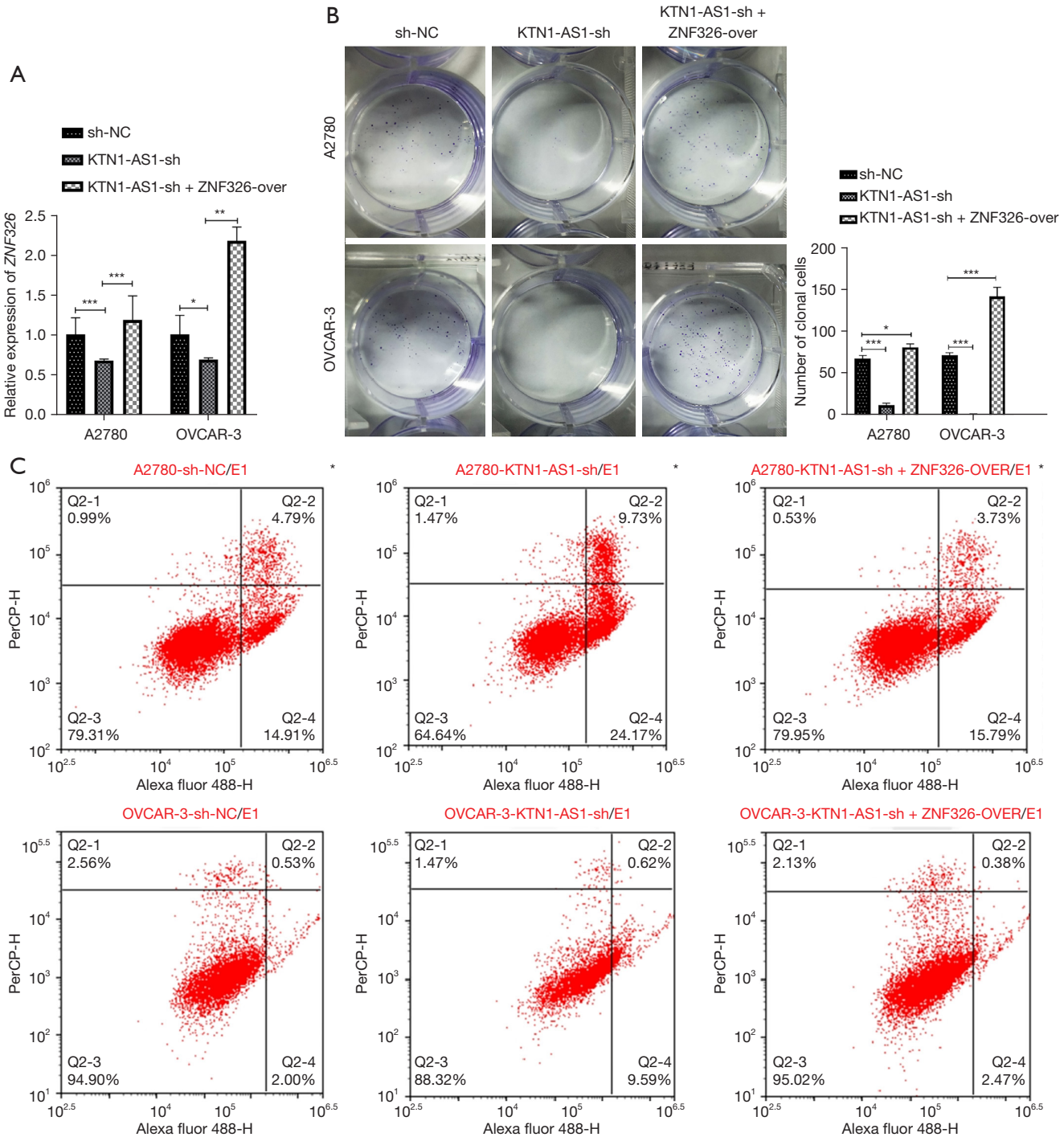


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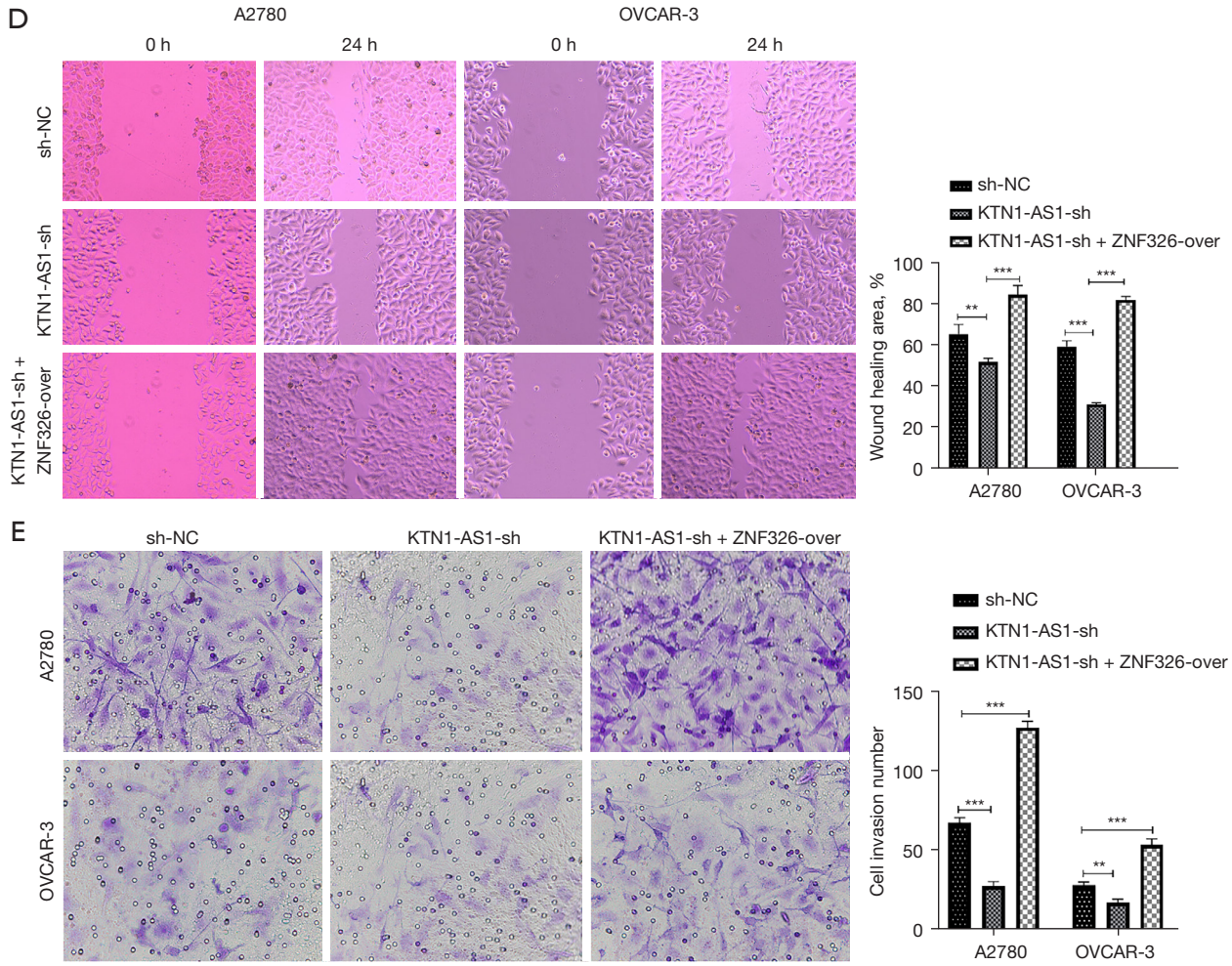


**Figure 5** KTN1-AS1 regulated OC cell proliferation and invasion by *ZNF326*. (A) *ZNF326* expression in OC cells transfected with pcDNA3.1/*ZNF326* was detected by RT-qPCR. (B) Proliferation of OC cells transfected with indicated plasmids was evaluated by colony formation assay. (C) Flow cytometry analysis was applied to evaluate apoptosis of OC cells with indicated transfection. (D,E) The migration and invasion of OC cells with indicated transfection was determined by wound healing assay and Transwell assay. The percentage of wound healing area and the number of cell clones in different groups at 24 hours were counted. Only use 1% crystal violet staining solution for staining. Photographs were taken using an inverted microscope. The magnification is 100× (wound healing assay) and 400× (transwell assay). *, P<0.05; **, P<0.01; ***, P<0.001. NC, negative control; OC, ovarian cancer; RT-qPCR, real-time quantitative polymerase chain reaction.

*ZNF326* abolished the inhibited proliferation caused by silencing *KTN1-AS1* (*Figure 5B*). Results of the flow cytometry analysis revealed that *KTN1-AS1* downregulation could fortify the apoptosis rate of OC cells, but *ZNF326* overexpression impaired the increase in the apoptosis rate (*Figure 5C*). Furthermore, we discovered that the migration suppressed by *KTN1-AS1* knockdown was restored by transfecting pcDNA3.1/*ZNF326* (*Figure 5D*). In addition, *ZNF326* overexpression also rescued the inhibitive effect of silenced *KTN1-AS1* on the invasion of OC cells (*Figure 5E*). In summary, *KTN1-AS1* increased the *ZNF326* level to enhance OC cell growth.

## Discussion

OC is the main cause of global female cancer incidence and cancer-related mortality (Webb and Jordan, 2017). (1). Most ovarian malignancies originate from the less coelomic epithelium follicular cells and germ cells (26). As the most lethal malignancy in females, only 15% of OC cases are

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found at the low-grade stage, and patients with high-grade OC usually relapse within about 16 months (27). Therefore, to prevent or treat OC, we need to find molecular networks that offer prognostic and targeted therapeutic values (28). In recent years, lncRNAs were found to be gene expression modulators of cancer cell phenotypes and are considered promising therapeutic targets for OC treatment (29). Our study confirmed that lncRNA *KTN1-AS1* was upregulated in OC. Functional experiments found that *KTN1-AS1* knockdown slowed the proliferation, migration, and invasion of OC cells, and accelerated apoptosis. This highlighted the oncogenic nature of *KTN1-AS1* in OC.

H3K27ac is known to modify the histone post-translation and transcriptionally activates gene expression by associating with the activity-enhancing regulatory elements (30,31). Existing research has shown that H3K27ac in the promoter region triggered the upregulation of some lncRNAs. For example, H3K27ac in the promoter region of LINC00519 caused the upregulated level of LINC00519 in lung squamous cell carcinoma (32). Furthermore, H3K27ac in the PLAC2 promoter region activated the expression of PLAC2 in oral squamous cell carcinoma (33). In this study, we found, through the UCSC website, that the *KTN1-AS1* promoter region was highly enriched with H3K27ac, which was further validated by ChIP assay. The decreased *KTN1-AS1* level by C646 further confirmed that H3K27ac modification caused the upregulation of *KTN1-AS1*. *CBP* is mainly responsible for histone acetylation and gene transcription (25,34). We confirmed that *CBP* bound to the *KTN1-AS1* promoter triggered the H3K27ac, thereby inducing *KTN1-AS1* upregulation.

Regarding mechanisms, lncRNAs were shown as ceRNAs that lncRNAs release mRNAs from posttranscriptional silence induced by miRNAs through sponging miRNAs in cancer progression (35,36). MiRNAs are small noncoding RNAs (18~24 nucleotides) that bind to target mRNA 3′-UTR to suppress protein translation and/or reversely modulate mRNA stability (37). Importantly, miRNAs play significant roles in cancer progression by serving as tumor facilitators or suppressors (38,39). In our research, *miR-505-3p* was identified as a downregulated miRNA and interacted with *KTN1-AS1*. Previously, *miR-505-3p* was shown to be expressed in a low amount and exerted an inhibitive role in pancreatic cancer (40) and breast cancer (41). Moreover, we identified *ZNF326* as the downstream gene of *miR-505-3p*. Former study implied that *ZNF326* increased HDAC7 expression and activated the Wnt pathway to promote a malignant phenotype in glioma (42). In non-small cell lung cancer, *ZNF326* facilitated cell proliferation by modulating ERCC1 expression (43). Finally, restoration experiments suggested that *KTN1-AS1* enhanced proliferation, migration, and invasion, and attenuated apoptosis by increasing *ZNF326*.

In summary, our study shows that H3K27ac-induced lncRNA *KTN1-AS1* accelerated proliferation, migration, and invasion of OC cells, and impeded apoptosis of OC cells through the *miR-505-3p/ZNF326* axis. These findings provide potential for a new molecular-targeted treatment method for OC patients.

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## Footnote

*Reporting Checklist:* The authors have completed the MDAR reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-443/rc

*Data Sharing Statement:* Available at https://atm.amegroups.com/article/view/10.21037/atm-22-443/dss

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-443/coif). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was approved by the Ethics Committee of Guangzhou Women and Children's Medical Center, Guangzhou Medical University (approval No. 2020-KY-021-03). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). An informed consent form was signed by each patient.

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