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RESEARCH ARTICLE

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LncRNA LIFR-AS1 overexpression suppressed the progression of serous ovarian carcinoma

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Abstract

Background: Serous ovarian carcinoma (SOC) is a common malignant tumor in female reproductive system. Long noncoding RNA (lncRNA) LIFR-AS1 is a tumor suppressor gene in colorectal cancer, but its effect and underlying mechanism in SOC are still unclear. Therefore, this study focuses on unveiling the regulatory mechanism of LIFR-AS1 in SOC.

Methods: The relationship between LIFR-AS1 expression and prognosis of SOC patients was analyzed by TCGA database and Starbase, and then, the LIFR-AS1 expression in SOC tissues and cells was detected by quantitative real-time PCR (qRT-PCR) and in situ hybridization (ISH). Besides, the relationship between LIFR-AS1 and clinical characteristics was analyzed. Also, the effects of LIFR-AS1 on the biological behaviors of SOC cells were measured by Cell Counting Kit-8, colony formation, and wound-healing and Transwell assays, respectively. Western blot and qRT-PCR were employed to determine the protein expressions of genes related to proliferation (PCNA), apoptosis (cleaved caspase-3), epithelial-mesenchymal transition (E-cadherin, N-cadherin, and Snail).

Results: LIFR-AS1 was lowly expressed in SOC, which was correlated with the poor prognosis of SOC patients. Low expression of LIFR-AS1 in SOC was associated with the tumor size, clinical stage, lymph node metastasis, and distant metastasis. LIFR-AS1 overexpression promoted the expressions of cleaved caspase-3 and E-cadherin while suppressing the malignant behaviors (proliferation, migration, and invasion) of SOC cells, the expressions of PCNA, N-cadherin, and Snail. Besides, silencing LIFR-AS1 exerted the effects opposite to overexpressed LIFR-AS1.

Conclusion: LIFR-AS1 overexpression inhibits biological behaviors of SOC cells, which may be a new therapeutic method.

KEYWORDS
epithelial–mesenchymal transition, LIFR-AS1, proliferation, serous ovarian carcinoma

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1 | INTRODUCTION

Ovarian cancer (OC) is one of the most common malignancies of the female reproductive system, and the WHO estimates that there are approximately 225, 500 newly diagnosed cases of OC and approximately 140, 200 newly emerged deaths worldwide each year.[1] Although the diagnosis and treatment of malignant tumors have gradually improved in recent years, the mortality rate of OC still ranks first in gynecological malignancies.[2] The most common pathological type of OC is epithelial ovarian cancer, 75% of which are serous ovarian carcinomas (SOCs).[3] SOC has been classified into various stages, including ovarian serous cystadenoma, borderline ovarian serous, low-grade SOC, and high-grade SOC, with different expressions of specific tumor markers at various stages.[3] SOC lacks a reliable early diagnostic indicator and typical early symptoms.[4] As such, about 75% of patients, at the time of diagnosis, are at clinically advanced stage III or IV.[5] Due to the susceptibility to recurrence and the postoperative resistance to chemotherapy drugs, the prognosis of patients is very poor and 5-year survival rate is low.[6] Therefore, exploring the molecular mechanisms associated with the biological behavior of SOC is essential for the early diagnosis and prognosis evaluation of SOC.

Long noncoding RNAs (lncRNAs) are RNAs that cannot encode proteins due to the lack of a meaningful open reading frame.[7] Several recent studies have discovered that lncRNAs have powerful gene regulatory function and participate in a variety of pathophysiological processes,[8,9] playing vital roles in cancer progression,[10] as well as the progression of SOC. For instance, lncRNA MAGI2-AS3 leads to the tumor suppression of high-grade SOC,[11] and lncRNA CTD-2020K17.1 promotes metastasis and proliferation of SOC cells.[12] In addition, lncRNA NEAT1 facilitates the malignant phenotype of SOC by mediating miR-506.[13] There is also evidence that the mechanism of action of some lncRNAs is dependent on their genomic location (sense, antisense, bidirection, intron, and intergene), particularly the positional relationship with neighboring genes,[14,15] in which antisense lncRNAs are noncoding RNAs encoded by genes located on the opposite strand of protein-coding genes and often completely or partially complementary to protein-coding genes.[16] A large body of evidence has indicated that antisense lncRNAs are pervasive, abundantly present within cells, and have specific cellular localizations, heralding that such molecules may have important biological implications.[17,18] However, less attention has been paid to this part, and the specific role of antisense lncRNAs in SOC has not been clearly elucidated. Combined with relevant literature and bioinformatics analysis, our study singled out LIFR-AS1 to unveil its effects on SOC.

LIFR-AS1 is a newly discovered lncRNA and has a strong association with tumor progression, which is transcribed in an antisense fashion from the leukemia inhibitory factor receptor (LIFR) gene.[19] LIFR could regulate cell proliferation, differentiation, and phenotype in various cancers, such as colorectal cancer and breast cancer.[20–22] Few literatures have indicated that LIFR-AS1 exerts suppressive effects on the initiation and progression of assorted cancers, such as breast cancer,[23] lung cancer,[24] and glioma.[25] Nevertheless, the effect and underlying mechanism of LIFR-AS1 in SOC are still dim, which is the direction of this current study.

2 | MATERIALS AND METHODS

2.1 | Ethics statement and specimen collection

The diagnostic and staging criteria of SOC were based on the study of Steffen Hauptmann et al.[26] The SOC (n = 87) and adjacent healthy fallopian tube tissues (n = 38, more than 2 cm away from tumor tissues) were obtained from SOC patients in the Second Affiliated Hospital of Jiaxing University. The clinical characteristics of SOC patients are depicted in Table 1. Tissue samples were cut from tumor and adjacent tissues about 0.5 cm in diameter, and taken back immediately after the operation. Following liquid–nitrogen cryogenic treatment, tissue samples were stored in a−80°C refrigerator. This research was conducted on the premise

TABLE 1 Clinical characteristics of SOC patients

	LIFR-AS1 expression		
Parameters	**Low**	**High**	***p* value**
Age (years old)			0.158
<55	21	27	
≥55	23	16	
Histological subtype			0.599
Mucinous	28	25	
Serous	16	18	
Tumor size (cm^3)			0.038
<10	12	21	
≥10	32	22	
Tumor location			0.452
Unilateral	30	26	
Bilateral	14	17	
Differentiation			0.331
Well and moderate	14	18	
Poor	30	25	
Clinical stage			0.002
I/II	13	27	
III/IV	31	16	
Lymph node metastasis			<0.001
No	10	29	
Yes	34	14	
Distant metastasis			0.020
Absent	27	36	
Present	17	7	
Recurrence			0.238
No	15	20	
Yes	29	23	

that patients agreed to provide their tissue for clinical research, and the clinical trial program was reviewed and approved by the Ethics Committee of The Second Affiliated Hospital of Jiaxing University (JXEY-ZFYJ045).

2.2 | Bioinformatics assay

Data on LIFR-AS1 expression in SOC tissues were retrieved from TCGA database (https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga), and the relationship between LIFR-AS1 high ($n = 187$) or low expression ($n = 187$) and overall survival (150 months) of SOC patients was analyzed by Starbase (http://starbase.sysu.edu.cn/).

2.3 | Cell culture

Human Ovarian Surface Epithelial (HOSE) cells (7310, Yuhengfeng biotech,) were grown in Ovarian Epithelial Cell Medium (OEpiCM, 7311, Yuhengfeng biotech). A2780 cells (CBP60283, Cobioer,) were cultivated in RPMI-1640 medium (R0883, Sigma-Aldrich,) with 10% fetal bovine serum (FBS, 12007C, Sigma-Aldrich). OV-56 cells (96020759, ECACC,) were cultured in the Dulbecco's Modified Eagle Medium (DMEM, 56499C, Sigma-Aldrich,) and HAMS F12 (51651C, Sigma-Aldrich,) (1:1) supplemented with 2 mM glutamine, 5% FBS, 0.5 μg/ml hydrocortisone, and 10 μg/ml insulin. OVCAR3 cells (HTB-161, ATCC,) were incubated in RPMI-1640 medium containing 20% FBS and 0.01 mg/mL bovine insulin. SK-OV-3 cells (HTB-77, ATCC) were maintained in McCoy's 5a Medium Modified (M9309, Sigma-Aldrich) added with 10% FBS. The above cells were cultured in 37°C with 5% CO_2.

2.4 | In situ hybridization (ISH)

The tissue sections were put into the mixture of 0.8% Pepsin/Hydrochloric Acid solution (EHJ-CAS0164999, JiaHui Biotech,) and digested in a water bath (TSGP28, Thermo Scientific,) at 37°C for 10 min (min). Then tissues were washed with tris-buffered saline (TBS, 28358, Thermo Scientific,) for 3 times (5 min (min) for each time), dehydrated by gradient ethanol, and dried at room temperature. The DNA probe of LIFR-AS1 (5'-GCGCGCGGGTGCTCCAAG-3′) was dripped into the section, covered with cover glass, denatured at 98°C for 10 min, annealed in ice bath, and hybridized in 37°C water bath for 1 h. Next, tissues were washed with TBS for additional 3 times (5 min each time). After addition of digoxigenin (DIG) antibody (1:1000, 11,093,274, Roche,) dropwise, the tissues were incubated with Alkaline Phosphatase (IVGN2208, Invitrogen,) at room temperature for 30 min. Following the washing with phosphate-buffered saline (PBS, 10010049, Gibco,) twice for 5 min, the tissues were supplemented with DAB (8801–4965-72, Invitrogen,) to develop for 5 min in the dark. Subsequently, the tissues were routinely dehydrated and transparently sealed. Finally, the coloration of LIFR-AS1 in tissues was observed under a microscope (×200, Eclipse 80i, Nikon,).

2.5 | Transfection

The overexpression plasmid of LIFR-AS1 was constructed using pCMV6-Entry vector (PS100001, Origene), and short hairpin RNA targeting LIFR-AS1 (shLIFR-AS1, C02003, 5'-TGGGACTTTGCGAATTACCTAAA-3′) were purchased from GenePharma. LIFR-AS1 overexpression plasmid, shLIFR-AS1, and empty vector (negative control) were transfected into the A2780 and SK-OV-3 cells under the help of Lipofectamine 3000 Reagent (L3000001, Thermo Fisher,). Briefly, cells were seeded onto 6-well plates at a density of 3×10^5 cells/well until the cells reached 70%–90% confluence. Then, the serum-free medium was utilized to dilute transfection reagent and LIFR-AS1 overexpression plasmid, empty vector, or shLIFR-AS1 to form the reagent/sample mixture, followed by 48 h culture of SOC cells and mixture. The success of transfection was tested by quantitative real-time polymerase chain reaction (qRT-PCR).

2.6 | QRT-PCR

Cell/Tissue Total RNA Isolation Kit (RK02009, Biomarker,) was utilized to isolate the total RNAs, and cDNA synthesis was then operated using RT Master Mix (HY-K0510A, MedChemExpress,). QRT-PCR was utilized for detecting the mRNA expression levels of LIFR-AS1, proliferating cell nuclear antigen (PCNA),

TABLE 2 Primers for quantitative real-time polymerase chain reaction

Gene names	Forward primer (5′–3′)	Reverse primer (5′–3′)
LIFR-AS1	GCAAATACTGTGTATTAGTCC	CCGCTTCCTTGTGAAGAAGGT
PCNA	ACCGCTGCGACCGCAATTTG	ACGTGCAAATTCACCAGAAGGCATC
E-Cadherin	GGTTTTCTACAGCATCACCG	GCTTCCCCATTTGATGACAC
N-Cadherin	TGAAACGGCGGGATAAAGAG	GGCTCCACAGTATCTGGTTG
Snail	GACCCACTCAGATGTCAAGAAG	CTTGTGGAGCAGGGACATT
GAPDH	ACCACAGTCCATGCCATCAC	TCCACCACCCTGTTGCTGTA

E-cadherin, N-cadherin, and Snail using SYBR Green Fast qPCR Mix (RK02001, Biomarker,) in a D10 PCR gene amplification instrument (XuSensmart,). PCR conditions were as follows: 40 cycles of denaturation at 95°C for 20 s(s), annealing at 58°C for 20 s, and extension at 72°C for 20 s. The sequences of the primers are listed in Table 2. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was chosen as the internal control to normalize the gene expressions using the $2^{-\Delta\Delta CT}$ method.[27]

2.7 | Cell counting kit-8 (CCK-8) assay

A2780 and SK-OV-3 cells inoculated on 96-well plates (5×10^3 cells/well) were cultured for 24, 48, and 72 h(h) after various treatments, followed by the incubation with CCK-8 reagent (96,992, 10 μl, Sigma-Aldrich,) for 4 h. The optical density (OD) value was recorded at a wavelength of 450 nm with the RNE-90002 microplate reader (Reagen,).

2.8 | Colony formation assay

For the determination of colony formation, 1×10^3 SOC cells that suspended in culture media with 10% FBS were placed in 6-well plates, which were subsequently subjected to the incubation at 37 °C with 5% CO_2 for 14 days. Thereafter, the fixation (15 min) and staining (20 min) of A2780 and SK-OV-3 cells were performed with 4% paraformaldehyde (M009, Gefanbio,) and 0.5% crystal violet solution (C0121, Beyotime,). Finally, the condition of cell colony formation was observed under a DMi8 microscope (×100, Leica).

2.9 | Wound-healing assay

A2780 and SK-OV-3 cells were seeded in a 6-well plate at a density of 5×10^4 cells/well until the cells reached 80% confluence. Then, wounds were created every 0.5 cm with a pipette tip. After being rinsed with PBS, the images of wound closure were obtained at 0 and 24 h with the microscope (×100).

2.10 | Transwell assay

The 24-well Transwell chamber (8 μm pores, Corning,) covered with Matrigel (354,234, Corning) was applied in the invasion assay. In brief, SOC cells maintained in serum-free medium (100 μl, 5×10^5 cells/mL) were put into the upper chamber, while those cultured in 600 μl medium with 10% FBS were put into the lower chamber. Following the incubation for 24 h, cells on the lower surface of membrane were fixed with 4% paraformaldehyde (BL-G002, Sbjbio,), followed by being stained with 0.5% crystal violet solution (G1062, Solarbio,) and observed under the microscope (×250).

2.11 | Western blot

RIPA lysis buffer (C500007, Sangon,) was applied to extract the total proteins from SOC cells. Following that, the measurement of protein concentration was performed using the BCA kit (E112-01, Vazyme,). Thereafter, 50 μg of total proteins and 5 μl of prestained protein marker (MP102-01, Vazyme,) were loaded into the SDS-PAGE gel (P0688, Beyotime,) and then shifted onto the PVDF membranes (FFP32, Beyotime,). Later, the membranes were blocked with 5% non-fat milk and cultured with primary antibodies at 4°C overnight, followed by being washed with Tris-buffered Saline with Tween-20 (TBST; BI-WB025, Sbjbio,) for 30 min. Subsequently, the membranes were incubated with secondary antibodies goat anti-rabbit IgG (1:1000, ab6702, Abcam,) and goat anti-mouse (1:2000, ab150113, Abcam,) at room temperature for 2 h. Next, the membranes were immersed in ECL luminescence reagent (R30199-100 ml, Pierce,) to observe its completion after being developed and photographed in the dark with a GEL-PRO-ANALYZER software (Bethesda,). GAPDH was selected as the internal reference. The primary antibodies involved in this assay mainly comprised those against PCNA (1:1000; Mouse; ab29, Abcam, 29 kDa), cleaved caspase-3 (1:500; Rabbit; ab2302, Abcam, 17 kDa), E-cadherin (1:1000; Mouse; ab76055, Abcam, 97 kDa), N-cadherin (1:1000; Rabbit; ab18203, Abcam, 130 kDa), Snail (1:1000; Rabbit; ab216347, Abcam, 29 kDa), and GAPDH (1:10000; Rabbit; ab181602, Abcam, 36 kDa).

2.12 | Statistical analysis

Based on the statistical analysis that conducted by Graphpad prism 8.0, measurement data were expressed as mean ± standard deviation (SD). The comparison on expression difference in LIFR-AS1 in adjacent and SOC tissues was conducted by paired sample *t* test, and comparisons among multiple groups were performed using one-way analysis of variance (ANOVA) and followed by Bonferroni post hoc test. The enumeration data in Table 1 were compared by chi-square test. The statistical significance was indicated by $p < 0.05$.

3 | RESULTS

3.1 | Low expression of LIFR-AS1 in SOC was associated with the poor prognosis of SOC patients

As depicted in Figure 1A, TCGA-OV database indicated that LIFR-AS1 expression was remarkably lowered in tumor samples as compared to that in healthy samples, and Starbase revealed that the low expression of LIFR-AS1 was associated with poor survival of SOC (Figure 1A, $p = 0.018$). In addition, the result of qRT-PCR further confirmed that LIFR-AS1 expression was lower in SOC tissues than that in adjacent tissues (Figure 1B, $p < 0.001$). Moreover, compared with SOC tissues, the adjacent tissues were obviously stained brown (Figure 1C), demonstrating that LIFR-AS1 expression was largely

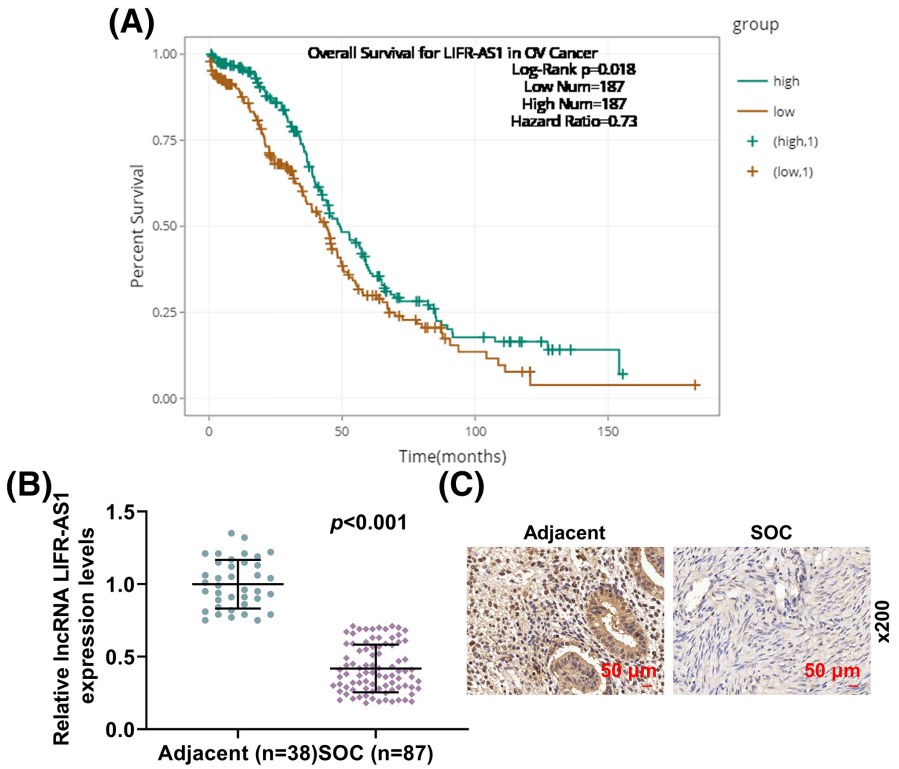


FIGURE 1 Expression of LIFR-AS1 in SOC. (A) The analysis of LIFR-AS1 expression was performed using TCGA database (https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga), and Starbase (http://starbase.sysu.edu.cn/) analyzed the relationship between LIFR-AS1 high ($n = 187$) or low expression ($n = 187$) and the overall survival of SOC patients ($p = 0.018$). (B) LIFR-AS1 expression in adjacent ($n = 38$) and SOC ($n = 87$) tissues was detected by qRT-PCR. GAPDH was served as the internal reference ($p < 0.001$). (C) ISH was used to detect the expression of LIFR-AS1 in SOC and adjacent normal tissues (magnification ×200). The data were presented as the mean ± SD of three independent experiments. Abbreviation: SOC, serous ovarian carcinoma; TCGA, the Cancer Genome Atlas; qRT-PCR, quantitative real-time PCR; ISH, in situ hybridization; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SD, standard deviation

decreased in SOC tissues. Besides, low expression of LIFR-AS1 in SOC was associated with higher levels of tumor size, clinical stage, lymph node metastasis, and distant metastasis ($p < 0.05$, Table 1).

3.2 | LIFR-AS1 overexpression inhibited viability and proliferation of SOC cells while silencing LIFR-AS1 had the opposite effect

Subsequently, we detected the LIFR-AS1 expression in normal (HOSE) and SOC cells (A2780, OV-56, SK-OV-3, OVCAR3). In SOC cell lines, LIFR-AS1 expression was lower in SOC cells than that in HOSE cells, and among these SOC cells, LIFR-AS1 in OV-56 cells exhibited the highest expression ($p < 0.01$) and SK-OV-3 cells presented the lowest expression ($p < 0.001$, Figure 2A). Because SK-OV-3 and A2780 cells showed the most significant difference, we selected these two kinds of cells in the following experiments. Thereafter, the transfection of LIFR-AS1 overexpression plasmid or sh-LIFR-AS1 into A2780 and SK-OV-3 cells was successfully conducted that overexpression or silencing vector observably up-regulated or down-regulated the expression of LIFR-AS1 ($p < 0.05$, Figure 2B). It is worth noting that LIFR-AS1 overexpression contributed to the decrease in cell viability and proliferation, while LIFR-AS1 knockdown exerted the opposite effects ($p < 0.05$, Figure 2C–E).

3.3 | LIFR-AS1 overexpression inhibited the migration and invasion of SOC cells while silencing LIFR-AS1 had the opposite effect

As the data suggested, the migration rates of A2780 and SK-OV-3 cells were reduced by overexpressed LIFR-AS1 and elevated by sh-LIFR-AS1 ($p < 0.001$, Figure 3A,B). Additionally, LIFR-AS1 overexpression weakened the invasive ability of A2780 and SK-OV-3 cells ($p < 0.01$, Figure 3C,D), but LIFR-AS1 silencing exerted the opposite effect and enhanced the ability of cell invasion ($p < 0.001$, Figure 3C,D).

3.4 | LIFR-AS1 regulated the expressions of PCNA, cleaved caspase-3, E-cadherin, N-cadherin, and Snail in SOC cells

It can be observed in Figure 4 that LIFR-AS1 overexpression decreased the mRNA expressions of PCNA, N-cadherin, and Snail in

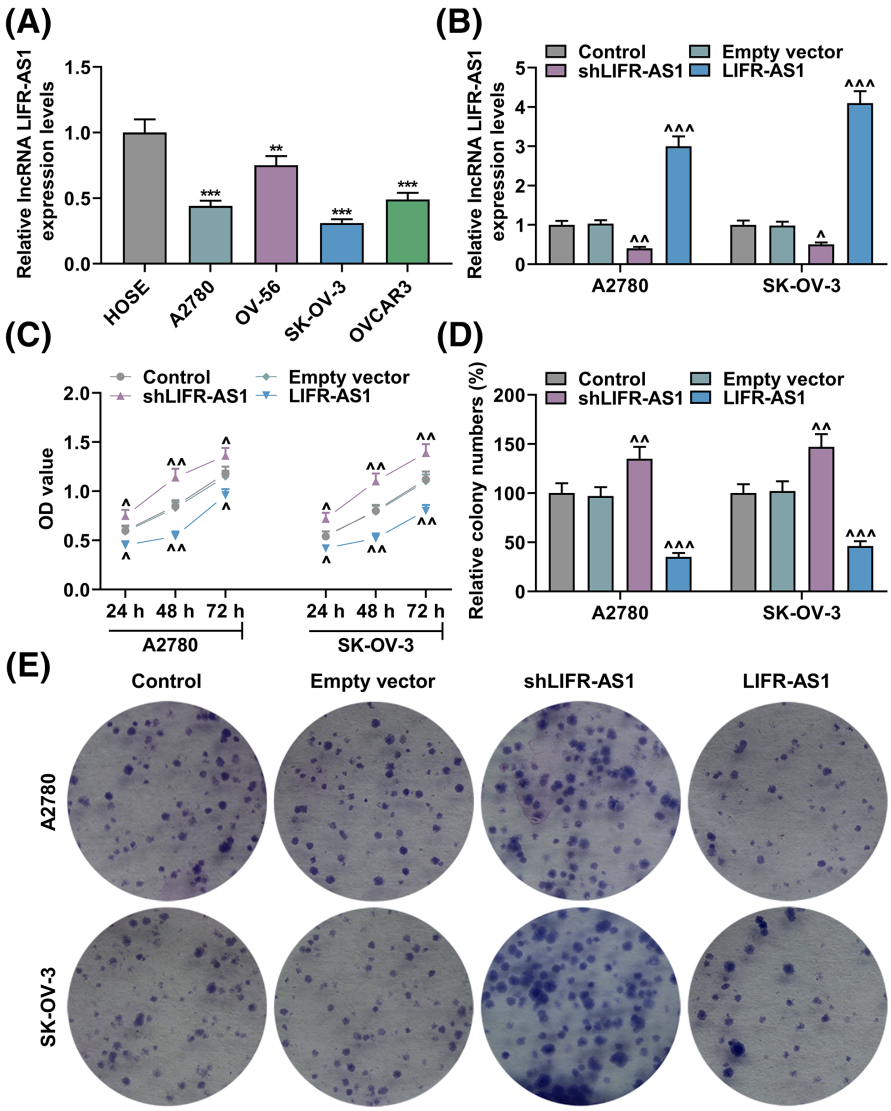


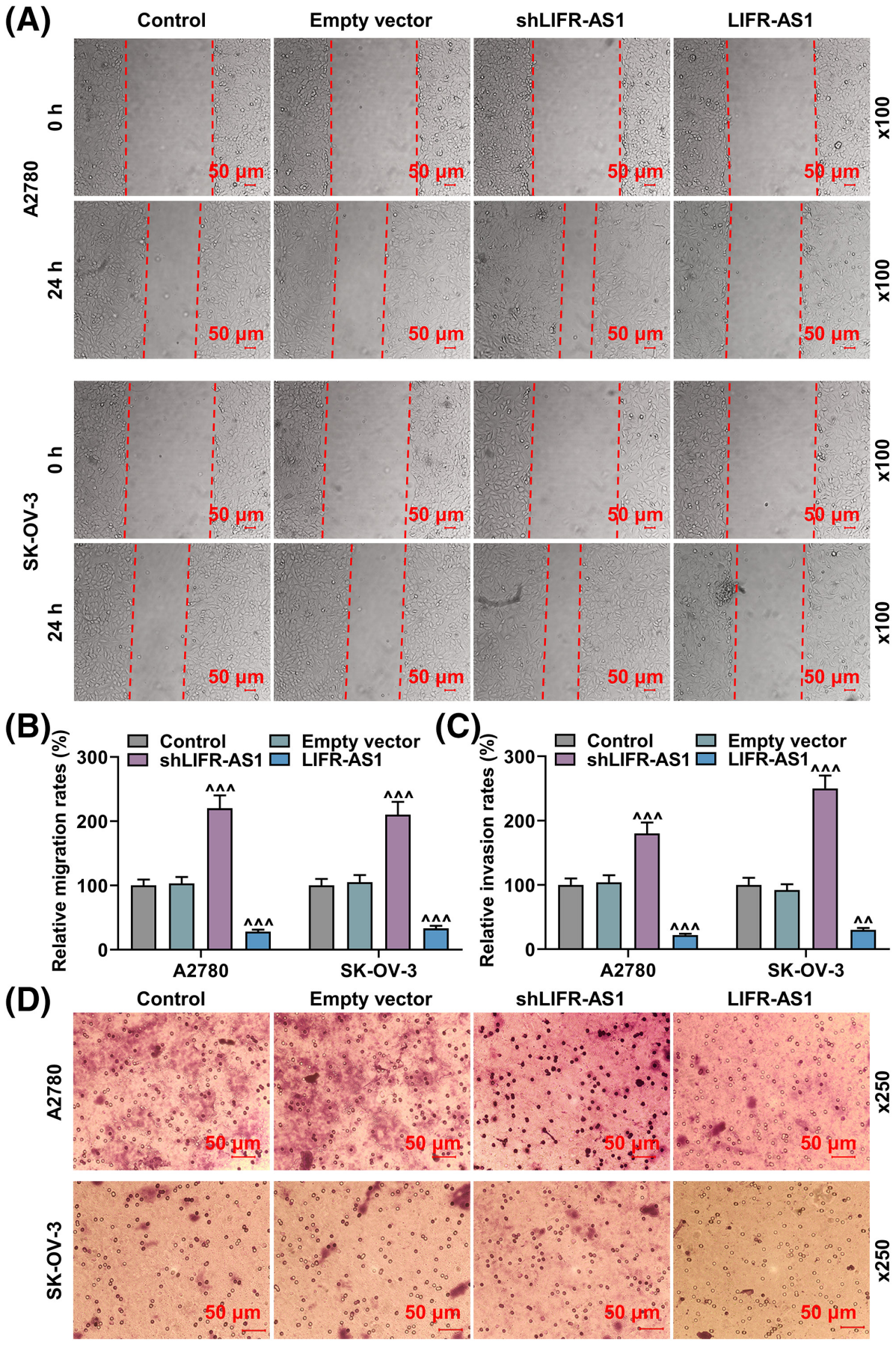
FIGURE 2 Effects of LIFR-AS1 on SOC cell viability and proliferation. (A) The expression of LIFR-AS1 in SOC cells was quantified by qRT-PCR. GAPDH was served as the internal reference. (B) QRT-PCR was utilized to measure the LIFR-AS1 expression in control, empty vector, sh-LIFR-AS1, and LIFR-AS1 groups. GAPDH was served as the internal control. (C) The viability of A2780 and SK-OV-3 cells was accessed by CCK-8 assay. (D,E) A2780 and SK-OV-3 cell proliferation was analyzed using colony formation assay. The data from three independent experiments were presented as the mean ± SD; $^{**}p < 0.01$; $^{***}p < 0.001$ vs. HOSE; $^{\wedge}p < 0.05$; $^{\wedge\wedge}p < 0.01$; $^{\wedge\wedge\wedge}p < 0.001$ vs. Empty vector. Abbreviation: qRT-PCR, quantitative real-time PCR; CCK-8, Cell Counting Kit-8; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SD, standard deviation

A2780 and SK-OV-3 cells ($p < 0.01$, Figure 4A and D), while increasing E-cadherin expression ($p < 0.001$, Figure 4A and D). Conversely, sh-LIFR-AS1 up-regulated the mRNA expressions of PCNA, N-cadherin, and Snail ($p < 0.01$, Figure 4A and D), but down-regulated the expression of E-cadherin in A2780 and SK-OV-3 cells ($p < 0.05$, Figure 4A and D). Simultaneously, the cleaved caspase-3 and E-cadherin protein expressions were increased ($p < 0.05$, Figure 4B,C and 4E,F), while PCNA, N-cadherin, and Snail expressions were decreased in LIFR-AS1 group when compared to empty vector group ($p < 0.05$, Figure 4B,C and 4E,F). Besides, LIFR-AS1 knockdown reversely regulated the protein expressions in A2780 and SK-OV-3 cells, which brought about the elevation of PCNA, N-cadherin, and Snail expressions ($p < 0.05$, Figure 4B,C and 4E,F), and the reduction in cleaved caspase-3 and E-cadherin expressions ($p < 0.05$, Figure 4B,C and 4E,F).

4 | DISCUSSION

As an important regulator, lncRNA has become a hotspot in researching SOC. Although lncRNAs could not encode protein, they can exert some effects on cellular behaviors such as cell proliferation, apoptosis, senescence, and migration by regulating the expressions of relevant functional genes.[15] The study of Ni et al. showed that down-regulated LINC00515 is correlated with the tumor growth of SOC.[28] Guo et al. proved that lncRNA SOCAR aggravates the development of SOC via the Wnt/β-catenin pathway.[29] Additionally, Gokulnath et al. indicated that lncRNA HAND2-AS1 has the function to suppress the tumor growth of SOC.[30] LIFR-AS1, which is an antisense lncRNA for the LIFR coding gene, has been noted that its aberrant expression is closely related to the pathogenesis and prognosis of patients with some tumors. The study by Okamura et al. found that LIFR-AS1 can

FIGURE 3 Effects of LIFR-AS1 on migration and invasion of SOC cells. (A,B) The migration rates of A2780 and SK-OV-3 cells were detected by wound healing assay (magnification ×100). (C,D) Transwell assay was used to determine the invasion of A2780 and SK-OV-3 cells (magnification ×250). All experiments were repeated three times to average. The data from three independent experiments were presented as the mean ± SD; $^{\wedge\wedge}p < 0.01$; $^{\wedge\wedge\wedge}p < 0.001$ vs. Empty vector. Abbreviation: SOC, serous ovarian carcinoma; SD, standard deviation


(A)
Control
Empty vector
shLIFR-AS1
LIFR-AS1
A2780
SK-OV-3
0 h
24 h
x100
50 μm
(B)
Relative migration rates (%)
Control
Empty vector
shLIFR-AS1
LIFR-AS1
300
200
100
0
^^^
A2780
SK-OV-3
(C)
Relative invasion rates (%)
Control
Empty vector
shLIFR-AS1
LIFR-AS1
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A2780
SK-OV-3
(D)
Control
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A2780
SK-OV-3
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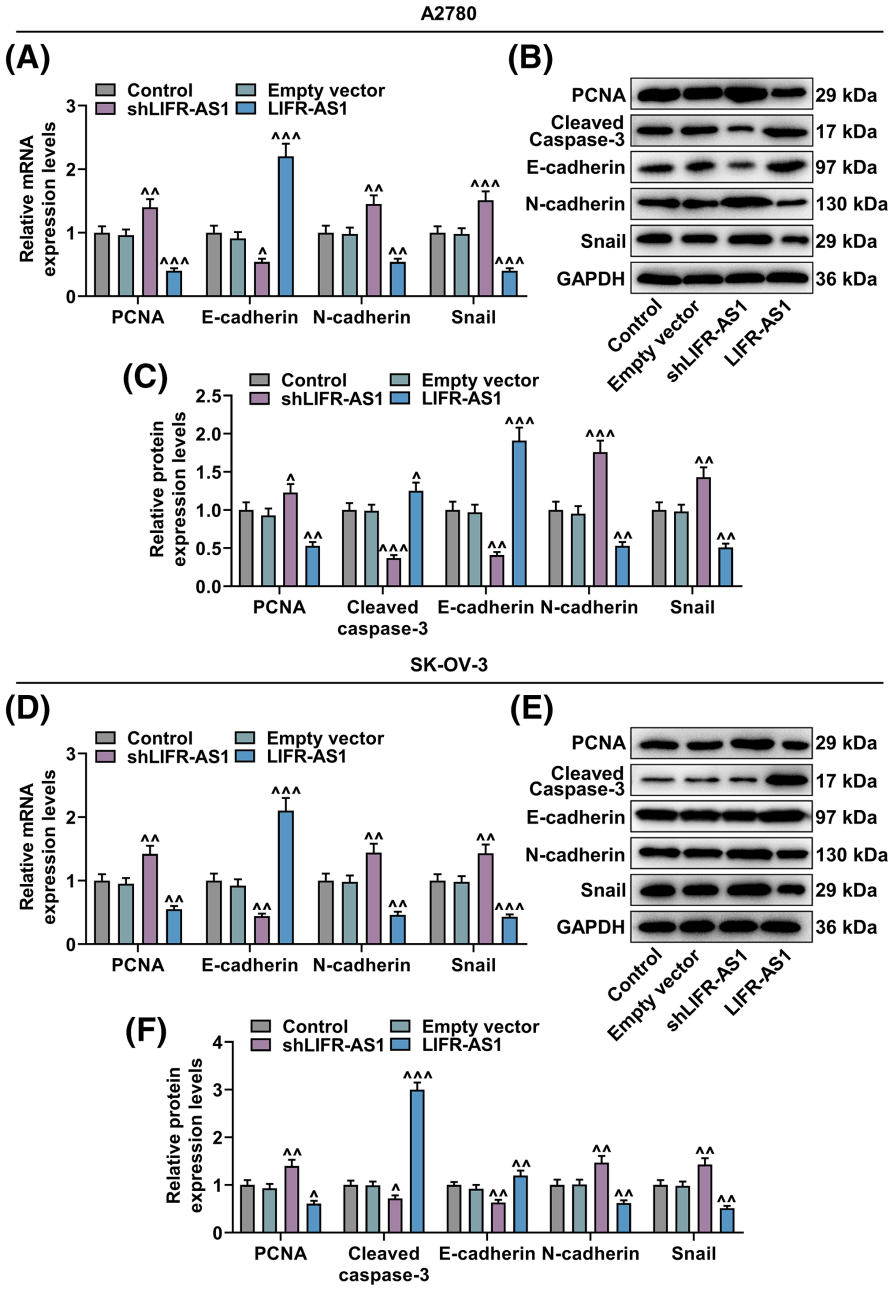

FIGURE 4 Effects of LIFR-AS1 on the expressions of genes related to proliferation and epithelial-mesenchymal transition in SOC cells. (A) The mRNA expressions of PCNA, E-cadherin, N-cadherin, and Snail in A2780 cells were measured by qRT-PCR. GAPDH was served as the internal reference. (B,C) The protein expressions of PCNA, cleaved caspase-3, E-cadherin, N-cadherin, and Snail in A2780 cells were detected by Western blot. GAPDH was employed as the internal reference. (D) The mRNA expressions of genes related to proliferation and epithelial-mesenchymal transition in SK-OV-3 cells were measured by qRT-PCR. GAPDH was served as the internal reference. (E,F) The protein expressions of genes related to proliferation and epithelial-mesenchymal transition in SK-OV-3 cells were determined by Western blot. GAPDH was served as the internal reference. All experiments were repeated three times to average. The data from three independent experiments were presented as the mean ± SD; $^{\wedge}p<0.05$; $^{\wedge\wedge}p<0.01$; $^{\wedge\wedge\wedge}p<0.001$ vs. Empty vector. Abbreviation: qRT-PCR, quantitative real-time PCR; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PCNA, proliferating cell nuclear antigen; SD, standard deviation

inhibit the proliferation and migration of hepatocellular carcinoma (HCC) cells and is an important indicator of poor prognosis in HCC.[31] Another study revealed that LIFR-AS1 up-regulation is positively correlated with more advanced and aggressive gastric cancer features.[32] Based on previous studies, this study explored the expression of LIFR-AS1 in SOC cells and its role in SOC progression. The results of this study signified that LIFR-AS1 was lowly expressed in SOC tissues and cells (A2780, OV-56, SK-OV-3, and OVCAR3). LIFR-AS1 overexpression could inhibit the cell viability, proliferation, migration, and invasion in A2780 and SK-OV-3 cells.

The indefinite proliferation and hampered apoptosis of the cells are the main characteristics of the tumor cells, such as SOC cells.[33,34] PCNA is a nuclear protein widely expressed in S phase of cell cycle and is only synthesized and expressed in proliferative cells. The positive expression of PCNA indicates that the cell is in proliferative state.[35,36] As a tumor marker protein, PCNA can reflect the synthesis and metabolism of RNA and DNA in tumor cells, its expression level can be measured to assess the proliferative activity of tissues and various cancer cells, contributing to better determining progression, aggressiveness, and prognosis of the lesions.[36] It has proved that down-regulation of the expression of PCNA repressed G1/S cell cycle transition of human OC cells.[37] Additionally, lncRNA DLX6-AS1 inhibited the proliferation of OC cells via reducing the expression of PCNA.[38] Caspase-3 is the most important final executor of apoptosis in caspase family and has a strong ability to induce apoptosis.[39] Studies evidenced that lncRNA NNT-AS1 knockdown or lncRNA PCAT-1 overexpression weakens the activity of caspase-3 to impede the apoptosis of human OC cells.[40,41] Similar to these studies, our

study got the knowledge that LIFR-AS1 overexpression decreased the expression of PCNA and increased the cleaved caspase-3 expression of SOC cells, and silencing of LIFR-AS1 reversely regulated these expressions, indicating that LIFR-AS1 overexpression hindered proliferation and induced apoptosis of SOC cells by regulating the levels of related molecules.

Invasion and metastasis are important contributors to the vast majority of tumor-associated metastasis and recurrence. Epithelial–mesenchymal transition (EMT) is an important mechanism of invasion and metastasis.[42] It refers to the pathophysiological process of epithelial cells with polar and tight adhesion into non-polar and highly mobile stromal cells.[42] EMT has been observed in a variety of human malignant tumors,[43,44] including SOC.[45] In this study, we detected the expressions of three EMT-related proteins, including E-cadherin, N-cadherin, and Snail. E-cadherin participates in the adhesion and connection between homotypic cells and maintains cell polarity, which plays an important role in maintaining the integrity of epithelial cell morphology and tissue structure. The overexpression of E-cadherin protein may inhibit tumor occurrence and metastasis, while the effect of N-cadherin is opposite to that of E-cadherin, and its expression can promote tumor invasion and metastasis.[46] Besides, Snail, as one of EMT-related transcription factors, could regulate the expression level of E-cadherin, and is also a key factor in EMT.[47] Massive amounts of evidence proved that lncRNAs enhance the migratory and invasive abilities of OC or SOC cells via regulating the EMT-regulated genes. For instance, lncRNA OIP5-AS1 up-regulates Snail expression to promote OC cell invasion and migration,[48] lncRNA EBIC promotes metastasis of OC cells through up-regulating the E-cadherin expression,[49] and lncRNA HAL suppresses the metastasis of SOC cells by regulating the expressions of E-cadherin and N-cadherin to inhibit EMT signaling pathway.[50] Consistent with the results described in the previous literature, our study discovered that LIFR-AS1 overexpression facilitated an anti-metastatic phenotype in SOC by regulating EMT-related genes. Concretely, LIFR-AS1 overexpression increased the expression of E-cadherin, but decreased N-cadherin and Snail expressions. Moreover, silencing LIFR-AS1 regulated these expressions in a reverse way.

To sum up, our study demonstrates that LIFR-AS1 expression is down-regulated in SOC, and LIFR-AS1 overexpression inhibits SOC cell viability, proliferation, invasion, and migration by regulating the expressions of PCNA, cleaved caspase-3, and EMT-related genes. Our findings may provide the potential of LIFR-AS1, as a therapeutic target for SOC.

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Not applicable.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

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REFERENCE

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin*. 2015;65(2):87-108.
2. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer*. 2015;136(5):E359-E386.
3. Hatano Y, Hatano K, Tamada M, et al. A comprehensive review of ovarian serous carcinoma. *Adv Anat Pathol*. 2019;26(5):329-339.
4. Kuhn E, Kurman RJ, Shih IM. Ovarian cancer is an imported disease: fact or fiction? *Curr Obstet Gynecol Rep*. 2012;1(1):1-9.
5. Gilbert L, Basso O, Sampalis J, et al. Assessment of symptomatic women for early diagnosis of ovarian cancer: results from the prospective DOvE pilot project. *Lancet Oncol*. 2012;13(3):285-291.
6. Lisio MA, Fu L, Goyeneche A, Gao ZH, Telleria C. High-grade serous ovarian cancer: basic sciences, clinical and therapeutic standpoints. *Int J Mol Sci*. 2019;20(4):952.
7. Maruyama R, Suzuki H. Long noncoding RNA involvement in cancer. *BMB Rep*. 2012;45(11):604-611.
8. Scheuermann JC, Boyer LA. Getting to the heart of the matter: long non-coding RNAs in cardiac development and disease. *EMBO J*. 2013;32(13):1805-1816.
9. Cabili MN, Trapnell C, Goff L, et al. Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. *Genes Dev*. 2011;25(18):1915-1927.
10. Gutschner T, Diederichs S. The hallmarks of cancer: a long non-coding RNA point of view. *RNA Biol*. 2012;9(6):703-719.
11. Gokulnath P, de Cristofaro T, Manipur I, et al. Long non-coding RNA MAGI2-AS3 is a new player with a tumor suppressive role in high grade serous ovarian carcinoma. *Cancer*. 2019;11(12):2008.
12. Zhu L, Guo Q, Lu X, et al. CTD-2020K17.1, a novel long non-coding RNA, promotes migration, invasion, and proliferation of serous ovarian cancer cells in vitro. *Med Sci Monit*. 2018;24:1329-1339.
13. Yong W, Yu D, Jun Z, et al. Long noncoding RNA NEAT1, regulated by LIN28B, promotes cell proliferation and migration through sponging miR-506 in high-grade serous ovarian cancer. *Cell Death Dis*. 2018;9(9):861.
14. Tian D, Sun S, Lee JT. The long noncoding RNA, Jpx, is a molecular switch for X chromosome inactivation. *Cell*. 2010;143(3):390-403.
15. Ørom UA, Derrien T, Beringer M, et al. Long noncoding RNAs with enhancer-like function in human cells. *Cell*. 2010;143(1):46-58.
16. Mattick JS. The genetic signatures of noncoding RNAs. *PLoS Genet*. 2009;5(4):e1000459.
17. Balbin OA, Malik R, Dhanasekaran SM, et al. The landscape of antisense gene expression in human cancers. *Genome Res*. 2015;25(7):1068-1079.
18. Nolasco S, Bellido J, Gonçalves J, Tavares A, Zabala JC, Soares H. The expression of tubulin cofactor A (TBCA) is regulated by a noncoding antisense Tbca RNA during testis maturation. *PloS one*. 2012;7(8):e42536.
19. Liu K, Yao H, Wen Y, et al. Functional role of a long non-coding RNA LIFR-AS1/miR-29a/TNFAIP3 axis in colorectal cancer resistance to pohotodynamic therapy. *Biochim Biophys Acta Mol Basis Dis*. 2018;1864(9 Pt B):2871-2880.
20. Chen D, Sun Y, Wei Y, et al. LIFR is a breast cancer metastasis suppressor upstream of the Hippo-YAP pathway and a prognostic marker. *Nat Med*. 2012;18(10):1511-1517.
21. Johnson RW, Finger EC, Olcina MM, et al. Induction of LIFR confers a dormancy phenotype in breast cancer cells disseminated to the bone marrow. *Nat Cell Biol*. 2016;18(10):1078-1089.

22. Wu HX, Cheng X, Jing XQ, et al. LIFR promotes tumor angiogenesis by up-regulating IL-8 levels in colorectal cancer. *Biochim Biophys Acta Mol Basis Dis*. 2018;1864(9 Pt B):2769-2784.
23. Xu F, Li H, Hu C. LIFR-AS1 modulates Sufu to inhibit cell proliferation and migration by miR-197-3p in breast cancer. *Biosci Rep*. 2019;39(7):BSR20180551.
24. Wang Q, Wu J, Huang H, et al. lncRNA LIFR-AS1 suppresses invasion and metastasis of non-small cell lung cancer via the miR-942-5p/ZNF471 axis. *Cancer Cell Int*. 2020;20:180.
25. Ding H, Cui L, Wang C. Long noncoding RNA LIFR-AS1 suppresses proliferation, migration and invasion and promotes apoptosis through modulating miR-4262/NF-κB pathway in glioma. *Neurol Res*. 2020;1-10:210-219.
26. Hauptmann S, Friedrich K, Redline R, Avril S. Ovarian borderline tumors in the 2014 WHO classification: evolving concepts and diagnostic criteria. *Virchows Arch*. 2017;470(2):125-142.
27. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C[T]) Method. *Methods*. 2001;25(4):402-408.
28. Ni MW, Zhou J, Zhang YL, et al. Downregulation of LINC00515 in high-grade serous ovarian cancer and its relationship with platinum resistance. *Biomark Med*. 2019;13(7):535-543.
29. Guo Q, Wang L, Zhu L, et al. The clinical significance and biological function of lncRNA SOCAR in serous ovarian carcinoma. *Gene*. 2019;713:143969.
30. Gokulnath P, de Cristofaro T, Manipur I, et al. Long non-coding RNA HAND2-AS1 acts as a tumor suppressor in high-grade serous ovarian carcinoma. *Int J Mol Sci*. 2020;21(11):4059.
31. Okamura Y, Nomoto S, Kanda M, et al. Leukemia inhibitory factor receptor (LIFR) is detected as a novel suppressor gene of hepatocellular carcinoma using double-combination array. *Cancer Lett*. 2010;289(2):170-177.
32. Zhao Z, Ji M, Wang Q, He N, Li Y. miR-16-5p/PDK4-mediated metabolic reprogramming is involved in chemoresistance of cervical cancer. *Mol Ther Oncolytics*. 2020;17:509-517.
33. Justiniano SE, Mathew A, Mitra S, Manivannan SN, Simcox A. Loss of the tumor suppressor Pten promotes proliferation of Drosophila melanogaster cells in vitro and gives rise to continuous cell lines. *PloS one*. 2012;7(2):e31417.
34. Qiu JJ, Wang Y, Ding JX, Jin HY, Yang G, Hua KQ. The long noncoding RNA HOTAIR promotes the proliferation of serous ovarian cancer cells through the regulation of cell cycle arrest and apoptosis. *Exp Cell Res*. 2015;333(2):238-248.
35. Ulrich HD, Takahashi T. Readers of PCNA modifications. *Chromosoma*. 2013;122(4):259-274.
36. Nafarzadeh S, Seyedmajidi M, Jafari S, Bijani A, Rostami-Sarokolaei A. A comparative study of PCNA and Ki-67 expression in dental follicle, dentigerous cyst, unicystic ameloblastoma and ameloblastoma. *Int J Mol Cell Med*. 2013;2(1):27-33.
37. Zhang Q, Chen X, Zhang X, Zhan J, Chen J. Knockdown of TMEM14A expression by RNAi inhibits the proliferation and invasion of human ovarian cancer cells. *Biosci Rep*. 2016;36(1):e00298.
38. Kong L, Zhang C. LncRNA DLX6-AS1 aggravates the development of ovarian cancer via modulating FHL2 by sponging miR-195-5p. *Cancer Cell Int*. 2020;20:370.
39. Zhu J, Zhang W, Zhang Y, Wang Y, Liu M, Liu Y. Effects of Spica prunellae on caspase-3-associated proliferation and apoptosis in human lung cancer cells in vitro. *J Cancer Res Ther*. 2018;14(4):760-763.
40. Huang Y, Shi J, Xu Y. Long non-coding RNA NNT-AS1 contributes to cell proliferation, metastasis and apoptosis in human ovarian cancer. *Oncol Lett*. 2018;15(6):9264-9270.
41. Min F, Chu G. Long noncoding RNA PCAT-1 knockdown prevents the development of ovarian cancer cells via microRNA-124-3p. *J Cell Biochem*. 2020;121(2):1963-1972.
42. Chen T, You Y, Jiang H, Wang ZZ. Epithelial-mesenchymal transition (EMT): A biological process in the development, stem cell differentiation, and tumorigenesis. *J Cell Physiol*. 2017;232(12):3261-3272.
43. Colas E, Pedrola N, Devis L, et al. The EMT signaling pathways in endometrial carcinoma. *Clin Transl Oncol*. 2012;14(10):715-720.
44. Yeung KT, Yang J. Epithelial-mesenchymal transition in tumor metastasis. *Mol Oncol*. 2017;11(1):28-39.
45. Cheng JC, Auersperg N, Leung PC. TGF-beta induces serous borderline ovarian tumor cell invasion by activating EMT but triggers apoptosis in low-grade serous ovarian carcinoma cells. *PloS one*. 2012;7(8):e42436.
46. Gravdal K, Halvorsen OJ, Haukaas SA, Akslen LA. A switch from E-cadherin to N-cadherin expression indicates epithelial to mesenchymal transition and is of strong and independent importance for the progress of prostate cancer. *Clin Cancer Res*. 2007;13(23):7003-7011.
47. Wang Y, Shi J, Chai K, Ying X, Zhou BP. The role of snail in EMT and tumorigenesis. *Curr Cancer Drug Targets*. 2013;13(9):963-972.
48. Jiang X, Ye Z, Jiang Y, Yu W, Fang Q. LncRNA OIP5-AS1 upregulates Snail expression by sponging miR-34a to promote ovarian carcinoma cell invasion and migration. *Biol Res*. 2020;53(1):49.
49. Xu QF, Tang YX, Wang X. LncRNA EBIC promoted proliferation, metastasis and cisplatin resistance of ovarian cancer cells and predicted poor survival in ovarian cancer patients. *Eur Rev Med Pharmacol Sci*. 2018;22(14):4440-4447.
50. Wu K, Li L, Li L, Wang D. Long non-coding RNA HAL suppresses the migration and invasion of serous ovarian cancer by inhibiting EMT signaling pathway. *Biosci Rep*. 2020;40(3):BSR20194496.

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