

# LncRNA LUCAT1 promotes proliferation of ovarian cancer cells by regulating miR-199a-5p expression

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**Abstract.** – **OBJECTIVE:** To investigate the biological effect of long non-coding ribonucleic acid (lncRNA) lung cancer-associated transcript 1 (LUCAT1) in the development of ovarian cancer.

**MATERIALS AND METHODS:** Real-time quantitative polymerase chain reaction (RT-qPCR) was utilized to detect the expression levels of lncRNA LUCAT1 in three human ovarian cancer cell lines (CaoV-3, SK-OV-3 and HO-8910) and the normal human ovarian surface epithelial cell line (IOSE80). Small interfering RNAs against lncRNA LUCAT1 (si-LUCAT1) were transfected into SK-OV-3 cells. Transfection efficiency of si-LUCAT1 was verified *via* RT-qPCR. Cell Counting Kit-8 (CCK-8) and colony formation assays were performed to test the effect of silencing lncRNA LUCAT1 on SK-OV-3 cell proliferation. The apoptosis was measured by flow cytometry. The miRcode database was searched to predict potential microRNAs (miRNAs) binding lncRNA LUCAT1. It was found that lncRNA LUCAT1 contained a highly conserved binding site of miR-199a-5p in the 3'-untranslated region (3'-UTR). Subsequently, the targeting relationship between them was determined through Dual-Luciferase reporter gene assay and RT-qPCR analysis.

**RESULTS:** LncRNA LUCAT1 was highly expressed in three human ovarian cancer cell lines compared to that in normal ovarian surface epithelial cell line ( $p < 0.05$ ). The cell proliferation rate in SK-OV-3 cells with lncRNA LUCAT1 knockdown was remarkably lower in comparison to that in control group. Moreover, colony formation assay also revealed that the number of cell clones decreased significantly after knockdown of lncRNA LUCAT1 compared to that in control group ( $p < 0.05$ ). In addition, the apoptosis rate was distinctly elevated in the lncRNA LUCAT1 silencing group ( $p < 0.05$ ). Furthermore, a highly conserved binding site of miR-199a-5p was found in the 3'-UTR of lncRNA LUCAT1. Dual-Luciferase reporter gene assay exhibited that the Luciferase activity of LUCAT1-wt was significantly reduced after overexpression of miR-199a-5p ( $p < 0.05$ ), while that of LUCAT1-mut

was unchangeable. Further analysis *via* RT-qPCR suggested that miR-199a-5p overexpression significantly decreased the expression level of lncRNA LUCAT1 ( $p < 0.05$ ).

**CONCLUSIONS:** LncRNA LUCAT1 is overexpressed in ovarian cancer cells, which may target miR-199a-5p to exert its effects on driving the malignant development of ovarian cancer.

*Key Words:*

LncRNA LUCAT1, Ovarian cancer, MiR-199a-5p, Proliferation, Apoptosis.

## Introduction

Ovarian cancer is the seventh most common cancer among women worldwide, accounting for approximately 4% of all new cases of female cancers<sup>1</sup>. According to the latest data, there were 22,240 new cases diagnosed with ovarian cancer and 14,070 deaths in 2018<sup>2</sup>. Several factors have been identified that can induce tumorigenesis of ovarian cancer in women, such as early or late stage of menarche and the number of menstrual cycles in a lifetime<sup>3</sup>. Given that ovarian cancer is mostly diagnosed at the advanced stage due to the lack of effective screening tests, it is necessary to diagnose disease as early as possible and detect its asymptomatic nature at an early stage. Although the first-line chemotherapy is effective in about 80% of patients with ovarian cancer, relapse occurs in more than 70% of patients with advanced ovarian cancer<sup>4</sup>. Increasing studies have focused on the roles of long non-coding ribonucleic acids (lncRNAs) in the occurrence of ovarian cancer.

LncRNA is a type of nucleotide sequence with more than 200 nucleotides and it has no protein-coding capability<sup>5</sup>. Several reports have indicated that lncRNAs are widely involved in various biological processes of tumor cells, such as cell proliferation, differentiation, and apoptosis<sup>6</sup>.

Moreover, lncRNAs can work by regulating transcription, modifying various post-transcriptional processes, and recruiting chromatin remodeling complexes<sup>7</sup>. It is important to identify cancer-related lncRNAs and explore their molecular and biological effects. By investigating the molecular mechanism of lncRNA lung cancer-associated transcript 1 (LUCAT1) in triple-negative breast cancer, Mou et al<sup>8</sup> found out that lncRNA LUCAT1 expression is upregulated in triple-negative breast cancer samples and cells. Moreover, the high expression of LUCAT1 is closely related to the advanced stage and poor prognosis of triple-negative breast cancer. LncRNA LUCAT1 induces the incidence and metastasis of triple-negative breast cancer by targeting miR-5702, which provides clues for improving the treatment of triple-negative breast cancer. However, the biological effects and regulatory mechanism of lncRNA LUCAT1 in proliferation and apoptosis of ovarian cancer cells remain unknown. Therefore, the function of lncRNA LUCAT1 in ovarian cancer and its potential relationship with microRNAs (miRNAs) were discussed and may provide new targets for the diagnosis and treatment of ovarian cancer.

## Materials and Methods

### Materials

Roswell Park Memorial Institute-1640 (RPMI-1640) medium and fetal bovine serum (FBS; Sigma-Aldrich, St. Louis, MO, USA), Apoptosis Detection Kit (Beyotime Biotechnology, Shanghai, China), Cell Counting Kit-8 (CCK-8) Reagent (Annoron, Beijing, China), Lipofectamine RNAiMAX Reagent and TRIzol Reagent (Invitrogen, Carlsbad, CA, USA), PrimeScript<sup>TM</sup> RT reagent Kit and SYBR Premix EX Taq Kit (TaKaRa, Otsu, Shiga, Japan), pmirGLO plasmid and Dual-Luciferase<sup>®</sup> Reporter Assay System (Promega, Madison, WI, USA).

### Cell Culture

Three human ovarian cancer cell lines (CaoV-3, SK-OV-3, and HO-8910) and the normal human ovarian surface epithelial cell line (IOSE80) were purchased from the Cell Bank of Type Culture Collection Committee of Chinese Academy of Sciences (Shanghai, China). All the cells were cultured in RPMI-1640 medium containing 10% FBS and 1% penicillin-streptomycin in a humidified incubator (37°C, 5% CO<sub>2</sub>).

### Cell Transfection

Small interfering RNAs against lncRNA LUCAT1 (si-LUCAT1) and miR-199a-5p mimics or inhibitors, as well as corresponding controls were designed and synthesized by Shanghai GenePharma Co., Ltd. (Shanghai, China). Lipofectamine RNAiMAX was adopted for transfection. The efficiency was verified *via* real-time quantitative polymerase chain reaction (RT-qPCR) at 24 h after transfection.

### RT-qPCR Analysis

The total RNA was extracted using TRIzol reagent. Subsequently, cDNA was reversely transcribed from 1 µg of total RNA using reverse transcription kit. The qPCR was performed on an ABI 7500 qPCR instrument using SYBR Premix EX Taq Kit. Three replicate wells were set for each sample. All primers were designed and synthesized by Shanghai GenePharma Co., Ltd. (Shanghai, China). Primer sequences were listed as follows: lncRNA LUCAT1: Forward: 5'-AAGGTCCATATTAACGTCCTACAA-3', Reverse: 5'-TAGCCATTAGACTGCCAGAGGA-3'. MiR-199a-5p: Forward: 5'-GCCAAGCCCAGTGTTTCAGAC-3', Reverse: 5'-GTGCAGGGTCCGAGGTATTC-3'. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH): Forward: 5'-CGACTTATACATGGCCTTA-3', Reverse: 5'-TTCCGATCACTGTTGGAAT-3'. U6: Forward: 5'-CTCGCTTCGGCAGCACACA-3', Reverse: 5'-AACGCTTCACGAATTTGCGT-3'. GAPDH and U6 were regarded as internal controls, and the relative expression levels of genes were calculated using the 2<sup>-ΔΔCt</sup> method.

### Cell Proliferation Analysis

The transfected cells (2×10<sup>4</sup>/well) were inoculated into a 96-well plate and incubated for 24 h, 48 h, and 72 h, separately. Next, 10 µL of CCK-8 reagent was added into each well, followed by incubation at 37°C for 3 h. Afterwards, the optical density (OD) value was measured at 450 nm on a microplate reader. Then, the viability curve was drawn with time of growth on the X-axis and OD on the Y-axis.

### Colony Formation Assay

The transfected cells (300/well) were inoculated into a 6-well plate and continuously cultured for 2 weeks. Colonies were fixed with formaldehyde for 10 min and stained with 1% crystal violet for 15 min. Afterwards, the colonies were photographed and counted.

### Apoptosis Analysis

The cells ( $5 \times 10^5$ ) at 48 h after transfection were collected and digested with trypsin. Then, the cells were suspended in binding buffer (500  $\mu$ L) and stained with Annexin V-FITC (5  $\mu$ L) and propidium iodide (PI; 5  $\mu$ L) in the dark at room temperature for 5 min. FACSCanto II Flow Cytometer was utilized to assess the apoptotic process.

### Detection of Luciferase Activity

The interaction between lncRNA LUCAT1 and miR-199a-5p was predicted by miRcode database. To detect the binding site of LUCAT1 and miR-375, wild-type LUCAT1 (LUCAT1-wt) and mutant LUCAT1 (LUCAT1-mut) (at the predicted complementary binding site of miR-199a-5p), Luciferase reporter plasmids were constructed. After 48 h of transfection, Luciferase activity in LUCAT1-wt or LUCAT1-mut was measured using the Dual-Luciferase Reporter Gene Assay System.

### Statistical Analysis

All data were expressed by mean  $\pm$  standard deviation ( $\bar{x} \pm s$ ). Differences between two groups were analyzed by the Student's *t*-test. Comparison between multiple groups was done using One-way ANOVA test, followed by Post-Hoc Test (Least Significant Difference). Besides, Bonferroni test was performed to analyze the data.  $p < 0.05$  suggested that the difference was statistically significant.

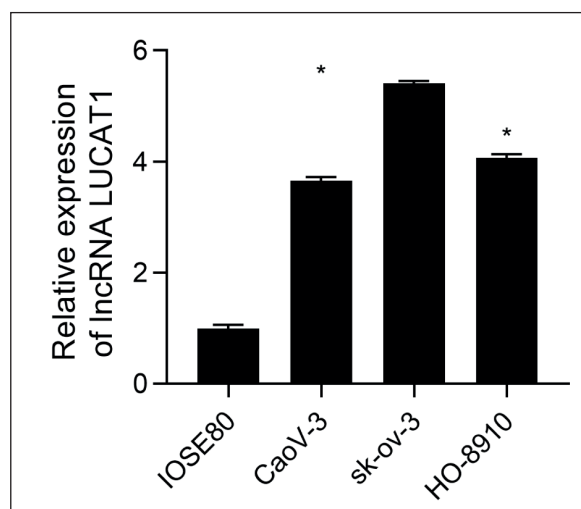
## Results

### Elevated Expression Level of LncRNA LUCAT1 In Ovarian Cancer Cells

First, RT-qPCR was utilized to detect the expression levels of lncRNA LUCAT1 in three human ovarian cancer cell lines (CaoV-3, SK-OV-3, and HO-8910) and the normal human ovarian surface epithelial cell line (IOSE80). It was found that lncRNA LUCAT1 was highly expressed in three human ovarian cancer cells compared to that in the normal ovarian surface epithelial cells (Figure 1).

### Effect of Silencing LncRNA LUCAT1 on SK-OV-3 Cell Proliferation

The function of lncRNA LUCAT1 in ovarian cancer was explored. Si-LUCAT1 was transfected into SK-OV-3 cells. Transfection efficiency of



**Figure 1.** Expression level of lncRNA LUCAT1 in ovarian cancer cells (\* $p < 0.05$ ).

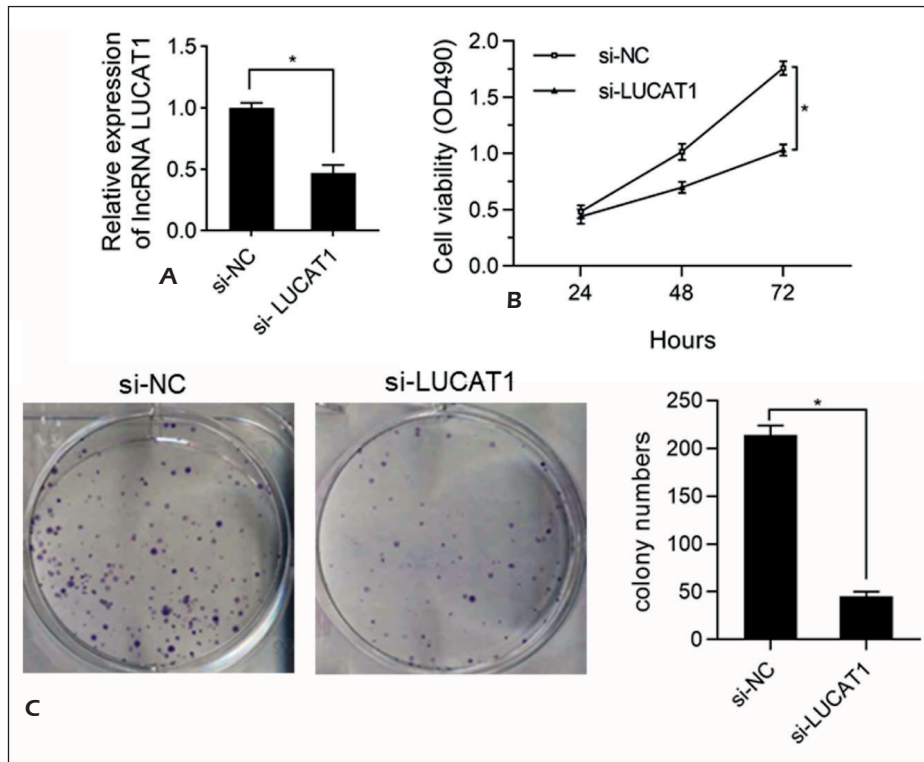
si-LUCAT1 was verified *via* RT-qPCR (Figure 2A). CCK-8 assay was performed to test the effect of silencing lncRNA LUCAT1 on SK-OV-3 cell proliferation. According to the results, the cell proliferation rate in the lncRNA LUCAT1 silencing group was remarkably lower in comparison with that in the control group (Figure 2B). Moreover, colony formation assay results also revealed that the number of cell clones in the lncRNA LUCAT1 silencing group decreased significantly compared to that in the control group (Figure 2C). The above results indicated that silencing lncRNA LUCAT1 inhibited the proliferation of ovarian cancer cells.

### Effect of Silencing LncRNA LUCAT1 on SK-OV-3 Cell Apoptosis

The apoptosis was measured by flow cytometry after SK-OV-3 cells were transfected with si-LUCAT1 for 48 h. The results revealed that the apoptosis rate was distinctly elevated in the lncRNA LUCAT1 silencing group compared to that in control group (Figure 3). Combined with the above results, it is suggested that lncRNA LUCAT1 may function as a tumor promoter in ovarian cancer.

### MiR-199a-5p Targeted 3'-Untranslated Region (3'-UTR) of LncRNA LUCAT1

To elucidate the molecular mechanism of lncRNA LUCAT1 as a tumor-promoting factor in ovarian cancer, miRcode database was searched to predict potential miRNAs binding lncRNA LUCAT1. It was found that lncRNA LUCAT1

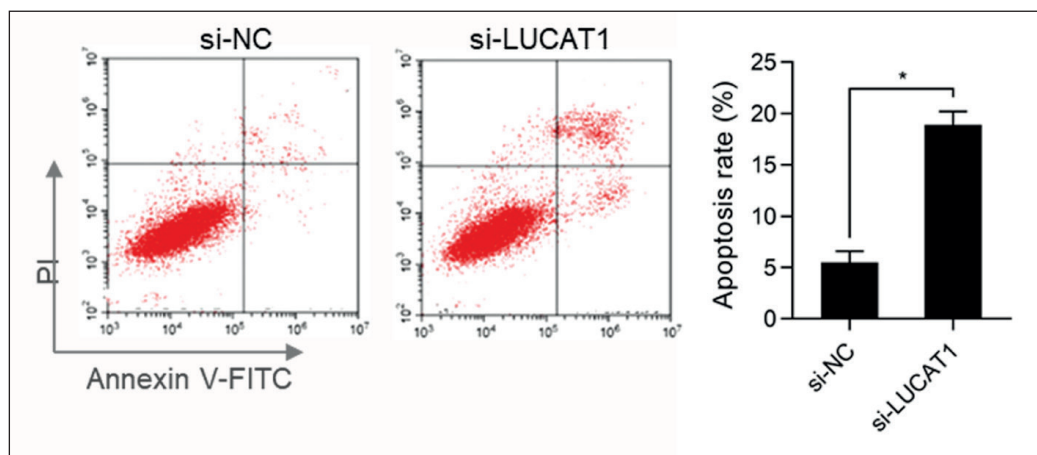


**Figure 2.** Effect of silencing lncRNA LUCAT1 on SK-OV-3 cell proliferation. **A**, Transfection efficiency of si-LUCAT1 verified via RT-qPCR. **B**, Effect of silencing lncRNA LUCAT1 on SK-OV-3 cell proliferation tested via CCK-8 assay. **C**, Effect of silencing lncRNA LUCAT1 on SK-OV-3 cell cloning ability analyzed via colony formation assay (magnification 40×) (\* $p < 0.05$ ).

contained a highly conserved binding site of miR-199a-5p in the 3'-UTR (Figure 4A). Furthermore, Dual-Luciferase reporter gene assay results exhibited that the Luciferase activity of LUCAT1-wt was significantly reduced in the miR-199a-5p mimic group, while that of LUCAT1-mut remained unchangeable (Figure 4B). The further analysis *via* RT-qPCR suggested that miR-199a-5p overexpression significantly decreased the expression level of lncRNA LUCAT1 (Figure 4C).

## Discussion

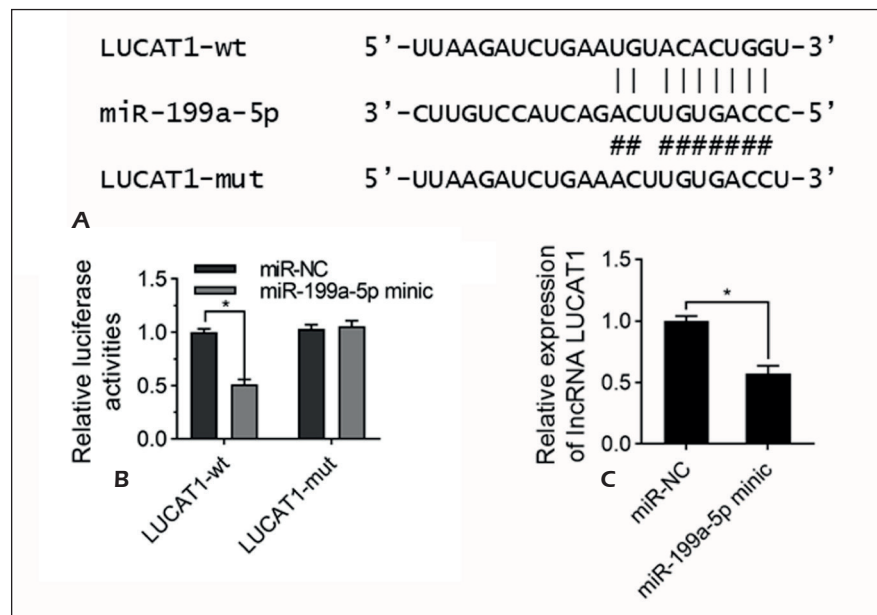
Ovarian cancer is the fifth leading cause of cancer-related mortality in female populations. It belongs to epithelial and germ cell-sex cord-stromal tumor based on its histological and genetic features. With the continuous deepening of the molecular mechanisms of the biological behaviors of malignant tumors, various new therapeutic methods have emerged. Targeted therapy that



**Figure 3.** Effect of silencing lncRNA LUCAT1 on SK-OV-3 cell apoptosis measured via flow cytometry (\* $p < 0.05$ ).



**Figure 4.** MiR-199a-5p targets the 3'-UTR of lncRNA LUCAT1. **A**, LncRNA LUCAT1 has a complementary binding site for miR-199a-5p in the 3'-UTR. **B**, Relative Luciferase activity in the two groups determined via Luciferase reporter gene assay. **C**, Changes in expression levels of lncRNA LUCAT1 after transfection of miR-199a-5p mimic measured via RT-qPCR.



is specific to tumor cell development, cell cycle regulation, apoptosis induction, and angiogenesis, has been well concerned<sup>9</sup>. It has been noted that there are multiple molecular therapeutic targets in the treatment of ovarian cancer, but the exact value remains to be confirmed by large-scale clinical studies. Meanwhile, the high heterogeneity, as well as multi-gene and multi-step occurrence and development of ovarian cancer, are also difficulties in the study of targeted drugs for ovarian cancer<sup>10</sup>. Therefore, to find more reasonable and effective molecular targets has become a key issue in the treatment of ovarian cancer.

LncRNAs have been proven to be involved in the pathogenesis of many cancers. The clinical significance and mechanism of lncRNA FLJ33360 in ovarian cancer was studied by Yang et al<sup>11</sup>. They demonstrated that FLJ33360 expression is significantly down regulated in ovarian cancer tissues and closely related to tumor staging and relapse. FLJ33360 may have potential values in detecting ovarian cancer. Functional analysis shows that FLJ33360 serves as a molecular sponge of miR-30b-3p to regulate the expression of target genes. These genes are mainly involved in the positive regulation of smooth muscle cell migration, unsaturated fatty acid metabolism, and the positive regulation of epithelial-mesenchymal transition. It is indicated that FLJ33360 functions as a biomarker in the early diagnosis and prognosis evaluation of ovarian cancer. Tong et al<sup>12</sup> evaluated the expression and function of human long non-coding RNA

activated by DNA damage (NORAD) in human epithelial ovarian cancer. In epithelial ovarian cancer cell lines, NORAD is significantly up-regulated. Lentivirus-mediated downregulation of NORAD has significant anti-cancer effects. Knockdown of NORAD inhibits cell proliferation, reduces the chemical resistance of bufalin, prevents cell cycle transitions, and suppresses the growth of xenografts. NORAD is probably repressed through endogenous competition with hsa-miR-155-5p, which may be a new tumor suppression strategy in epithelial ovarian cancer.

LUCAT1, first found in the airway epithelium of smokers, is a lncRNA located on chromosome 5<sup>13</sup>. LncRNA LUCAT1 over-expression can promote the proliferation of human non-small cell lung cancer by inhibiting p21 and p57 expressions<sup>14</sup>. LncRNA LUCAT1 inhibits the expressions of tumor suppressors by regulating the stability of DNMT113 and leads to the formation and invasion of esophageal squamous cell carcinoma<sup>15</sup>. In addition, the up regulation of lncRNA LUCAT1 is remarkably correlated with the malignant degree of clear cell renal cell carcinoma (ccRCC) and suggests a poor prognosis in ccRCC patients<sup>16</sup>. In this study, it was found that lncRNA LUCAT1 expression was significantly upregulated in ovarian cancer cells. In addition, silence of lncRNA LUCAT1 inhibited the proliferation of ovarian cancer cells and promoted their apoptosis. The above results indicated that lncRNA LUCAT1 promotes the incidence of ovarian cancer and may serve as an oncogene.

MiR-199a-5p has been detected to be involved in the regulation of multiple biological processes, including cell proliferation, apoptosis, migration, and invasion<sup>17,18</sup>. In recent years, miR-199a-5p is considered to be a tumor suppressor, which is down-regulated in prostatic cancer, gastric cancer, and non-small cell lung cancer<sup>19,20</sup>. In this study, miR-199a-5p targeted the 3'-UTR of lncRNA LUCAT1. Overexpression of miR-199a-5p markedly inhibited the expression of lncRNA LUCAT1.

## Conclusions

To sum up, we first observed that lncRNA LUCAT1 is highly expressed in ovarian cancer cells. Knockdown of lncRNA LUCAT1 inhibits the proliferation of ovarian cancer cells and promotes their apoptosis. lncRNA LUCAT1 may target miR-199a-5p to exert its effects.

## Conflict of Interests

The authors declare that they have no conflict of interests.

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