Abstract. – OBJECTIVE: Ovarian cancer is one of the leading causes of cancer-related death in women, but treatment remained unsatisfactory. Studies have shown that lncRNA colon cancer-associated transcript 1 (CCAT1) plays an important regulatory role in different cancers, but its role in ovarian cancer remained largely unclear.

PATIENTS AND METHODS: Quantitative Real-time polymerase chain reaction (qRT-PCR) was applied to detect the expression of lncRNA CCAT1 in ovarian cancer and adjacent tissue, and analysis was applied to explore the relationship between expression and clinical characteristic. After lncRNA CCAT1 suppression, Cell Counting Kit-8 (CCK8) and wound-healing assay were used to detect the proliferation and metastasis ability of ovarian cancer, respectively.

RESULTS: qRT-PCR showed that lncRNA CCAT1 was highly expressed in ovarian cancer tissue, compared with adjacent tissue. Moreover, we found that the expression of lncRNA CCAT1 was closely related to prognosis, tumor size, and lymph node metastasis. We also found that lncRNA CCAT1 could sponge miR-1290 in ovarian cancer.

CONCLUSIONS: In this study, we found that lncRNA CCAT1 could sponge miR-1290 in ovarian cancer, and was closely related to prognosis, proliferation, and metastasis.

Key Words: lncRNA CCAT1, Ovarian Cancer, miR-1290.

Introduction

According to a recent report¹, ovarian cancer (OC) is one of the most frequent cancers related death in women. It has been reported that about 238,700 new cases are diagnosed each year, and 151,900 patients use to die of this disease¹. Despite great advancement in surgery and chemotherapy have been made in past decades, the survival of advanced OC patients is less than 30%, compared with 90% in early stage OC patients²,³. One of the possible reasons might be the metastasis and abnormal proliferation of OC cells⁴. Therefore, it is important to explore the molecular mechanism of metastasis and proliferation in OC, which may provide a new target for therapy and diagnosis. Long non-coding RNA (lncRNA) is a kind of RNA with more than 200 nucleotides that do not code proteins. Recent researches showed that lncRNA could regulate different cell processes. What’s more, accumulating evidence showed that abnormal expression of lncRNAs was also found in different kinds of cancer and was involved in malignant activities (such as proliferation, metastasis, drug resistance, and so on) via diverse mechanisms. Qu et al⁵ showed that lncRNA was activated in renal cell carcinoma with sunitinib resistance (ARSR) and could bind with miR-34/miR-449 to facilitate axial length (AXL) and tyrosine-protein kinase Met (c-MET) expression in renal cell carcinoma, disseminating sunitinib resistance. Li et al⁶ observed that highly upregulated lncRNA in liver cancer (HULC) could bind to Y-box-binding protein 1 (YB-1), which could promote the phosphorylation of YB-1, leading to the release of YB-1 from target mRNA and activation of oncogenic mRNAs. Arab et al⁷ found that lncRNA transcription factor 21 (TCF21) antisense RNA inducing demethylation (TARID) could bind with TCF21 promoter and growth arrest and DNA-damage-inducible alpha (GADD45A), which could, in turn, recruit components of DNA demethylation, promoting demethylation of TCF21 promoter and activating the expression of TCF21. These papers suggested that lncRNA could regulate cancer processes at both genetic and epigenetic level, providing new targets for cancer treatment. However, the role of lncRNA in OC remained largely unknown.

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LncRNA CCAT1 promotes proliferation and metastasis of ovarian cancer via miR-1290

The lncRNA colon cancer-associated transcript 1 (CCAT1) has been reported to be highly expressed in colon cancer, gastric cancer, gallbladder cancer, hepatocellular carcinoma, breast cancer, and so on. lncRNA CCAT1 could enhance metastasis and proliferation of different cancer via interacting with different factors, such as cellular-myelocytomatosis (c-Myc), sprouty homolog 4 (SPRY4) and various miRs. These results suggested that lncRNA CCAT1 could be used as a novel biomarker to predict prognosis of patients. However, the mechanism and function of CCAT1 in OC remained largely unclear.

In this study, we first detected the expression of lncRNA CCAT1 in OC tissue and cell lines by quantitative Real-time polymerase chain reaction (qRT-PCR). Then, correlation analysis was used to show the relationship between the lncRNA CCAT1 and clinical significance. We also measured the expression of lncRNA CCAT1 in the proliferation and metastasis of OC cell lines. Finally, we showed that lncRNA CCAT1 could promote the malignant activity of OC by sponging miR-1290.

Patients and Methods

Tissue Collection
A total of 40 ovarian cancer tissue samples were collected from Lishui’s Central Hospital from May 2015 to June 2016. This research was approved by the Ethical Institutional Review Board of Lishui’s Central Hospital. All the patients were well informed for the use of samples and also signed the consent forms. No patients had received chemotherapy or radiotherapy before the surgery. Ovarian cancer and normal ovarian tissue specimens were divided into small piece after they were washed with RNase-free PBS (phosphate buffered solution) and, then, stored in liquid nitrogen until use.

Cell Culture
Human ovarian cancer cell lines OVCAR-8 and SKOV-3 were obtained from American Type Culture Collection (Manassas, VA, USA). IOSE386 and OMC685 were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). OMC685, OVCAR-8, and SKOV-3 were ovarian cancer cell lines, IOSE386 was normal human ovarian epithelial cell line, which was used as the control in this investigation. All cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM, HyClone, South Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, Rockville, MD, USA), 100 U/ml penicillin and 100 µg/ml streptomycin (Beyotime Biotechnology, Beijing, Beijing, China) at 37°C, 5% CO2.

qRT-PCR Procedure
Total RNA was extracted from tissue samples and cultured cells using the TRizol reagent (Invitrogen, Carlsbad, CA, USA) according to manufacturer’s instructions. The concentration of RNA was detected and the RNA solution was stored at -80°C for further use. Then cDNA was obtained by reverse transcription using the TaKaRa Reverse Transcriptase kit (TaKaRa, Dalian, Liaoning, China). The CCAT1 expression level was detected and quantified using Real-time qPCR with SYBR RT-PCR kit (TaKaRa, Dalian, Liaoning, China). The values were normalized to GAPDH, which was used as an endogenous control.

Cell Counting Kit-8 (CCK8) Assay
Cell proliferation was evaluated by CCK8 assay according to the manufacturer’s instructions. The cells were plated in 96-well plates at a density of 2000 cells per well with 200 ul cell suspension. Data were collected for 5 days and 3 replicate wells were set in each group. After 24 h, 10 ul of cell counting kit 8 (Dojundo, Kumamoto, Japan) were added into 100 µl of DMEM medium in each well. The plate was kept for 2 h at 37°C and the absorbance value was measured at 450 nm. The whole experiment was repeated 3 times.

Wound Healing Assay
Target cells were seeded into 6-well plate. When cells grew to 90% confluent, cells monolayers were scraped with a sterile micropipette tip. The wounded monolayers were washed by PBS to remove cell debris. The distance between the two edges of wound was measured in three different dimensions. 24 h later, the distance between the two edges were measured again.

Plasmid Transfection
ShRNA of lncRNA CCAT1 plasmid was purchased from GeneChem Company (Shanghai, Shanghai, China). Cells in 6-well plates at 80% confluency were placed in a fresh culture medium without FBS for 2 h before transfection. The plasmid was transfected into cells with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) for
8 h following the manufacturer’s protocol. 1 μg of plasmid and 1 μl of lipofectamine 2000 were added into 250 μl of medium without FBS respectively and incubated for 5 min. Diluted plasmid and lipofectamine 2000 were mixed and incubated for 30 min. Finally, the plasmid-lipid complex was added to the cells.

**Statistical Analysis**

All the experiments were repeated at least three times, and the data were presented as means ± standard deviation (SD). The Student’s *t*-test or one-way ANOVA (“Brown-Forsythe test” to validate ANOVA) was used to analyze the data to assess whether there is a significant difference among the groups. *p* < 0.05 was considered statistically significant. All statistical analyses were performed using the GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA).

**Results**

**lncRNA CCAT1 was Highly Upregulated in OC Tissue and Cell Lines**

To explore the role of lncRNA CCAT1 in OC, qRT-PCR was used to detect the expression of lncRNA CCAT1 in the 40 OC tissues, the adjacent tissue, the cultured OC cell line and the normal epithelia cell line. We found that the expression of lncRNA CCAT1 was highly expressed in OC tissue, compared with that in adjacent tissue (Figure 1A, *p* < 0.01). Meanwhile, the qRT-PCR result also showed that lncRNA CCAT1 was upregulated in OC cell lines (Figure 1B, *p* < 0.01). These results showed that lncRNA CCAT1 was upregulated in OC tissue and cell lines, but the effect of lncRNA CCAT1 in OC remained unclear.

**The Expression of lncRNA CCAT1 was Closely Related to Survival of OC Patients**

According to the results of qRT-PCR assay, we analyzed the correlation between expression of lncRNA CCAT1 and survival of OC patients. We found that patients with higher expression of lncRNA CCAT1 were associated with shorter survival time (Figure 2A, *p* < 0.01). Then, we also found that the expression of lncRNA CCAT1 was associated with the tumor size and lymph node metastasis of OC (Figure 2B and Figure 2C). Collectively, these results suggested that high expression of lncRNA CCAT1 could be used as an indicator of the survival time of OC patients.

**Knockdown of lncRNA CCAT1 Inhibit OC Cell Proliferation and Migration**

Previously, we found that lncRNA CCAT1 was closely related to tumor size and lymph node metastasis of OC patients, suggesting that lncRNA CCAT1 might be closely related to the proliferation and metastasis of OC cells. We used siRNA to suppress the expression of lncRNA CCAT1 in OC cell line OVCAR-8 and SKOV-3 (Figure 3A). Then, CCK8 assay and wound-healing assay were applied to detect the effect of lncRNA
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CCAT1 on proliferation and metastasis of OC cell lines. We found that suppression of lncRNA CCAT1 in OC cell line OVCAR-8 and SKOV-3 could significantly inhibit the proliferation ability (Figure 3B). The wound-healing assay showed that migration ability of OVCAR-8 and SKOV-3 transfected with siRNA of lncRNA CCAT1 was significantly decreased compared with that of si-NC group (Figure 3C). Taken together, these findings demonstrated that the silence of lncRNA CCAT1 could inhibit cell proliferation and migration in vitro.

Figure 2. The expression of lncRNA CCAT1 was closely related with survival of OC patients. A, Survival analysis of patients with different expression of lncRNA CCAT1, \( p < 0.01 \). B-C, The expression of lncRNA CCAT1 was closely related to the tumor size and lymph node metastasis, \( p < 0.01 \).

Figure 3. Knockdown of lncRNA CCAT1 inhibited OC cell proliferation and migration. A, The expression of lncRNA CCAT1 was suppressed by siRNA. B-C, Suppression of lncRNA CCAT1 in OMC685 and SKOV3 could inhibit the proliferation of OMC685 and SKOV3. D, Suppression of lncRNA CCAT1 could inhibit migration ability of OMC685 and SKOV3.
**IncRNA CCAT1 Could Sponge miR-1290 in OC Cells**

Studies have found that IncRNA CCAT1 could sponge miRNA in different cancers. Next, we used LNCipedia website to select miRNAs that could be sponged by IncRNA CCAT1. We found that miR-1290, miR-3190, miR-3679, miR-4328 could be sponged by IncRNA CCAT1 (http://www.lncipedia.org/). Furthermore, recent researches showed that miR-1290 could act as a tumor suppressor gene in gynecologic tumor. We would like to detect whether IncRNA CCAT1 could sponge miR-1290 in OC. Interestingly, we found that the expression of miR-1290 in OVCAR-8 and SKOV-3 was increased while IncRNA CCAT1 was suppressed (Figure 4A). Then, we transfected the inhibitor of miR-1290 into OVCAR-8 and SKOV-3 cells, CCK8 assay showed that inhibition of miR-1290 could restore the effect of IncRNA CCAT1 (Figure 4B). These results indicated that IncRNA CCAT1 could inhibit the proliferation of OVCAR-8 and SKOV-3 via miR-1290.

**Discussion**

OC is the leading cause of cancer-related death in women worldwide. Despite progress in therapy has been made in past decades, the mortality is still high, suggesting that new target should be found to improve the situation.

Many studies have shown that IncRNAs were closely implicated in regulating tumor processes, such as proliferation, metastasis, and apoptosis. There are also some reports showing that the expression of IncRNA could regulate the progression of OC. Zhang et al observed that IncRNA maternally expressed 3 (MEG3) could sponge miR-214 to downregulate the transfer mediated by extracellular vesicles, thereby reducing drug resistance in OC cells. Similarly, Yang et al reported that IncRNA urothelial carcinoma-associated 1 (UCAT1) could function as an endogenous sponge by directly binding to miR-485-5p, resulting in upregulating the expression of matrix metalloproteinase 14 (MMP14), thus promoting metastasis of OC. Chai et al found that IncRNA nuclear paraspeckle assembly transcript 1 (NEAT1) could be stabilized by human antigen R (HuR) protein and promote the progression of OC. In addition, some researches also showed that the expression of IncRNA was associated with the recurrence of OC, but the mechanism remained unclear, suggesting that IncRNA was important in regulating the procession of OC, and further study was needed.

In our work, we found that IncRNA CCAT1 was highly expressed in the OC tissue, compared with that in adjacent tissue using qRT-PCR assay. According to the analysis of IncRNA CCAT1 expression and clinical information from OC patients, we found that IncRNA CCAT1 was positively correlated with the tumor size and lymph node metastasis. Interestingly, this result was also confirmed by CCK8 assay and wound-healing assay in vitro. These studies suggested that IncRNA CCAT1 might act as an oncogene in OC.

Studies have demonstrated that IncRNA CCAT1 could act as sponge RNA in different cancer. Zhuang et al reported that IncRNA CCAT1 could promote proliferation and invasion of laryngeal squamous cell carcinoma by suppressing the expression of let-7 and promoting the expression of target genes such as high-mobility group AT-hook 2 (HMGA2) and c-Myc. Zhang et al showed that IncRNA CCAT1 could also interact with miR-218-5p in human retinoblastoma. A recent study showed that IncRNA CCAT1

**Figure 4.** IncRNA CCAT1 could sponge miR-1290 in OC cells. **A**, Suppression of IncRNA CCAT1 could increase the expression of miR-1290. **B-C**, Inhibition of miR-1290 could restore the proliferation ability of OC cell lines, $p < 0.05$. 

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could sponge let-7c, promoting the expression of c-Myc, which in turn upregulated the expression of CCAT1. These researches suggested that lncRNA CCAT1 may act as a sponge RNA in promoting tumor progression. However, whether lncRNA CCAT1 could regulate the expression of miRNA in OC remained unclear.

We found that miR-1290, along with miR-3190, miR-3679 and miR-4328, was the potential target for lncRNA CCAT1, and we intended to verify that miRNA could be sponged by lncRNA target for lncRNA CCAT1. The study demonstrated that miR-1290 acted as a tumor suppressor gene in gynecologic tumor. We then detected that inhibition of lncRNA CCAT1 could increase the expression of miR-1290. In addition, the suppression of miR-1290 could also promote the proliferation of OC cells, which was suppressed by si-CCAT1. These results showed that lncRNA CCAT1 might promote the progression of OC by sponging miR-1290.

Conclusions

We observed that lncRNA CCAT1 was highly expressed in OC, and closely related with survival of OC patients, tumor size and metastasis. We also found that lncRNA CCAT1 might sponge miR-1290 in OC cells to promote proliferation of OC. These results showed that lncRNA CCAT1 might be a potential target for OC treatment in future.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References


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