LINC00665 Affects the Malignant Biological Behavior of Ovarian Cancer via the miR-148b-3p/KLF5

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Research Article

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Abstract

Objectives: This study was to investigate the expression and clinical significance of long intergenic noncoding RNA 00665 (LINC00665) in ovarian cancer , as well as its effect on malignant biological behavior of ovarian cancer cells.

Methods: The expressions of LINC00665, miR-148b-3p and KLF5 in ovarian cancer (OC) tissues and cells were detected by RT-qPCR. Western blotting was used to detect KLF5 expression. The expression patterns of LINC00665, U6 and GAPDH in nuclear and cytoplasm fractions were detected using qRT-PCR. Besides, CCK-8 assay, clone formation assay, transwell, scratch test, and flow cytometry were respectively used to detect the cell activity, proliferation, invasiveness, healing of cells, and apoptosis rate of OC cells. Furthermore, the interactions between LINC00665 and miR-148b-3p and between miR-148b-3p and KLF5 were verified by luciferase reporter assay, and the correlations among these three genes were analyzed.

Results: LINC00665 expression was up-regulated in ovarian cancer cell lines and tissues. Si-LINC00665 inhibited cell proliferation, invasion and migration, and induced the apoptosis to a certain extent. Subcellular fraction assay revealed the location of LINC00665 was mainly situated in the cytoplasm. Besides, miR-148b-3p was a target of LINC00665, and KLF5 was directly targeted by miR-148b-3p. Si-LINC00665 inhibited KLF5 expression, miR-148b-3p inhibitor promoted KLF5 expression, and si-KLF5 inhibited LINC00665 expression. Interestingly, expressions of LINC00665 and miR-148b-3p, and expressions of miR-148b-3p and KLF5 were negatively correlated, while expressions of LINC00665 and KLF5 were positively correlated. In addition, si-KLF5 inhibited the malignant biological behavior of ovarian cancer cells, while miR-148b-3p inhibitor had the opposite effect. Most importantly, the si-LINC00665 could reverse the promotion effect of miR-148b-3p inhibitor on the malignant biological behavior of ovarian cancer cells.

Conclusions: LINC00665 can be used as an effective prognostic indicator of ovarian cancer, which has the potential to be a new drug target.

Introduction

Ovarian cancer is one of the three malignancies of the female reproductive system, which is the leading cause of female death ^[1, 2]. The ovary is located deep in the pelvic cavity, so early ovarian cancer is not easy to find. Many patients go to the clinic with symptoms at an advanced stage. Ovarian cancer is easy to metastasize and widely spread. The 5-year survival rate still lingers at 30%~40%, and the mortality rate ranks the first among gynecological malignant tumors ^[3]. Therefore, it is of great significance to explore the pathogenesis of ovarian cancer.

It has been reported that long-non-coding RNAs (lncRNAs) can present different types of high or low expression states in cancer. Moreover, lncRNAs play their roles through a variety of complex molecular mechanisms ^[4], such as affecting the epigenetic activity of tumor cells in the nucleus, gene transcription

and post-transcriptional modification of mRNA [5]. Long intergenic noncoding RNA 00665 (LINC00665) has been reported to be up-regulated in ovarian cancer [6]. Besides, a report on the potential mechanism of lncRNAs in lymphocyte infiltration showed that LINC00665 was positively correlated with lymphocyte infiltration of ovarian cancer [7]. What counts is that LINC00665 is reported to be a key regulator in many malignancies. Studies have shown that LINC00665 promotes breast cancer cell metastasis by triggering EMT [8]. Besides, dysfunction of LINC0066 plays a critical role in the progression of NSCLC, and its down-regulation can reduce cell proliferation and invasion [9]. Furthermore, LINC00665 is up-regulated in osteosarcoma, and its high expression is associated with poor prognosis of osteosarcoma [10]. Although LINC00665 plays an important role in cancer, its regulatory mechanism in the occurrence and development of ovarian cancer remains unclear. Through bioinformatics analysis, we found that LINC00665 may have a potential binding relationship with miR-148b-3p.

MiR-148b-3p is a miRNA that plays a key role in disease progression. It has been reported that miR-148b-3p plays an anticancer role in human cancers including gastric carcinoma [11], pituitary adenoma [12] and breast carcinoma [13]. Through bioinformatics analysis, we found that KLF5 is the potential target gene of miR-148b-3p. KLF5 is highly expressed in a variety of cancers [14, 15], and ovarian cancer is not excluded. We speculated that the LINC00665/ miR-148b-3p /KLF5 axis may exist in ovarian cancer and play an important regulatory role in the development of ovarian cancer.

In this study, the interaction of LINC00665/ miR-148b-3p /KLF5 axis in ovarian cancer tissues were detected, and the effect of LINC00665 on the biological function of ovarian cancer cells by regulating miR-148b-3p /KLF5 was also detected, which laid the experimental foundation for the later in-depth study.

Materials And Methods

2.1 Patients and tissue samples

30 pairs of ovarian cancer tissue samples and normal para-tumor tissue samples were taken from patients diagnosed with ovarian cancer at the Fifth People's Hospital of Qinghai Province. We excluded patients with other malignancies and those who had not received preoperative chemoradiotherapy. After surgical removal, tissues were collected and stored in liquid nitrogen until further use. According to the regulations of the International Federation of Obstetrics and Gynecology (FIGO), EOC's diagnosis, stage and risk status were determined. This study was permitted by the Ethics Committee of The Fifth People's Hospital of Qinghai Province .

2.2 Cell culture

The human EOC cell lines HO-8910, SKOV3, A2780 and normal human ovary surface epithelial cells (HOSE) were cultured in DMEM containing 10% fetal bovine serum. The above cell lines were inoculated at the atmosphere of 37 °C and 5% CO₂.

2.3 Transfection of ovarian cancer cells

Small interfering RNA (siRNA) targeting LINC00665 (si-LINC00665), si-NC, small interfering RNA (siRNA) targeting KLF5 (si-KLF5), miR-148b-3p mimic, NC-mimic, miR-148b-3p inhibitor and NC inhibitor sequences were synthesized by Shanghai GenePharma Co., Ltd. These plasmids (each 50 nM) were transfected into SKOV3 and A2780 cell lines using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

2.4 RT-qPCR Analysis

Total RNA was extracted by conventional RNA extraction according to the TRIzol Kit. Then follow the reverse transcription and PCR kit instructions. RT-qPCR amplification was performed using cDNA as template. Amplification condition was as follows: 95°C for 30 s, 95°C for 5 s, and 60°C for 45 s (40 cycles). Data analysis was made up of $2^{-\Delta\Delta Ct}$ method and GAPDH mRNA was used as the reference gene. The primer sequences used were as follows: LINC00665 Forward: 5'-TAAGCAGAGGGCTACAGAA-3', Reverse: 5'-CAGGGAGATCAGGAGGAC-

3'; miR-148b-3p Forward: 5'-TCTCTCCAGTCTACTCAGTGCATC-3'; Reverse: 5'-TATGGTTTTTGACGACTGTGTGAT-3'; KLF5 Forward: 5'-ACACCAGACCGC

AGCTCCA-3', Reverse: 5'-TCCATTGCTGCTGTCTGATTTGTAG-3'; GAPDH Forward: 5'-TTGCCATCAATGACCCCTTCA-3', Reverse: 5'-CGCCCCACTTGA

TTTTGGA-3'; U6 Forward: 5'-GCTTCGGCAGCACATATACTAAAAT-3', Reverse: 5'-CGCTTCACGAATTTGCGTGTCA-3'; β -actin Forward: 5'-TTGCCGACAGG

ATGCAGAA-3', Reverse: 5'-GCCGATCCACACGGAGTACT-3'.

2.5 CCK-8 assay

The CCK-8 assay was performed to detect the proliferation of SKOV3 and A2780 cells. The specific determination method was as follows: the cells were inoculated into a 96-well plate, each well was added with 10 μ l CCK8 reagent and cultured for 24h, and the absorbance value of 450nm was determined by a microplate microscope.

2.6 Colony formation assay

After SKOV3 and A2780 cells were transfected for 48h, cells of each group were seeded in a 60mm cell culture dish with 1×10^3 cells/well. After 14 days, the cells were stained with crystal violet staining solution, and colonies containing ≥ 30 cells were counted. Representative colonies were photographed and counted with Olympus inverted fluorescence microscope.

2.7 Flow cytometry assay

The semi-fused SKOV3 and A2780 cells were transfected and cultured for 48h. After these ovarian cancer cells were washed with PBS solution, 100uL PBS solution was added and resuspended. Subsequently, 5uL Annexin V-FITC and 5uL PI were added. After mixing evenly, the mixture was incubated at room temperature and away from light for 15min. Then add 400uL binding buffer, mixed well and thus placed on ice for 1h for upflow cytometry detection. The apoptotic cells were measured by BD Caliber flow cytometry (Becton Dickinson).

2.8 Transwell invasion assay

50ul matrix glue was added to Transwell chamber to solidify at 37°C for 3h. Serum-free medium was added to the upper chamber and complete medium was added to the lower chamber. SKOV3 and A2780 cells of each group were seeded in the upper chamber of Transwell chamber and cultured for 48h. Erase all cells that had not crossed the membrane. Paraformaldehyde was used to fixed the residual cells, and then 0.5% crystal violet (Solarbio, Beijing, China) was used to stain and take photos. Assays were independently repeated three times.

2.9 Wound Healing Assay

3×10^5 cells/ml SKOV3 and A2780 cells were inoculated in a 6-well plates. The medium lance was used to line the cells vertically and horizontally when the cells covered 80% of the plates. After the cells were washed with PBS solution, the cells were cultured for 2 h and 24 h in a humidified incubator at 37°C and 5% CO₂, and the scratch healing was observed under a microscope.

2.10 Nuclear and cytoplasmic RNA fraction isolation

Adherent SKOV3 and A2780 cells were placed in an ice bath and cell lysis buffer was added for 10min. The whole experiment process was carried out according to the Ambion PARISTM kit. The lysate was collected and centrifuged at 3000r/min for 10min (centrifugation radius was 10cm). The supernatant was the cytoplasm and the precipitation part was mainly the nucleus. The supernatant was placed in the ice bath. The precipitate was further lysed and placed in an ice bath. Then RNA was extracted and RT-qPCR was performed.

2.11 Luciferase reporter assay

LINC00665 (wild-type and mutant) and KLF5 3'-UTR (wild-type and mutant) were constructed into pGL3 Luciferase reporter vectors (Promega, Madison, WI, USA) to generate wildtype reporters (LINC00665-WT and KLF5-WT) or mutant reporters (LINC00665-MUT and KLF5-MUT). In the first experiment, SKOV3 and A2780 cells were co-transfected with miR-148b-3p mimics/ mimic-NC and LINC00665-WT / LINC00665-MUT using Lipofectamine 2000 for 24h. In another experiment, SKOV3 and A2780 cells were co-transfected with miR-148b-3p mimics/ mimic-NC and KLF5-WT / KLF5-MUT using Lipofectamine 2000. Finally, luciferase activity of transfected cells was assayed after 48 hours of transfection using a Dual-Luciferase reporter assay system.

2.12 Western blot

Total protein of each group was extracted with RIPA solution and quantified with BAC kit. The samples were sampled at 30ug per well and the proteins were separated by 10% SDS-PAGE. The protein was imprinted on the PVDF membrane by semi-dry transfer method and sealed with 5% skim milk for 2h. Then rabbit anti-KLF5 (ab137676; 1:1,000; abcam, USA) was incubated. β -actin was used as inner loading control. The protein expression bands on the membrane were detected by chemiluminescence. The second antibody was added to incubate for 2h, and the ECL color developing solution was added drop for development.

2.13 Statistical analysis

SPSS19.0 statistical software was used for Student's t-test and one-way ANOVA. GraphPad Prism 6.0 software was used for graphing. Measurement data were expressed as mean $\pm$ standard deviation.

Results

3.1 LINC00665 was upregulated in ovarian cancer tissues and cells

Primarily, 30 pairs of OC tissue samples and normal para-tumor tissue samples were collected. The results showed that LINC00665 was significantly up-regulated in OC tissues compared with normal para-tumor tissues (Fig. 1A). RT-qPCR results also showed that LINC00665 was significantly up-regulated in OC cell lines (HO-8910, SKOV3 and A2780) compared with normal ovarian epithelial cell line (HOSE) (Fig. 1B).

3.2 Knockdown of LINC00665 inhibited the proliferation, invasion, migration and promoted the apoptosis of ovarian cancer cells

To investigate the biological roles of LINC00665 in the behavior of ovarian cancer cells, we used small interfering RNA (siRNA) to perform the following functional deficiency tests. LINC00665 expression was silenced when si-LINC00665 (knockdown of LINC00665) was transfected into SKOV3 and A2780 cells (Fig.2A). Furthermore, comparing with si-NC group, CCK-8 assay revealed that si-LINC00665 in the si-LINC00665 group significantly reduced the proliferative ability of OC cells (Fig.2B). Colony formation assay showed that si-LINC00665 in the si-LINC00665 group significantly reduced the number of cloning in the si-LINC00665 group (Fig.2C and D). Flow cytometry analysis revealed that si-LINC00665 in the si-LINC00665 group significantly increased apoptotic rate of SKOV3 and A2780 cells (Fig.2E and F). Transwell invasive assay illustrated that si-LINC00665 in the si-LINC00665 group significantly inhibited the invasive ability of SKOV3 and A2780 cells (Fig.2G and H). The scratch test revealed that si-LINC00665 in the si-LINC00665 group significantly inhibited the migration ability of SKOV3 and A2780 cells (Fig.2I, and H). Thus, we conclude that knockdown of LINC00665 inhibited the proliferation, invasion, migration and promoted the apoptosis of ovarian cancer cells.

3.3 miR-148b-3p was a target of LINC00665

To determine the subcellular location of LINC00665, the locations of LINC00665 in SKOV3 and A2780 cells were analyzed by the subcellular of RNA fractions analysis. The RT-qPCR results showed that, the location of LINC00665 was mainly situated in the cytoplasm. This finding sent out the idea that LINC00665 might function as the 'sponge' of miRNAs. To indentify the interaction between LINC00665 and miR-148b-3p, in the previous stage, we predicted that LINC00665 may have a potential binding relationship with miR-148b-3p through bioinformatics online tools analysis (TargetScan, http://www.targetscan.org/vert_71/). Luciferase gene reporter assay confirmed that the activity was decreased when the LINC00665 wild qequence (LINC00665-WT) was co-transfected with miR-148b-3p mimics (Fig.3C). Besides, the a putative binding site between LINC00665 and miR-148b-3p were showed in Fig.3D. In addition, RT-qPCR results showed a significant downward trend of miR-148b-3p in ovarian cancer tissue compared to normal paracancer tissue (Fig.3E), and also a significant downward trend of miR-148b-3p in OC cell lines (HO-8910, SKOV3 and A2780) compared with normal ovarian epithelial cell line (HOSE) (Fig.3F). Besides, miR-148b-3p level was found to passively correlated with LINC00665 level (Figure 3G). Besides, RT-qPCR results revealed that si-LINC00665 in the si-LINC00665 group significantly increased the expression of miR-148b-3p in SKOV3 and A2780 cells (Figure 3H). These data suggested that LINC00665 was a sponge of miR-148b-3p.

Furthermore, the mRNA expression level of KLF5 was significantly reduced in the si-LINC00665 transfected SKOV3 and A2780 cells compared with the si-NC group (Figure 3I). Sequentially, the function of miR-148b-3p in SKOV3 and A2780 cells was investigated in KLF5 level. Western blot analyses displayed that the si-LINC00665 group transfected with si-LINC00665 significantly reduced the KLF5 level compared with the si-NC group transfected with si-NC, and the si-LINC00665+miR-148b-3p inhibitor group co-transfected with si-LINC00665 and miR-148b-3p inhibitor significantly upregulated the KLF5 level compared with the si-LINC00665 group (Figure 3J and K). What's more, RT-qPCR results revealed that miR-148b-3p mimic group transfected with miR-148b-3p mimic significantly downregulated the KLF5 level compared with the si-NC group, while miR-148b-3p inhibitor group transfected with miR-148b-3p inhibitor significantly upregulated the KLF5 level compared with the NC-inhibitor group (Figure 3L).

3.4 KLF5 was directly targeted by miR-148b-3p

Given the above introductions, we further searched for the possible targets of miR-148b-3p. The a putative binding site between LINC00665 3'-UTR and miR-148b-3p was predicted by TargetScan (Fig. 4A). Luciferase reporter gene assay confirmed that the activity of KLF5 wild qequence (KLF5-WT) was decreased after co-transfection with miR-148b-3p mimics (Fig.4B). RT-qPCR results showed a significant upward trend of KLF5 in ovarian cancer tissue compared to normal paracancer tissue (Fig. 4C), and also a significant upward trend of KLF5 in OC cell lines (HO-8910, SKOV3 and A2780) compared with normal ovarian epithelial cell line (HOSE) (Fig. 4D). Besides, KLF5 level was found to passively correlated with miR-148b-3p level (Figure 4E), and positively correlation with LINC00665 (Figure 4F).

3.5 Regulatory effects of miR-148b-3p on KLF5 and KLF5 on LINC00665

To further confirm the regulatory relationship between KLF5 and miR-148b-3p, RT-qPCR assay was used to detect the regulatory effects of miR-148b-3p on KLF5. miR-148b-3p expression was significantly upregulated when miR-148b-3p mimic (overexpression of miR-148b-3p) was transfected (Fig.5A) into SKOV3 and A2780 cells. Western blot result showed that KLF5 expression was suppressed by miR-148b-3p mimic (Fig.5B and C). Furthermore, KLF5 expression was significantly downregulated when si-KLF5 (knockdown of KLF5) was transfected into SKOV3 and A2780 cells (Fig.5D). RT-qPCR assay revealed that LINC00665 expression was significantly inhibited by si-KLF5 (Fig.5E).

3.6 The LINC00665/miR-148b-3p/KLF5 axis regulated the malignant biological behavior of ovarian cancer cells

To determine if the regulating effect of LINC00665/miR-148b-3p/KLF5 axis on malignant biological behavior of ovarian cancer cells, si-NC alone, si-NC with miR-148b-3p inhibitor, si-LINC00665 with miR-148b-3p inhibitor, or si-KLF5 alone were transfected into SKOV3 and A2780 cell lines. In both SKOV3 and A2780 cells, comparing with si-NC group, CCK-8 assay revealed that miR-148b-3p inhibitor in si-NC+miR-148b-3p inhibitor group significantly increased the proliferative ability of OC cells, and si-KLF5 in si-KLF5 group significantly decreased the proliferative ability of OC cells. While comparing with si-NC+miR-148b-3p inhibitor group, si-LINC00665 in si-LINC00665+miR-148b-3p inhibitor group significantly reduced the proliferative ability of OC cells (Fig.6A). Colony formation assay showed that, comparing with si-NC group, si-NC+miR-148b-3p inhibitor group significantly increased the quantity of clones, and si-KLF5 group significantly decreased the quantity of clones. While comparing with si-NC+miR-148b-3p inhibitor group, si-LINC00665+miR-148b-3p inhibitor group significantly reduced the quantity of clones (Fig.6B and C). Flow cytometry analysis revealed that, comparing with si-NC group, si-NC+miR-148b-3p inhibitor group significantly decreased the apoptotic rate of cells, and si-KLF5 group significantly increased the apoptotic rate of cells. While comparing with si-NC+miR-148b-3p inhibitor group, si-LINC00665+miR-148b-3p inhibitor group significantly increased the apoptotic rate of cells (Fig.6D and E). Transwell invasive assay illustrated that comparing with si-NC group, si-NC+miR-148b-3p inhibitor group significantly increased the invasive ability of cells, and si-KLF5 group significantly decreased the invasive ability of cells. While comparing with si-NC+miR-148b-3p inhibitor group, si-LINC00665+miR-148b-3p inhibitor group significantly reduced the invasive ability of cells (Fig.6F and G). The scratch test revealed that, comparing with si-NC group, si-NC+miR-148b-3p inhibitor group significantly increased the migration ability of cells, and si-KLF5 group significantly decreased the migration ability of cells. While comparing with si-NC+miR-148b-3p inhibitor group, si-LINC00665+miR-148b-3p inhibitor group significantly reduced the migration ability of cells (Fig.6H-J). Furthermore, efficacy tests showed that, miR-148b-3p expression was inhibited when miR-148b-3p inhibitor was transfected into SKOV3 and A2780 cells (Fig.6K).

Discussion

Targeted therapy is a new method for the treatment of malignant tumors, which can inhibit the proliferation of cancer cells through specific factors or even directly kill cancer cells. Therefore, it is of great importance to study the molecular mechanism of the occurrence and development of ovarian

cancer for the molecular targeted therapy of ovarian cancer^[16]. As a new regulatory factor, lncRNA is currently a hot research field in oncology, and its biological functions are mainly expressed as the regulation of gene activation or gene expression inhibition^[17, 18]. In this study, we found that LINC00665 expression was up-regulated in ovarian cancer cell lines and tissues. The abnormal expression of LINC00665 in ovarian cancer cells indicates that LINC00665 may trigger a series of pathophysiological processes as an oncogene. The knockdown of LINC00665 inhibited the proliferation, invasion and migration of ovarian cancer cells, and promoted the apoptosis of ovarian cancer cells to a certain extent.

Previously, multiple lncRNAs have been reported to regulate various biological processes in ovarian cancer cells^[19-21]. For instance, lncRNA MEG3 regulates proliferation, invasion, and migration of ovarian cancer cells via regulating PTEN^[19]. lncRNA TP73-AS1 promotes proliferation and metastasis of ovarian cancer cells by regulating the expression of MMP2 and MMP9^[22]. As a poorly researched lncRNA, LINC00665 has been identified as an oncogene of human diseases in a variety of cancer cells, such as breast cancer cells^[8], non-small cell lung cancer cells^[9], osteosarcoma^[10], and prostate cancer^[23]. Our results are consistent with previous reports.

In addition, miRNAs play an important role in regulating gene expression and cell function, and there are many reports on their involvement in tumor genesis and development. Regulatory networks of non-coding RNA are involved in the development of many types of tumors^[24]. Studies have shown that lncRNAs and other transcriptional substances can bind to miRNAs through miRNA recognition elements and play the role of ceRNA^[25]. For instance, studies have reported that, HOTAIR is a ceRNA in gastric cancer cells through modulating miR-331-3p^[26]. For another example, OIP5-AS1 as ceRNA positively regulates PAPPA expression through the sponging of miRNA-152-3p^[27]. These findings stimulated the idea that LINC00665 might function as the 'sponge' of miRNAs. Therefore, we analyzed the potential targets of LINC00665 through bioinformatics, and found that miR-148b-3p was a potential target to LINC00665. Furthermore, luciferase gene reporter assay confirmed that LINC00665 was a sponge of miR-148b-3p. Our hypothesis was confirmed that LINC00665 could act as the "sponge" of miRNA. Our research reported that LINC00665 acted as a ceRNA to drive proliferation, invasion, migration and apoptosis of ovarian cancer cells via the sponging of miR-148b-3p. Moreover, we identified the down-expression level of miR-148b-3p in ovarian cancer. It is worth mentioning that, miR-148b-3p was found to inhibit the development of a variety of cancers, such as gastric carcinoma^[11], pituitary adenoma^[12] and breast carcinoma^[13]. Our results are consistent with these findings.

In this study, we found that LINC00665 was mainly localized in the cytoplasm, suggesting its post-transcriptional regulation. Subsequently, we further verified that KLF5 mRNA and its product proteins were targets of miR-148b-3p, which was consistent with previous research results^[28], thus we had constructed the LINC00665 / miR-148b-3p / KLF5 axis. KLF5 is one of the most studied members of the KLFs family. It belongs to the family of DNA-binding transcriptional regulators and has various biological functions. In recent years, studies have found that KLF5 is closely related to the occurrence and development of tumors. KLF5 is abnormally expressed in different types of tumors and plays important

roles in different tumors ^[29, 30]. In this study, we also found that a significant up-regulation of KLF5 were shown in ovarian cancer. Interestingly, a negative correlation between LINC00665 and miR-148b-3p expression, a positive correlation between LINC00665 and KLF5 expression, and a negative correlation between miR-148b-3p and KLF5 expression were found in ovarian cancer tissues. Thus, we further confirmed the existence of LINC00665 / miR-148b-3p /KLF5 axis. KLF5 expression is often abnormal in human cancers, and there is growing evidence that KLF5 represents a novel therapeutic target for cancer treatment ^[31]. Our findings are further supported by the fact that KLF5 is an important transcription factor involved in the survival, migration, invasion and apoptosis of osteosarcoma cells ^[32]. Importantly, we found that down-regulation of LINC00665 inhibited the expression of KLF5 by promoting miR-148b-3p in ovarian cancer cells, thus inhibiting the malignant biological behavior of ovarian cancer.

As mention above, our data suggested that LINC00665, by sponging miR-148b-3p /KLF5 axis, depressed cell proliferation, invasion and migration, and induced the apoptosis to a certain extent, suggesting an important regulation of LINC00665 on the ovarian cancer tumorigenesis.

Conclusions

LINC00665 can be used as an effective prognostic indicator of ovarian cancer. By elucidating the mechanism of LINC00665 in the malignant biological behavior of ovarian cancer, we demonstrate that LINC00665 has the potential to be a new drug target.

Declarations

Authors' contributions

SLW, CCL, ARZ, YCL, and YXX conceived and designed the experiments. SLW, CCL, JWQ and XLC performed the experiments. JB and RL analyzed the data and contributed to the acquisition of reagents and materials. SLW and YXX wrote the manuscript.

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Availability of data and materials

All data used or analyzed during the current study are available from the corresponding author on reasonable request. The datasets supporting the conclusions of this article are included in the article.

Competing interests

The authors declare that they have no competing interests.

Data Availability Statement

Availability of data and material: All data generated or analyzed in this study are included in this published article.

Ethics statement

Ethics approval and consent to participate: This study was conducted after obtaining Fifth People's Hospital of Qinghai Province (no. 2020427A).

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Figures

Figure 1

LINC00665 was upregulated in ovarian cancer tissues and cells. (A) Total 30 histologically verified ovarian cancer tissue and normal para-tumor tissue samples were collected. RT-qPCR assay was used to measure LINC00665 expression in tissues. $**p<0.01$. (B) RT-qPCR assay was used to measure LINC00665 expression in OC cell lines (HO-8910, SKOV3 and A2780) and normal ovarian epithelial cell line (HOSE). $*p<0.05$, $**p<0.01$, compared with HOSE group.

Figure 2

Knockdown of LINC00665 inhibited the proliferation, invasion, migration and promoted the apoptosis of ovarian cancer cells. (A) Loss-of-functional assays were performed using LINC00665 small interfering RNA (siRNA) in SKOV3 and A2780 cells. (B) CCK-8 assay revealed the proliferative ability in the si-LINC00665 transfected SKOV3 and A2780 cells. (C-D) Colony formation assay revealed the colony numbers of clones in the si-LINC00665 transfected SKOV3 and A2780 cells. (E-F) Flow cytometry

analysis revealed apoptotic rate in the si-LINC00665 transfected SKOV3 and A2780 cells. (G-H) Transwell invasive assay illustrated the invasive ability in the si-LINC00665 transfected SKOV3 and A2780 cells. (I-K) Wound healing assay showed the wound areas at 2h and 24h after wounding in the si-LINC00665 transfected SKOV3 and A2780 cells. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared with si-NC group.

Figure 3

miR-148b-3p was a target of LINC00665. (A-B) The subcellular of RNA fractions analysis revealed the location of LINC00665 in the cytoplasm or nuclear of SKOV3 or A2780 cells. (C) Luciferase gene reporter assay showed the activity when co-transfected with the LINC00665 wild/mutant sequences and miR-148b-3p mimics/NC-mimics. *** $p < 0.001$, compared with NC-mimic group. (D) The a putative binding site

between LINC00665 3'-UTR and miR-148b-3p. (E) Total 30 histologically verified ovarian cancer tissue and normal para-tumor tissue samples were collected. RT-qPCR assay was used to measure miR-148b-3p expression in tissues. * $p < 0.05$. (F) RT-qPCR assay was used to measure miR-148b-3p expression in OC cell lines (HO-8910, SKOV3 and A2780) and normal ovarian epithelial cell line (HOSE). * $p < 0.05$, compared with HOSE group. (G) RT-qPCR assay for the correlation between miR-148b-3p level and LINC00665 level. (H) RT-qPCR assay revealed that si-LINC00665 significantly increased the expression of miR-148b-3p in SKOV3 and A2780 cells. * $p < 0.05$, compared with si-NC group. (I) RT-qPCR assay for revealed that si-LINC00665 significantly decrease the expression of KLF5 in SKOV3 and A2780 cells. * $p < 0.05$, compared with si-NC group. (J-K) Western blot analyses displayed the protein levels of KLF5 under LINC00665 or miR-148b-3p detection in SKOV3 and A2780 cells. ** $p < 0.01$, *** $p < 0.001$, compared with si-NC group; # $p < 0.05$, compared with si-LINC00665 group. (L) RT-qPCR assay displayed the mRNA levels of LINC00665 under miR-148b-3p overexpression or detection in SKOV3 and A2780 cells. * $p < 0.05$, ** $p < 0.01$, compared with NC mimic group; # $p < 0.05$, compared with NC-inhibitor group.

Figure 4

KLF5 was directly targeted by miR-148b-3p. (A) The a putative binding site between KLF5 3'-UTR and miR-148b-3p. (B) Luciferase gene reporter assay showed the activity when co-transfected with the KLF5 wild/mutant sequences and miR-148b-3p mimics/NC-mimics. *** $p < 0.001$, compared with NC-mimic group. (C) Total 30 histologically verified ovarian cancer tissue and normal para-tumor tissue samples were collected. RT-qPCR assay was used to measure KLF5 expression in tissues. *** $p < 0.001$. (D) RT-qPCR assay was used to measure KLF5 expression in OC cell lines (HO-8910, SKOV3 and A2780) and normal

ovarian epithelial cell line (HOSE). * $p < 0.05$, compared with HOSE group. (E) RT-qPCR assay for the correlation between miR-148b-3p level and KLF5. (F) RT-qPCR assay for the correlation between LINC00665 level and KLF5.

Figure 5

Regulatory effects of miR-148b-3p on KLF5 and KLF5 on LINC00665. (A) Overexpressions of miR-148b-3p in miR-148b-3p mimic transfected SKOV3 and A2780 cells were identified by RT-qPCR. (B-C) Western blot assay revealed that miR-148b-3p mimic significantly decreased the expression of KLF5 in SKOV3 and A2780 cells. * $p < 0.05$, ** $p < 0.01$, compared with si-NC group. (D) Loss-of-functional assays were performed using KLF5 siRNA in SKOV3 and A2780 cells. * $p < 0.05$, ** $p < 0.01$, compared with si-NC group. (E) RT-qPCR assay revealed that KLF5 siRNA significantly decreased the expression of LINC00665 in SKOV3 and A2780 cells. * $p < 0.05$, compared with si-NC group.

Figure 6

Regulating effect of LINC00665/miR-148b-3p/KLF5 axis on malignant biological behavior of ovarian cancer cells. Si-NC alone, si-NC with miR-148b-3p inhibitor, si-LINC00665 with miR-148b-3p inhibitor, or si-KLF5 alone were transfected into SKOV3 and A2780 cell lines. (A) CCK-8 assay revealed the proliferative ability in SKOV3 and A2780 cells. (B-C) Colony formation assay revealed the colony numbers of clones in SKOV3 and A2780 cells. (D-E) Flow cytometry analysis revealed apoptotic rate in SKOV3 and A2780 cells.

(F-G) Transwell invasive assay illustrated the invasive ability in SKOV3 and A2780 cells. (H-J) Wound healing assay showed the wound areas at 2h and 24h after wounding in SKOV3 and A2780 cells. (K) Loss-of-functional assays were performed using miR-148b-3p inhibitor transfected in SKOV3 and A2780 cells. (A-J): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared with si-NC group; # $p < 0.05$, ## $p < 0.01$, compared with si-NC+miR-148b-3p inhibitor group. (K) * $p < 0.05$, compared with NC-inhibitor group.