LncRNA OIP5-AS1 Affects the Malignant Biological Behavior of Ovarian Cancer via the miR-153-3p/KLF5 Axis

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Research

Keywords: Ovarian Cancer, LncRNA OIP5-AS1, cell proliferation, cell invasion, cell apoptosis

Posted Date: November 19th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-108470/v1>

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Abstract

Objectives: The purpose of this study was to investigate the expression and clinical significance of lncRNA OIP5-AS1 in ovarian cancer, as well as its effect on malignant biological behavior of ovarian cancer cells.

Methods: The expression of OIP5-AS1, miR-153-3p 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 OIP5-AS1, 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 interaction between OIP5-AS1 and miR-153-3p and between miR-153-3p and KLF5 were verified by luciferase reporter assay, and the correlations among these three genes were analyzed.

Results: OIP5-AS1 expression was up-regulated in ovarian cancer cell lines and tissues. Si-OIP5-AS1 inhibited cell proliferation, invasion and migration, and induced the apoptosis to a certain extent. Subcellular fraction assay revealed the location of OIP5-AS1 was mainly situated in the cytoplasm. In addition, miR-153-3p was a target of OIP5-AS1, and KLF5 was directly targeted by miR-153-3p. Si-OIP5-AS1 inhibited KLF5 expression, miR-153-3p inhibitor promoted KLF5 expression, and si-KLF5 inhibited OIP5-AS1 expression. Interestingly, expression of OIP5-AS1 and miR-153-3p, and expression of miR-153-3p and KLF5 were negatively correlated, while expression of OIP5-AS1 and KLF5 was positively correlated. In addition, si-KLF5 inhibited the malignant biological behavior of ovarian cancer cells, while miR-153-3p inhibitor had the opposite effect. Most importantly, the addition of si-OIP5-AS1 to miR-153-3p silenced cells could reverse the promotion effect of miR-153-3p inhibitor on the malignant biological behavior of ovarian cancer cells.

Conclusions: OIP5-AS1 can be used as an effective prognostic indicator of ovarian cancer, which has the potential to be a new drug target.

1. Introduction

Ovarian cancer is one of the three malignancies of the female reproductive system, which is the leading cause of female death^[25, 19]. 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^[32]. 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^[20], such as affecting the epigenetic activity of tumor cells in the nucleus, gene transcription

and post-transcriptional modification of mRNA [2]. LncRNA OIP5-AS1 has been reported to inhibit the proliferation of multiple malignant tumors [29, 28]. It can regulate a variety of biological activities of cells in some diseases and tumors. For example, lncRNA OIP5-AS1 regulates proliferation and apoptosis in multiple myeloma. LncRNA OIP5-AS1 can inhibit GAK expression [16], thus controlling mitosis at the cytoskeleton level. It also binds to the RNA-binding protein HuR [15], thereby inhibiting the proliferation of tumor cells [7]. Although OIP5-AS1 plays an important role in cancer, its role in ovarian cancer is still unknown. Through bioinformatics analysis, we found that OIP5-AS1 may have a potential binding relationship with miR-153-3p.

Mir-153-3p is a miRNA that plays a key role in disease progression. It has been reported that miR-153-3p plays an anticancer role in human cancers including melanoma [18], gastric cancer [12] and ovarian cancer [3]. Through bioinformatics analysis, we found that KLF5 is the potential target gene of miR-153-3p. KLF5 expression is up-regulated in many cancers [10, 4], and our previous studies have shown that KLF5 expression is also up-regulated in ovarian cancer tissues. However, its regulatory mechanism is still unclear. We speculated that the OIP5-AS1/miR-153-3p/KLF5 axis may exist in ovarian cancer and play an important regulatory role in the development of ovarian cancer.

In this study, the expression level of OIP5-AS1/miR-153-3p/KLF5 in ovarian cancer tissues were detected, and the effect of si-OIP5-AS1 on the proliferation, invasion and apoptosis ability of ovarian cancer cells by regulating miR-153-3p/KLF5 axis was also detected, which laid the experimental foundation for the later in-depth study.

2. 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

Si-OIP5-AS1, si-NC, KLF5 siRNA (si-KLF5), miR-153-3p mimic, NC-mimic, miR-153-3p inhibitor and NC-inhibitor sequences were synthesized by Shanghai GenePharma Co., Ltd. The interference segment information was as follows: si-OIP5-AS1: 5'-GCAGCAUGCUGUGUGCAAA-3', si-NC: 5'-GAAATGTACTTG AGCGTGGAGAC-3', si-KLF5: 5'-GACCACCGACAGATACGTG-3', miR-153-3p: 5'-UUGCAUAGUCACAAAAGUGAUC-3', NC mimic: 5'-UUUGUACUACACAA AAGUACUG-3', miR-153-3p inhibitor: 5'-GAUCACUUUUGUGACUAUGCAA-3', and NC-inhibitor: 5'-UUUGUACUACACAAAAGUACUG-3'. These plasmids (each 50 nM) were transfected into SKOV3 and A2780 cell lines using Lipofectamine 2000.

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: OIP5-AS1 Forward: 5'-TGCGAAGATGGCG GAGTAAG-3', Reverse: 5'-TAGTTCCTCTCCTCTGGCCG-3'; miR-153-3p Forward: 5'-TTGCATAGTCACAAAAGTGATCG-3', Reverse: 5'-GTGTCGTGGAGTCCGC AA-3'; KLF5 Forward: 5'-ACACCAGACCGCAGCTCCA-3', Reverse: 5'-TCCATTGCTGCTGTCTGATTTGTAG-3'; GAPDH Forward: 5'-TTGCCATCAATGACCCCTTCA-3', Reverse: 5'-CGCCCCACTTGATTTTGGGA-3'; U6 Forward: 5'-GCTTCGGCAGCACATATACTAAAAT-3', Reverse: 5'-CGCTTCACGAATTTGCGTGTCAT-3'; β -actin Forward: 5'-TTGCCGACAGGATGCAGAA-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 24 h, and the absorbance value of 450 nm was determined by a microplate microscope.

2.6 Colony formation assay

After SKOV3 and A2780 cells were transfected for 48 h, cells of each group were seeded in a 60 mm 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 48 h. After these ovarian cancer cells were washed with PBS solution, 100 μ l PBS solution was added and resuspended. Subsequently, 5 μ l Annexin V-FITC and 5 μ l PI were added. After mixing evenly, the mixture was incubated at room temperature and away from light for 15 min. Then added 400 μ l binding buffer, mixed well and thus placed on ice for 1 h for upflow cytometry detection. The apoptotic cells were measured by BD Caliber flow cytometry (Becton Dickinson).

2.8 Transwell invasion assay

50 ul matrix glue was added to Transwell chamber to solidify at 37°C for 3 h. 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 48 h. Erased 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 10 min. The whole experiment process was carried out according to the Ambion PARISTM kit. The lysate was collected and centrifuged at 3000r/min for 10 min (centrifugation radius was 10 cm). 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

The fragments of OIP5-AS1 3'-UTR or KLF5 3'-UTR (wild-type and mutant) containing the miR-153-3p binding site plasmids were constructed into pGL3 vector (Promega, Madison, WI, USA) to generate wildtype reporters (OIP5-AS1-WT and KLF5-WT) or mutant reporters (OIP5-AS1-MUT and KLF5-MUT). In the first experiment, SKOV3 and A2780 cells were co-transfected with miR-153-3p mimics/ mimic-NC and OIP5-AS1 3'-UTR/OIP5-AS1-MUT using Lipofectamine 2000 for 24 h. In another experiment, SKOV3 and A2780 cells were co-transfected with miR-153-3p mimics/ mimic-NC and KLF5 3'-UTR/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 2 h.

Then incubated with rabbit anti-KLF5 (ab137676; 1:1,000; abcam, USA). 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 2 h, 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.

3. Results

3.1 LncRNA OIP5-AS1 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 OIP5-AS1 was significantly up-regulated in OC tissues compared with normal para-tumor tissues (Fig. 1A). RT-qPCR results also showed that OIP5-AS1 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 OIP5-AS1 inhibited the proliferation, invasion, migration and promoted the apoptosis of ovarian cancer cells

To investigate the biological roles of OIP5-AS1 in the behavior of ovarian cancer cells, we used small interfering RNA (siRNA) to perform the following functional deficiency tests. OIP5-AS1 expression was silenced when si-OIP5-AS1 (knockdown of OIP5-AS1) was transfected into SKOV3 and A2780 cells (Fig. 2A). Furthermore, comparing with si-NC group, CCK-8 assay revealed that si-OIP5-AS1 in the si-OIP5-AS1 group significantly reduced the proliferative ability of OC cells (Fig. 2B). Colony formation assay showed that si-OIP5-AS1 in the si-OIP5-AS1 group significantly reduced the number of cloning in the si-OIP5-AS1 group (Fig. 2C and D). Flow cytometry analysis revealed that si-OIP5-AS1 in the si-OIP5-AS1 group significantly increased apoptotic rate of SKOV3 and A2780 cells (Fig. 2E and F). Transwell invasive assay illustrated that si-OIP5-AS1 in the si-OIP5-AS1 group significantly inhibited the invasive ability of SKOV3 and A2780 cells (Fig. 2G and H). The scratch test revealed that si-OIP5-AS1 in the si-OIP5-AS1 group significantly inhibited the migration ability of SKOV3 and A2780 cells (Fig. 2I, and H). Thus, we conclude that knockdown of OIP5-AS1 inhibited the proliferation, invasion, migration and promoted the apoptosis of ovarian cancer cells.

3.3 MiR-153-3p was a target of LncRNA OIP5-AS1

To determine the subcellular location of OIP5-AS1, the locations of OIP5-AS1 in SKOV3 and A2780 cells were analyzed by the subcellular of RNA fractions analysis. The RT-qPCR results showed that, the location of OIP5-AS1 was mainly situated in the cytoplasm. This finding sent out the idea that OIP5-AS1 might function as the 'sponge' of miRNAs. To indentify the interaction between OIP5-AS1 and miR-153-3p, in the previous stage, we predicted that OIP5-AS1 may have a potential binding relationship with miR-153-3p through bioinformatics online tools analysis (TargetScan, http://www.targetscan.org/vert_71/). Luciferase gene reporter assay confirmed that the activity was decreased when the lncRNA OIP5-AS1 wild qequence (OIP5-AS1-WT) was co-transfected with miR-153-3p mimics (Fig. 3C). Besides, the a putative binding site between OIP5-AS1 and miR-153-3p were showed in Fig. 3D. In addition, RT-qPCR results showed a significant downward trend of miR-153-3p in ovarian cancer tissue compared to normal paracancer tissue (Fig. 3E), and also a significant downward trend of miR-153-3p in OC cell lines (HO-8910, SKOV3 and A2780) compared with normal ovarian epithelial cell line (HOSE) (Fig. 3F). Besides, miR-153-3p level was found to passively correlated with OIP5-AS1 level (Fig. 3G). Besides, RT-qPCR results revealed that si-OIP5-AS1 in the si-OIP5-AS1 group significantly increased the expression of miR-153-3p in SKOV3 and A2780 cells (Fig. 3H). These data suggested that OIP5-AS1 was a sponge of miR-153-3p.

Furthermore, the mRNA expression level of KLF5 was significantly reduced in the si-OIP5-AS1 transfected SKOV3 and A2780 cells compared with the si-NC group (Fig. 3I). Sequentially, the function of miR-153-3p in SKOV3 and A2780 cells was investigated in KLF5 level. Western blot analyses displayed that the si-OIP5-AS1 group transfected with si-OIP5-AS1 significantly reduced the KLF5 level compared with the si-NC group transfected with si-NC, and the si-OIP5-AS1 + miR-153-3p inhibitor group co-transfected with si-OIP5-AS1 and miR-153-3p inhibitor significantly upregulated the KLF5 level compared with the si-OIP5-AS1 group (Fig. 3J and K). What's more, RT-qPCR results revealed that miR-153-3p mimic group transfected with miR-153-3p mimic significantly downregulated the KLF5 level compared with the si-NC group, while miR-153-3p inhibitor group transfected with miR-153-3p inhibitor significantly upregulated the KLF5 level compared with the NC-inhibitor group (Fig. 3L).

3.4 KLF5 was directly targeted by miR-153-3p

Given the above introductions, we further searched for the possible targets of miR-153-3p. The a putative binding site between OIP5-AS1 3'-UTR and miR-153-3p 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-153-3p 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-153-3p level (Fig. 4E), and positively correlation with OIP5-AS1 (Fig. 4F).

3.5 Regulatory effects of miR-153-3p on KLF5 and KLF5 on OIP5-AS1

To further confirm the regulatory relationship between KLF5 and miR-153-3p, RT-qPCR assay was used to detect the regulatory effects of miR-153-3p on KLF5. miR-153-3p expression was significantly upregulated when miR-153-3p mimic (overexpression of miR-153-3p) was transfected (Fig. 5A) into SKOV3 and A2780 cells. Western blot result showed that KLF5 expression was suppressed by miR-153-3p 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 OIP5-AS1 expression was significantly inhibited by si-KLF5 (Fig. 5E).

3.6 The OIP5-AS1/miR-153-3p/KLF5 axis regulated the malignant biological behavior of ovarian cancer cells

To determine if the regulating effect of OIP5-AS1/miR-153-3p/KLF5 axis on malignant biological behavior of ovarian cancer cells, si-NC alone, si-NC with miR-153-3p inhibitor, si-OIP5-AS1 with miR-153-3p 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-153-3p inhibitor in si-NC + miR-153-3p 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-153-3p inhibitor group, si-OIP5-AS1 in si-OIP5-AS1 + miR-153-3p 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-153-3p inhibitor group significantly increased the quantity of clones, and si-KLF5 group significantly decreased the quantity of clones. While comparing with si-NC + miR-153-3p inhibitor group, si-OIP5-AS1 + miR-153-3p 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-153-3p 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-153-3p inhibitor group, si-OIP5-AS1 + miR-153-3p 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-153-3p 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-153-3p inhibitor group, si-OIP5-AS1 + miR-153-3p 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-153-3p 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-153-3p inhibitor group, si-OIP5-AS1 + miR-153-3p inhibitor group significantly reduced the migration ability of cells (Fig. 6H-J). Furthermore, efficacy tests showed that, miR-153-3p expression was inhibited when miR-153-3p inhibitor was transfected into SKOV3 and A2780 cells (Fig. 6K).

3. 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^[22]. In recent years, many studies have pointed out the abnormal expression of lncRNA in various types of tumors^[21, 6, 11]. 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^[27, 13]. lncRNA OIP5-AS1 is a highly conserved lncRNA in mammals, which is mainly expressed in the cytoplasm^[26]. It has been reported that OIP5-AS1 may act as a biological sponge, acting as competing endogenous RNAs (ceRNA) to inhibit the binding mRNA of RNA-binding protein HuR (HuR was a member of the human embryo lethal abnormal visual RBP family), and then inhibited the expression of HuR, thus participating in the regulation of phenotypes^[15]. 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^[34]. Studies have shown that lncRNAs and other transcriptional substances can bind to miRNAs through miRNA recognition elements and play the role of ceRNA^[14, 17]. For instance, studies have reported that OIP5-AS1 is a ceRNA in Hepatoblastoma cells through modulating miR-186a-5p/ZEB1^[35]. For another example, OIP5-AS1 as ceRNA positively regulates PAPP expression through the sponging of miRNA-152-3p^[31]. Our research team reported that lncRNA OIP5-AS1 acted as a ceRNA to drive proliferation, invasion, migration and apoptosis of ovarian cancer cells via the sponging of miR-153-3p/KLF5 axis.

In this study, we found that OIP5-AS1 expression was up-regulated in ovarian cancer cell lines and tissues. The abnormal expression of OIP5-AS1 in ovarian cancer cells indicates that OIP5-AS1 may trigger a series of pathophysiological processes as an oncogene. The knockdown of OIP5-AS1 inhibited the proliferation, invasion and migration of ovarian cancer cells, and promoted the apoptosis of ovarian cancer cells to a certain extent. OIP5-AS1 has been identified as an oncogene of human diseases in a variety of cancer cells, such as breast cancer cells^[30], non-small cell lung cancer cells^[5], pancreatic ductal carcinoma cells^[29], and multiple myeloma cells^[28].

In order to further study the deep mechanism of OIP5-AS1 regulating the occurrence of ovarian cancer tumors, the locations of OIP5-AS1 in SKOV3 and A2780 cells were analyzed by the subcellular of RNA fractions analysis. We found that OIP5-AS1 was mainly localized in the cytoplasm, suggesting its post-transcriptional regulation. This finding stimulated the idea that lncRNA OIP5-AS1 might function as the 'sponge' of miRNAs. To identify the interaction between OIP5-AS1 and miR-153-3p, in the previous stage, we predicted that OIP5-AS1 may have a potential binding relationship with miR-153-3p through bioinformatics online tools analysis. Luciferase gene reporter assay confirmed that OIP5-AS1 was a sponge of miR-153-3p. Our hypothesis was confirmed that lncRNA CASC15 could act as the "sponge" of miRNA. Subsequently, on the basis of previous studies, we further verified that KLF5 mRNA and its product proteins were targets of miR-153-3p through luciferase gene reporter assay, which was consistent with previous research results^[33], thus constructed the OIP5-AS1/ miR-153-3p/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 different roles in different tumors^[36, 23, 1]. Thus, on the other hand, RT-qPCR was also used to detect the expressions of miR-153-3p and KLF5 in ovarian cancer cell lines and tissues, and we found that a significant down-regulation of miR-153-3p and a significant up-regulation of KLF5 were shown in ovarian cancer. Interestingly, a negative correlation between OIP5-AS1 and miR-153-3p expression, a positive correlation between OIP5-AS1 and KLF5 expression, and a negative correlation between miR-153-3p and KLF5 expression were found in ovarian cancer tissues.

Through the above luciferase reporter gene validation, we confirmed that miR-153-3p was a target of lncRNA OIP5-AS1, and KLF5 was directly targeted by miR-153-3p. Next, we found that the interaction of OIP5-AS1/miR-153-3p/KLF5 axis related genes played an important role in the malignant biological behavior of ovarian cancer cells. Si-KLF5 inhibited the malignant biological behavior of ovarian cancer cells, as shown in our study. 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^[8]. What's more, our study also found that miR-153-3p inhibitor promoted the malignant biological behavior of ovarian cancer cells, while the addition of si-OIP5-AS1 to miR-153-3p silenced cells could reverse the promotion effect of miR-153-3p inhibitor on the malignant biological behavior of ovarian cancer cells, suggesting that OIP5-AS1 acted as a ceRNA to drive proliferation, invasion, migration and apoptosis of ovarian cancer cells via the sponging of miR-153-3p/KLF5 axis. A new study reports that, OIP5-AS1 promoted the progression of gastric cancer cells through miR-153-3p/ZBTB2 axis, and its research target between OIP5-AS1 and miR-153-3p was surprisingly consistent with our results^[9]. Moreover, OIP5-AS1 knockdown inhibits breast cancer progression by competitively binding miR-216a-5p^[30]. Down-regulation of OIP5-AS1 can inhibit the proliferation and migration of hepatocellular carcinoma cells and promote apoptosis via regulating miR-3163/VEGFA^[24].

As mention above, our data suggested that lncRNA OIP5-AS1, by sponging miR-153-3p/KLF5 axis, depressed cell proliferation, invasion and migration, and induced the apoptosis to a certain extent, suggesting an important regulation of OIP5-AS1 on the ovarian cancer tumorigenesis.

Conclusions

OIP5-AS1 can be used as an effective prognostic indicator of ovarian cancer. By elucidating the mechanism of OIP5-AS1 in the malignant biological behavior of ovarian cancer, we demonstrate that OIP5-AS1 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.

Acknowledgments

Not applicable.

Funding

The present study was supported by the Qinghai Science and Technology Department (2018-ZJ-755).

Availability of data and materials

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

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

lncRNA OIP5-AS1 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 OIP5-AS1 expression in tissues. $**p<0.01$. (B) RT-qPCR assay was used to measure OIP5-AS1 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.

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Figure 2

Knockdown of OIP5-AS1 inhibited the proliferation, invasion, migration and promoted the apoptosis of ovarian cancer cells. (A) Loss-of-functional assays were performed using OIP5-AS1 small interfering RNA (siRNA) in SKOV3 and A2780 cells. (B) CCK-8 assay revealed the proliferative ability in the si-OIP5-AS1 transfected SKOV3 and A2780 cells. (C-D) Colony formation assay revealed the colony numbers of clones in the si-OIP5-AS1 transfected SKOV3 and A2780 cells. (E-F) Flow cytometry analysis revealed

apoptotic rate in the si-OIP5-AS1 transfected SKOV3 and A2780 cells. (G-H) Transwell invasive assay illustrated the invasive ability in the si-OIP5-AS1 transfected SKOV3 and A2780 cells. (I-K) Wound healing assay showed the wound areas at 2h and 24h after wounding in the si-OIP5-AS1 transfected SKOV3 and A2780 cells. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared with si-NC group.

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Figure 3

MiR-153-3p was a target of LncRNA OIP5-AS1. (A-B) The subcellular of RNA fractions analysis revealed the location of LncRNA OIP5-AS1 in the cytoplasm or nuclear of SKOV3 or A2780 cells. (C) Luciferase gene reporter assay showed the activity when co-transfected with the OIP5-AS1 wild/mutant sequences and miR-153-3p mimics/NC-mimics. ***p<0.001, compared with NC-mimic group. (D) The a putative binding site between OIP5-AS1 3'-UTR and miR-153-3p. (E) Total 30 histologically verified ovarian cancer

tissue and normal para-tumor tissue samples were collected. RT-qPCR assay was used to measure miR-153-3p expression in tissues. * $p < 0.05$. (F) RT-qPCR assay was used to measure miR-153-3p 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-153-3p level and OIP5-AS1 level. (H) RT-qPCR assay revealed that si-OIP5-AS1 significantly increased the expression of miR-153-3p in SKOV3 and A2780 cells. * $p < 0.05$, compared with si-NC group. (I) RT-qPCR assay for revealed that si-OIP5-AS1 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 OIP5-AS1 or miR-153-3p detection in SKOV3 and A2780 cells. ** $p < 0.01$, *** $p < 0.001$, compared with si-NC group; # $p < 0.05$, compared with si-OIP5-AS1 group. (L) RT-qPCR assay displayed the mRNA levels of OIP5-AS1 under miR-153-3p 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.

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KLF5 was directly targeted by miR-153-3p. (A) The a putative binding site between KLF5 3'-UTR and miR-153-3p. (B) Luciferase gene reporter assay showed the activity when co-transfected with the KLF5 wild/mutant sequences and miR-153-3p 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-153-3p level and KLF5. (F) RT-qPCR assay for the correlation between OIP5-AS1 level and KLF5.

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Figure 5

Regulatory effects of miR-153-3p on KLF5 and KLF5 on OIP5-AS1. (A) Overexpressions of miR-153-3p in miR-153-3p mimic transfected SKOV3 and A2780 cells were identified by RT-qPCR. (B-C) Western blot assay revealed that miR-153-3p 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 OIP5-AS1 in SKOV3 and A2780 cells. * $p < 0.05$, compared with si-NC group.

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Figure 6

The regulating effect of OIP5-AS1/miR-153-3p/KLF5 axis on malignant biological behavior of ovarian cancer cells. Si-NC alone, si-NC with miR-153-3p inhibitor, si-OIP5-AS1 with miR-153-3p 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.

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The regulating effect of OIP5-AS1/miR-153-3p/KLF5 axis on malignant biological behavior of ovarian cancer cells. Si-NC alone, si-NC with miR-153-3p inhibitor, si-OIP5-AS1 with miR-153-3p 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-153-3p 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-153-3p inhibitor group. (K) * $p < 0.05$, compared with NC-inhibitor group.