Long non-coding RNA OIP5-AS1 facilitates the progression of ovarian cancer via the miR-128-3p/CCNG1 axis

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Abstract. Long non-coding RNA (LncRNA) o-phthalaldehyde-interacting protein 5 antisense transcript 1 (OIP5-AS1) serves major roles in the progression of various types of cancer. The present study investigated its biological function in ovarian cancer (OC) and its mechanisms. The levels of OIP5-AS1, microRNA-128-3p (miR-128-3p) and cyclin G1 (CCNG1) were examined by reverse transcription-quantitative PCR. Cell viability, apoptosis, migration and invasion were detected to analyze cellular progression. Glycolytic metabolism was assessed by detecting the levels of glucose consumption and lactate production. CCNG1 and hexokinase 2 protein levels were measured by western blotting. Dual-luciferase reporter assay, RNA immunoprecipitation and RNA pull-down assays were performed to affirm the interaction between two molecules. OIP5-AS1 was found to be upregulated in OC tissues and cells. Knockdown of OIP5-AS1 suppressed cell viability, migration, invasion and glycolysis while promoting apoptosis in OC cells. OIP5-AS1 interacted with miR-128-3p and functioned as an oncogene by sequestering miR-128-3p. In addition, CCNG1 was a target gene for miR-128-3p and miR-128-3p regulated the CCNG1-induced effects on OC cells by downregulating CCNG1. OIP5-AS1 upregulated the expression of CCNG1 via targeting miR-128-3p. OIP5-AS1 knockdown also inhibited tumor growth of OC in vivo by modulating the expression of miR-128-3p and CCNG1. Collectively, these data illustrated that the oncogenic role of OIP5-AS1 in OC was associated with the miR-128-3p/CCNG1 axis at least in part. OIP5-AS1 might be a probable diagnostic and therapeutic biomarker for the treatment of OC patients.

Introduction

Ovarian cancer (OC) is the seventh most common cancer among females with an estimated 239,000 new cases being diagnosed worldwide annually; the incidence rate (11.4 per 100,000) is the highest in Central and Eastern Europe (1). Although the diagnosis and treatment for OC have been increasingly successful, the 5-year survival rate of the advanced patient with OC is only ~30% (1.2). Therefore, it is imperative to search more molecular targets for promoting the diagnostic and therapeutic effects on OC patients. Studies have reported that long non-coding RNAs (lncRNAs) can regulate biological behaviors in OC cells (3-5).

Aerobic glycolysis, known as the ‘Warburg effect’, is characterized by oxidizing glucose and producing lactate under the catalysis of diverse enzymes and normoxic conditions (6). Glycolysis can provide the energy for tumor cell growth and the promotion of glycolytic metabolism indicates the malignant progression of types of cancer (7). A number of lncRNAs have been shown to regulate the metabolic process of glycolysis in types of cancer, including OC (8-10). It may be pertinent to seek the useful targets in OC progression through the glycolytic metabolism. lncRNAs are common class of regulatory ncRNAs with length >200 nucleotides and lack of protein-encoding capacity (11).

lncRNAs can participate in the initiation and progression of various types of human tumors as oncoproteins or tumor inhibitors (12,13). O-phthalaldehyde-interacting protein 5 antisense transcript 1 (OIP5-AS1) has been found as the regulator by acting as the sponges of microRNAs (miRNAs) in various types of cancers. For instance, Wang et al. (14) revealed that OIP5-AS1 improves cell proliferation and results in poor prognosis through sponging miR-378a-3p in lung cancer. In addition, OIP5-AS1 knockdown represses CCAT enhancer-binding protein α and TNF receptor-associated factor 4 levels to retard glioma cell growth via binding to miR-367-3p (15). OIP5-AS1 is reported to be overexpressed in OC and facilitates the malignant tumor growth and metastasis of OC by the miR-137/ZNF217 or miR-324-3p/NFIB axis (16,17). However, the regulation in glycolytic process of OC and other regulatory mechanisms underpinning OIP5-AS1 remain to be elucidated.

Non-coding miRNAs can cause mRNA degradation and translation inhibition by combining with the 3' untranslated regions (3'-UTRs) of target mRNAs, leading to a significant

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involvement in biological processes (18,19). Studies have found an inhibitory effect of miR-128-3p on the development of a number of carcinomas (20-22). The aberrant downregulation of miR-128 is found in OC (23,24). As a subunit of miR-128, the present study hypothesized that miR-128-3p might also be associated with OC regulation.

Cyclin G1 (CCNG1), a member of cell cyclin protein, is dysregulated in leiomyoma and colorectal cancer (25,26). CCNG1 is associated with the regulation of miR-122a in hepatocellular carcinoma as a target of miR-122a (27). Studies also suggest that CCNG1 acts as an oncogene to enhance OC cell growth (28,29), but it is unknown if CCNG1 is a target of miR-128-3p in OC.

The present study was mainly devoted to investigating the role of OIP5-AS1 in OC and its functional mechanism with miR-128-3p and CCNG1, it also attempted to provide a novel pathogenesis for OC progression.

Materials and methods

Tissues acquisition and cell culture. A total of 41 patients with OC were recruited to this study between March 2015 and September 2018. A total of 41 paired tissues of OC and adjacent normal tissues (>3 cm from tumor sites) were acquired from these patients with OC (age range 18-60 years old) subjected to surgical excision in the Shengli Oilfield Central Hospital, after obtaining informed consents from the patients. These tissues were divided into two groups (I+II: n=23; III+IV: n=18) according to the tumor stage. All specimens were promptly extracted. The present study was approved and supported by the Shengli Oilfield Central Hospital.

Human ovarian surface epithelial cells (IOSE-80) and OC cell line OVCAR-3 were bought from American Type Culture Collection and another OC cell line, SKOV3, was obtained from the cell bank of the Chinese Academy of Sciences. The Shengli Oilfield Central Hospital. The present study was mainly devoted to investigating the role of OIP5-AS1 in OC and its functional mechanism with miR-128-3p and CCNG1, it also attempted to provide a novel pathogenesis for OC progression.

Cell transfection. Small interfering (si) RNA against OIP5-AS1 (si-OIP5-AS1, 5'-GGCTTTTGTCTTTTATCTACAGG-3'), siRNA against CCNG1 (si-CCNG1, 5'-TTGAAGTAAAGATCTTTCTGT-3'), siRNA negative control (si-control, 5'-TTCCCCAGGTTGTCAGT-3'), short hairpin (sh) RNA vector against OIP5-AS1 (sh-OIP5-AS1, 5'-CCG GGCTCTTAGATTCCGTATCTCAGGAGGCGAGGTAAGGGTTCGAGAGTTCATGTTCAGTGAGT-3'), shRNA negative control (sh-control, 5'-ACCAGTTTTGCACATGTTCAGGAGGTAAGGGTTCGAGAGTTCATGTTCAGTGAGT-3'), mimic negative control (miR-128-3p, 5'-UCACAGUGAACC GGUCUCUUU-3'), mimic negative control (miR-control, 5'-UUUGUACUCAACAAAGUACUG-3'), miR-128-3p inhibitor (anti-miR-128-3p, 5'--AAAGAAGCCGGUUGUACUG-3'), inhibitor negative control (anti-control, 5'-CAGUAC UUUUGUUGUACAA-3') and the overexpression vector pCE-RB-Mam-OIP5-AS1 (OIP5-AS1) vector or the empty vector pCE-RB-Mam were all purchased from Guangzhou RiboBio Co., Ltd. Cell transfection was performed using Lipofectamine® 3000 (Invitrogen; Thermo Fisher Scientific, Inc.). Cells were seeded into the 24-well plates and cultured overnight to 50% coverage, then transfected with different concentrations of RNAs or vectors (40 nM siRNA, 40 nM shRNA vector, 40 nM mimic, 20 nM inhibitor or 2 µg pCE-RB-Mam/OIP5-AS1 vector). Further experimentation was performed after cells were incubated at 37°C for 48 h.

RNA extraction and reverse transcription-quantitative (RT-q) PCR. The extraction of total RNA was performed using TRIzol® (Thermo Fisher Scientific, Inc.) from OC tissues and cells (2x10^5) in accordance with the manufacturer's protocol, followed by the quantification of RNA concentration. Then 1 µg RNA was reversely transcribed to synthesis the complementary DNA (cDNA) using Prime Script RT regent kit (Takara Bio, Inc.) according to the manufacturer's protocols. PCR reaction system was prepared in 384 well-plates using a TB Green Premix EX Taq II kit (Takara Bio, Inc.) following the manufacturer's protocol and amplified via ABI Step One Real-time PCR System (Thermo Fisher Scientific, Inc.). The reaction system was prepared on ice with a 20-µl volume: 10 µl TB Green Premix EX Taq II (2x), 0.8 µl forward primer, 0.8 µl reverse primer, 0.4 µl ROX Reference Dye, 2 µl cDNA, 6 µl sterile water. Three technical replications were set for each sample. The reaction protocols were as follows: Predenaturation at 95°C for 30 sec, 40 cycles of denaturation at 95°C for 5 sec and annealing at 60°C for 30 sec. GAPDH and U6 were used as respective internal control for IncRNA or mRNA and miRNA. Primers were synthesized by Sangon Biotech Co., Ltd. and the sequences were:

- OIP5-AS1 (forward: 5'-TGCGAGATGGGCGAGGTAAG-3', reverse: 5'-TAGTTCCCTCTCTCGGCCG-3');
- miR-128-3p (forward: 5'-GCCAGATCATCGAACCCT-3', reverse: 5'-CAGTGCAGGGTCGAGGTAAG-3');
- CCNG1 (forward: 5'-GTACCCGCTGTAGGCTCGTAGC-3', reverse: 5'-ATAGCCCATCTGAGTACTGAGTC-3');
- GAPDH (forward: 5'-GTCTCTGACTCTCAACACC-3', reverse: 5'-ACCCCTCGTTGCTGTAGC-3');
- U6 (forward: 5'-ATTGGAACGATACGAAAGATT-3', reverse: 5'-GGAACGTCACAGATTTG-3').

The collected data from three independent experiments were analyzed using the 2^-ΔΔCt method (30).

Flow cytometry. An FITC Annexin V Apoptosis Detection kit I (BD Biosciences) was used to distinguish the apoptotic cells. After 48 h post-transfection, OVCAR-3 and SKOV3 cells were collected following digestion by 0.25% trypsin.
(GIBCO), then cell pellets were washed by pro-cooled phosphate buffered saline (PBS; GIBCO) and resuspended in 1X binding buffer. Afterwards, cells were stained by 5 µl FITC Annexin V and 5 µl PI for 15 min in the dark at room temperature according to the manufacturer’s protocol. The apoptotic cells were examined using a BD Accuri C6 flow cytometer (BD Biosciences) and analyzed using the BD Accuri C6 system (32-bit) software (BD Biosciences). The apoptosis rate was calculated as the percentage of early apoptotic cells (Annexin+/PI-), late apoptotic cells (Annexin+/PI+), and late apoptotic cells (Annexin+/-/PI+).

Transwell migration and invasion assays. A Transwell 24-well chamber (Corning Life Sciences) was used to detect the abilities of cell migration and invasion. Cell suspension in serum-free medium was pipetted into the upper chamber at room temperature. Simultaneously, 600 µl RPMI 1640 medium with 10% FBS was added to the lower chamber at room temperature. The chamber was incubated at 37°C for 24 h, and cells that passed through the lower membranes were fixed with methyl alcohol (Sangon Biotech Co., Ltd.) for 10 min and stained with crystal violet (Sangon Biotech Co., Ltd.). The obtained proteins were quantified using BCA assay kit (cat. no. BC5120; BCA; cat. no. ab205718; 1:5,000) for 1 h at room temperature. The gray level of each band was obtained by the statistical analysis of ImageJ software (National Institutes of Health) using β-actin as the endogenous reference.

Dual-luciferase reporter assay. The online software applications StarBase (http://starbase.sysu.edu.cn) and TargetScan (http://www.targetscan.org) were used to seek the miRNA target of OIP5-AS1 and target gene for miR-128-3p, respectively. To construct the recombinant luciferase plasmids, the wild-type (WT) OIP5-AS1 and 3’UTR of CCNG1 sequences were respectively cloned into the pGL-3 luciferase basic vector (Promega Corporation) to acquire WT-OIP5-AS1 and WT-CCNG1, then their mutant-types (MUT) with the mutant sites for miR-128-3p were also constructed as MUT-OIP5-AS1 and MUT-CCNG1. Each constructed luciferase reporter plasmid was transfected into OVCAR-3 and SKOV3 cells with miR-128-3p or miR-control by lipofectamine 3000. After transfection for 48 h, cells were lysed and the luciferase intensity was determined by the dual-luciferase reporter system (Promega Corporation) following the manufacturer’s protocol. The luciferase activity ratio of firefly and Renilla was considered to be the relative luciferase activity.

RNA immunoprecipitation (RIP) assay. RIP assay was performed using Magna RNA immunoprecipitation kit (EMD Millipore). OC cells were centrifuged at 4°C and 16,000 x g for 10 min and 2x10^7 cells were lysed using RIPA lysis buffer (EMD Millipore). The cells were then incubated with magnetic beads pro-covered with antibodies against Argonaute2 (Anti-Ago2; Abcam; cat. no. ab32381) using anti-Immunoglobulin G (Anti-IgG; Abcam; cat. no. ab205718) as the negative reference. RNA was extracted using TRIzol...
and the RNA enrichment was detected via RT-qPCR. Finally, the levels of OIP5-AS1 and miR-128-3p in Anti-IgG and Anti-Ago2 groups were compared.

**RNA pull-down assay.** Pierce™ Biotinylated Protein Interaction Pull-Down kit (Thermo Fisher Scientific, Inc.) was used to perform the pull-down assay. OVCAR-3 and SKOV3 cells were transfected with biotin-labeled miR-128-3p (Bio-miR-128-3p) or Bio-NC (Guangzhou riboBio co., ltd) using Lipofectamine 3000. Following transfection at 37°C for 48 h, cells were harvested by centrifugation at 4°C and 16,000 g for 10 min. The cell lysates were then incubated with streptavidin-coupled agarose beads at 4°C overnight. Following the isolation of RNA from the washed beads by TRIzol, the enrichment of OIP5 was measured by RT-qPCR analysis.

**Xenograft tumor assay.** A total of 12 BALB/c nude mice (six-week-old, 20-25 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. and maintained in a specific-pathogen-free environment under temperature of 25°C, humidity of 70% and a 12 h light/dark cycle. These mice received food and water *ad libitum*. Mice were arbitrarily divided into two groups with 6 mice/group, comprising sh-control and sh-OIP5-AS1 groups. The xenograft tumor model was established through the subcutaneous injection of 2x10^6 OVCAR-3 cells stably expressing sh-OIP5-AS1 or sh-control into the left flank of mouse's back. The health and behavior of mice were monitored every 2 days and tumor volume (length x width^2 x 0.5) was measured using a digital caliper every week. After cell injection for 4 weeks, tumor volume reached 900 mm^3 (<1,000 mm^3, which was set as the humane endpoint) and the mice were sacrificed. The 30% air of environment (per min) was displaced using the flow rate of CO_2 according to the current guidelines of the American Veterinary Medical Association (31), then mice were verified to have succumbed by monitoring the breathing. No mice died during the 4-week experimental period. The tumor tissues were excised from mice and weighed on an electronic scale. The examination of OIP5-AS1, miR-128-3p and CCNG1 was performed via RT-qPCR or western blotting. This experiment was ratified by the Animal Ethics Committee of the Shengli Oilfield Central Hospital and all procedures followed the guidelines.
provided by the National Institutes of Health for the Care and Use of Laboratory Animals, 8th edition (32).

Statistical analysis. All data were given as the mean ± standard deviation from three independent experiments. SPSS 19.0 (IBM Corp.) was used to conduct statistical analysis and figure plotting was performed using GraphPad Prism 7 (GraphPad Software, Inc.). The differences of two groups and more than two groups were analyzed through Student’s t-test and a one-way analysis of variance followed by Tukey’s post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

OIP5-AS1 is upregulated in OC tissues and cells. RT-qPCR analysis was firstly conducted to examine the expression of OIP5-AS1. OIP5-AS1 expression was markedly increased in OC tissues compared with the adjacent normal tissues (Fig. 1A). Among 41 OC tissues, OIP5-AS1 was upregulated in tissues at III+IV stage compared with those tissues at I+II stage (Fig. 1B). OIP5-AS1 expression was higher in both OVCAR-3 and SKOV3 cells compared with normal human ovarian IOSE-80 cells (Fig. 1C). This dysregulation of OIP5-AS1 implied its vital role in OC.

Knockdown of OIP5-AS1 represses cell viability, migration, invasion and glycolysis while promoting apoptosis in OC cells. OVCAR-3 and SKOV3 cells were transfected with si-OIP5-AS1 or si-control to investigate the function of OIP5-AS1 in OC. As shown in Fig. 2A, the decreased OIP5-AS1 expression in si-OIP5-AS1 group compared with the si-control group demonstrated that OIP5-AS1 was successfully knocked down in the two cell lines. MTT assay demonstrated that cell viability of OVCAR-3 and SKOV3 cells was distinctly reduced following the introduction of si-OIP5-AS1 compared with si-control group (Fig. 2B), while the apoptosis rate was shown to be increased (Fig. 2C). Transwell assay suggested that the migrated and invaded cells were decreased after downregulation of OIP5-AS1 (Fig. 2D and E). Analysis of glycolysis was performed by the detection of glucose consumption and lactate production, as well as the protein level of HK2 (an important enzyme of glycolysis). The data revealed that the transfection of si-OIP5-AS1 suppressed the consumption of glucose (Fig. 2F), lactate production (Fig. 2G) and HK2 protein expression (Fig. 2H) in both OVCAR-3 and SKOV3 cells, indicating that OIP5-AS1 knockdown caused the inhibition of glycolysis. These results suggested that knockdown of OIP5-AS1 repressed the progression of OC.

OIP5-AS1 interacts with miR-128-3p. LncRNAs exert their regulatory effects through sponging miRNAs generally (33). As demonstrated in Fig. 3A, StarBase predicted that OIP5-AS1 contained the binding sequences (UCACUGUG) for miR-128-3p and these sites were mutated into AGUGACAC in mutant OIP5-AS1. After performing the dual-luciferase reporter assay, it was found that the luciferase activity of WT-OIP5-AS1 plasmid was definitely inhibited by miR-128-3p.
mimic (compared with the miR-control group) while there was no obvious change in MUT-OIP5-AS1 group of OVCAR-3 and SKOV3 cells (Fig. 3B). RIP assay demonstrated that OIP5-AS1 and mir-128-3p were enriched in Ago-2 pellet compared with the IgG group (Fig. 3C). Moreover, the enrichment of OIP5-AS1 was much higher in Bio-mir-128-3p group compared with the Bio-NC group, suggesting that OIP5-AS1 was pulled down by mir-128-3p (Fig. 3D). Subsequently, the mir-128-3p expression in OC cells was examined and the results indicated that mir-128-3p level was downregulated in OVCAR-3 and SKOV3 cells compared with normal IOSE-80 cells (Fig. 3E). After verifying the successful overexpression effects on mir-128-3p
and OIP5-AS1 by miR-128-3p mimic and OIP5-AS1 vector (Fig. 3F and G), it was noticed that miR-128-3p overexpression could recover the OIP5-AS1-induced miR-128-3p suppression (Fig. 3H). The results demonstrated that OIP5-AS1 could directly sponge miR-128-3p.

**Overexpression of OIP5-AS1 ameliorates the suppressive effects of miR-128-3p on the progression of OC cells.** To explore the regulatory mechanism of OIP5-AS1 and miR-128-3p, OVCAR-3 and SKOV3 cells were transfected with miR-128-3p, miR-128-3p+OIP5-AS1 or their matched controls. MTT and flow cytometry demonstrated that miR-128-3p had a suppressive effect on cell viability (Fig. 4A and B) and a promoting effect on apoptosis (Fig. 4C and D) in OVCAR-3 and SKOV3 cells, whereas the overexpression of OIP5-AS1 reversed these effects. Cell migration (Fig. 4E and F) and invasion (Fig. 4G and H) were inhibited by miR-128-3p transfection, which was abrogated following the upregulation of OIP5-AS1. OIP5-AS1 transfection counteracted the inhibition of glucose consumption (Fig. 4I and J) and lactate production (Fig. 4K and L) caused by miR-128-3p. The miR-128-3p-induced downregulation of HK2 protein level was clearly alleviated by the promotion of OIP5-AS1 expression (Fig. 4M and N). Hence, the inhibitory effect of miR-128-3p on OC progression was abated by OIP5-AS1 overexpression.

**CCNG1 is a target of miR-128-3p.** TargetScan software predicted the binding domain of miR-128-3p in the 3'UTR sequence of CCNG1 (Fig. 5A), indicating that CCNG1 might be a potential target of miR-128-3p. Dual-luciferase reporter assay proved the combination of miR-128-3p and CCNG1 because the overexpression of CCNG1 markedly repressed the luciferase activity of WT-CCNG1 group but failed to decrease that of Mut-CCNG1 group (Fig. 5B). Then RT-qPCR and western blotting revealed that the mRNA and protein expression levels of CCNG1 were higher in OVCAR-3 and SKOV3 cells than those in normal IOSE-80 cells (Fig. 5C and D). The RT-qPCR analysis showed that the repressive impact of anti-miR-128-3p on the expression of miR-128-3p was significant (Fig. 5E). Subsequently, the introduction of miR-128-3p was presented to suppress the CCNG1 mRNA and protein levels, while the opposite effects were observed following anti-miR-128-3p transfection (Fig. 5F and G). Collectively, miR-128-3p directly targeted CCNG1.

**Downregulation of miR-128-3p restored the si-CCNG1-induced effects on OC cells.** To explore whether CCNG1 was associated with the influence of miR-128-3p on OC development, transfection of si-CCNG1, si-CCNG1+anti-miR-128-3p or the relative controls was conducted in OVCAR-3 and SKOV3 cells. Western blotting indicated that si-CCNG1 transfection markedly decreased the protein level of CCNG1, while
miR-128 inhibitor relieved this expression downregulation in OVCAR-3 and SKOV3 cells (Fig. S1). Subsequent experiments demonstrated that anti-miR-128-3p partly abolished the si-CCNG1-induced cell viability repression (Fig. 6A and B), apoptosis enhancement (Fig. 6C and D) and migration (Fig. 6E and F) or invasion (Fig. 6G and H) inhibition. Additionally, the decline of glucose consumption (Fig. 6I and J), lactate production (Fig. 6K and L) and HK2 protein level (Fig. 6M and N) caused by CCNG1 knockdown was also weakened following the downregulation of miR-128-3p. Above data clarified that miR-128-3p inhibition lightened the effects of CCNG1 knockdown on OC cells, hinting that CCNG1 inhibition was responsible for the anti-cancer effect of miR-128-3p on OC.

Figure 6. Continued.
OIP5-AS1 upregulated CCNG1 expression via sponging miR-128-3p in OC cells. To ascertain whether CCNG1 could be regulated by OIP5-AS1, OVCAR-3 and SKOV3 cells were respectively transfected with miR-control, miR-128-3p, miR-128-3p+vector or miR-128-3p+OIP5-AS1, followed by the analysis of RT-qPCR and western blotting. As shown in...
**Fig. 7** miR-128-3p transfection resulted in reducing the CCNG1 mRNA and protein levels, which was ameliorated by ectopic high expression of OIP5-AS1. In sum, OIP5-AS1 could upregulate the CCNG1 level by targeting miR-128-3p.

**OIP5-AS1 depression inhibited tumor growth of OC by miR-128-3p/CCNG1 axis in vivo.** The xenograft tumor model was constructed to explore the effect of OIP5-AS1 on OC in vivo. As Fig. 8A and B demonstrate, tumor volume and weight were significantly lower in sh-OIP5-AS1 group compared with the sh-control group, implying that OIP5-AS1 knockdown restrained the OC carcinogenesis in vivo. In contrast to the sh-control group, the expression of OIP5-AS1 was downregulated (Fig. 8C) but miR-128-3p was upregulated (Fig. 8D) in sh-OIP5-AS1 group according to the results of RT-qPCR. CCNG1 protein level was decreased with the downregulation of OIP5-AS1 in western blot assay (Fig. 8E). Therefore, OIP5-AS1 knockdown impeded the oncogenesis of OC by the miR-128-3p/CCNG1 axis in vivo.

**Discussion**

OC is one of the most common and familiar diseases of the reproductive system with an estimated 239,000 new cases being diagnosed worldwide annually (1), which can lead to the severe injury and mortality. Present clinical therapies leave much to be desired due to the dissatisfactory outcomes for OC patients. In recent years, IncRNAs have been reported to participate in regulating various types of cancers, including OC (34,35). In the present study, OIP5-AS1 was considered as a diagnostic and therapeutic biomarker in OC and the OIP5-AS1/miR-128-3p/CCNG1 network was revealed.

A previous study, using the analysis of The Cancer Genome Consortium, suggested that OIP5-AS1 was overexpressed in human epithelial origin types of cancer, including lung, cervical and head and neck tumors (36). Consistent with this finding, the present study also found that the expression of OIP5-AS1 in OC tissues and cells was conspicuously upregulated. It has been reported that the proliferation and migration of glioma cells are inhibited after OIP5-AS1 knockdown (37). In addition, a study of cervical cancer revealed that silencing OIP5-AS1 reduced cell proliferation of HeLa cells (38). Bai and Li (39) stated that cell proliferation and migration were impeded, while more apoptotic cells were induced, following the silence of OIP5-AS1 in gastric cancer. Knockdown of OIP5-AS1 restrained cell viability, migration, invasion and induced apoptosis in OC cells during the current study, which agrees with the results of OIP5-AS1 in other OC research (16,17). The link between OIP5-AS1 and glycolysis has yet to be reported. The present study revealed the inhibitory effects of low OIP5-AS1 expression on glucose consumption, lactate production and HK2 level. The acceleration of glycolysis by OIP5-AS1 also reflected its oncogenic function in OC evolution.

Previous studies have reported that IncRNAs can inhibit miRNA activity as miRNA ‘sponges’ (40-42). OIP5-AS1 is reported to interact with miRNAs in different types of cancer, such as lung cancer and glioma (14,15). In the present study, miR-128-3p was identified as a target of OIP5-AS1 and OIP5-AS1 was involved in the cellular processes of OC by sponging miR-128-3p. Sun et al (43) found that OIP5-AS1 regulates Wnt-7b expression via targeting miR-410 in glioma cells. Nevertheless, the target of miR-128-3p in OC cells remains to be elucidated. Glycolytic metabolism has been found to be associated with G1/S transition in cell cycle progression of types of cancer (44,45). In 1996, Horne et al (46) first discovered the high expression of CCNG1 in skeletal muscle, kidney and ovary. Knockdown of CCNG1 can reduce the incidence of hepatic tumor (47). The present study found that downregulating CCNG1 repressed cell growth, migration, invasion and glycolysis, demonstrating the pro-cancer effect of CCNG1 on OC. Subsequently, CCNG1 was reported to promote OC progression as the target genes of miR-1271 and...
miR-23b (28,29). The data of the present study proved that CCNG1 functioned as a downstream target of miR-128-3p and the role of miR-128-3p in OC was attributed to the negative regulation of CCNG1.

Notably, OIP5-AS1 has already been proved to modulate glioma progression through the miR-410/Wnt-7b axis (43). The results of the present study clearly indicated that OIP5-AS1 indirectly regulated the CCNG1 level by sponging miR-128-3p. The oncogenic role of OIP5-AS1 in OC was dependent on the miR-128-3p/CCNG1 axis in vivo.

There are certain limitations to the current study. First, the effects of OIP5-AS1/miR-128-3p/CCNG1 on several signaling pathways remain to be elucidated. Second, other targets of miR-128-3p associated with glycolysis need to be discovered. Given the involvement of OIP5-AS1 and miR-128-3p in glycolytic metabolism, it would be worth exploring the potentials of glycolytic genes including phosphoglycerate dehydrogenase and glucose 6-phosphatase as the targets of miR-128-3p.

In conclusion, the present study revealed that OIP5-AS1 worked as a tumorigenic factor in OC development via the miR-128-3p/CCNG1 axis and revealed the OIP5-AS1/miR-128/CCNG1 modulatory network in OC. The present study might lay the foundation for OC progression at the lncRNA level.

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Availability of data and materials

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

Authors’ contributions

XF and XW were responsible for the conceptualization and methodology; YL, YUl and XS performed formal analysis and data curation; YUl and YAL were responsible for validation and investigation; and YU, XF and XW prepared the original draft of the manuscript, which they wrote, reviewed and edited. YUl and XF confirm the authenticity of all raw data. All authors reviewed and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the ethical review committee of the Shengli Oilfield Central Hospital.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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