

Long Non-Coding RNA OIP5-AS1 Contributes to Gallbladder Cancer Cell Invasion and Migration by miR-143-3p Suppression

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Objective: This study was designed to investigate the effect of long non-coding RNA (lncRNA) OIP5-AS1 on cell migration and invasion of gallbladder cancer (GBC) and its specific mechanism.

Methods: The expressions of lncRNA OIP5-AS1 and *miR-143-3p* in GBC cell lines (GBC-SD, SGC996 and NOZ) and gallbladder epithelial cells (HGBE cells) were measured by qRT-PCR. After loss- and gain-of-function experiments for OIP5-AS1 and *miR-143-3p* in GBC-SD cells, CCK-8 was applied to examine cell viability, cell scratch assay to measure cell migration, and transwell chamber to inspect cell invasion capacity. The interaction between OIP5-AS1 and *miR-143-3p* was predicted by StarBase. Then, luciferase reporter gene assay and RNA pull-down were used to verify the targeting relationship between *miR-143-3p* and OIP5-AS1.

Results: OIP5-AS1 was highly expressed and *miR-143-3p* was downregulated in GBC cell lines, when compared with HGBE cells. Overexpression of OIP5-AS1 or downregulation of *miR-143-3p* facilitated GBC-SD cell invasion, proliferation and migration, while different expression patterns were found in GBC-SD cells in response to OIP5-AS1 suppression or *miR-143-3p* overexpression. OIP5-AS1 negatively mediated *miR-143-3p*. *miR-143-3p* upregulation partially reversed the inhibitory effect of OIP5-AS1 knockdown on GBC-SD cell activities.

Conclusion: lncRNA OIP5-AS1 accelerates the progression of GBC by suppressing miR-143-3p.

Keywords: OIP5-AS1, *miR-143-3p*, gallbladder cancer, proliferation, migration, invasion

Introduction

Gallbladder cancer (GBC), with high risk of distant metastasis and lymph node metastasis at the initial diagnosis, has emerged as one of the most prevalent gastrointestinal cancers.¹ The poor prognosis of GBC may attribute to its aggressiveness and absence of symptoms in the early stages.² Surgical resection is the only possible curative treatment, but the success rate is variable, depending on the stage of GBC and completeness of resection.³ Therefore, understanding the molecular changes related to GBC is of great significance for the development of effective therapy and early diagnosis, thereby improving the prognosis of GBC patients.

Long non-coding RNAs (lncRNAs), which act as genomically transcribed non-coding transcripts over 200 nucleotides, confer momentous impact on the

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deterioration or remission of cancer.⁴ In recent years, the coordinating roles of lncRNAs have been frequently reported in multiple biological processes of GBC progression, including migration, invasion and proliferation. For example, lncRNA LINC01694 interferes with the growth and invasion of GBC cells by regulating *miR-340-5p* to restrain Sox4.⁵ In GBC, GATA6-AS may restrain cancer cell invasion and migration through *miR-421* knockdown.¹ OPA-interacting protein 5 antisense RNA 1 (OIP5-AS1), which transcribed in the antisense of gene encoding OIP5, is implicated in the cancer progression.⁶ In laryngeal squamous cell carcinoma, OIP5-AS1 modulates migration and growth of cells.⁷ Additionally, OIP5-AS1 is responsible for oral squamous cell carcinoma regarding cell migration, proliferation and invasion through mediation of *miR-338-3p*/NRP1 axis.⁸ Nonetheless, the functional roles and precise expression patterns of OIP5-AS1 in GBC cells have not been clearly defined. MicroRNAs (miRNAs), performing themselves in GBC, are non-coding single-stranded RNAs that can bind to the 3'untranslated regions of their target mRNAs to repress protein expression.⁹ Among miRNAs, *miR-143-3p* has been researched to suppress the growth and angiogenesis of GBC.¹⁰

Former studies proposed the possibility that lncRNA acts as a competing endogenous (ceRNA) for miRNA to regulate its targeting gene expression in the GBC.^{11,12} In consideration of the potential relationship of lncRNA and miRNA, we aimed to explore whether lncRNA OIP5-AS1 is engaged in the progression of GBC through targeting *miR-143-3p* in a ceRNA manner. This competitive role of lncRNA OIP5-AS1 is elicited to contribute to tumor progression. More evidence revealed that OIP5-AS1 promotes the progression of GBC through suppressing *miR-143-3p*.

Materials and Methods

Cell Culture

The Human GBC GBC-SD cell line was supplied by the Cell Bank of Chinese Academy of Sciences (Shanghai, China). SGC996 cell line was purchased from the Cancer Institute of Tongji University School of Medical (Shanghai, China). NOZ cell line was provided by Health Science Research Resources Bank (Osaka, Japan) and gallbladder epithelial cells (HGBE) were offered by ATCC (Gaithersburg, MD, USA). The GBC-SD, SGC996 and NOZ cell lines were soaked in 1640 culture medium (11879020, Gibco, New York, USA) supplemented with 10% fetal bovine serum (FBS, 16140071, Gibco,

New York, USA), and the gallbladder epithelial cells (HGBE) were immersed in Dulbecco's modified Eagle medium (DMEM, 11965092, Gibco, New York, USA) containing 10% FBS and 1% penicillin/streptomycin. Cell culture was conducted at 37°C gassed with 5% CO₂. The following experiments were conducted when cell confluence reached 80% ~90%. The use of cell lines was approved by the Ethics Committee of the Third Xiangya Hospital of Central South University.

Cell Transfection and Grouping

The si-OIP5-AS1, si-NC, pcDNA3.1-OIP5-AS1, pcDNA3.1-NC, *miR-143-3p* antagomir, NC antagomir, *miR-143-3p* agomir and NC agomir were designed and synthesized by Invitrogen (Shanghai, China). Twenty-four h before transfection, cells were aspirated into a six-well plate (1 × 10⁵ cells/well) with fresh serum-free medium. Following transfection based on the instruction of the Lipofectamine 3000 kit (L3000015, Invitrogen, New York, USA), cells were correspondingly assigned into the si-OIP5-AS1 group (2.5 µg), si-NC group (2.5 µg, negative control of si-OIP5-AS1), pcDNA3.1-OIP5-AS1 group (2.5 µg), pcDNA3.1-NC group (2.5 µg, negative control of pcDNA3.1-OIP5-AS1), *miR-143-3p* agomir group (200 nmol), NC agomir (200 nmol, negative control of *miR-143-3p* agomir), *miR-143-3p* antagomir group (200 nmol), NC antagomir (200 nmol, negative control of *miR-143-3p* antagomir), si-OIP5-AS1+*miR-143-3p* antagomir group, si-NC+NC antagomir group, si-OIP5-AS1+NC antagomir group, si-NC+NC antagomir group, si-NC+*miR-143-3p* antagomir group and si-NC+NC antagomir group. Blank group was set as the negative control (exposure to the Lipofectamine 3000 kit without any plasmid). Measurement or detection in each group was performed 48 h after cell transfection.

RNA Isolation and qRT-PCR

GBC cells were dissolved in 1 mL of TRIzol (Thermo Fisher Scientific, MA, USA) and the total RNA was extracted. The cDNA template was obtained by reverse transcription of total RNA by using M-MLV reverse transcriptase and random primers. The reaction system was configured according to the instructions of Premix Ex TaqTMII kit (Takara, Dalian, China), the reaction conditions corresponding to each primer were set and the ABI7500 quantitative PCR instrument (Applied Biosystems, Shanghai, China) was employed for RT-PCR experiments. The internal reference was presented as

GAPDH, and data analysis utilized $2^{-\Delta\Delta Ct}$ method.¹³ The formula is as follows: $\Delta\Delta Ct = [Ct_{(target\ gene)} - Ct_{(reference\ gene)}]_{experimental\ group} - [Ct_{(target\ gene)} - Ct_{(reference\ gene)}]_{control\ group}$. The primer sequences are shown in Table 1.

CCK-8 Assay

Cell proliferation rate was evaluated by using the CCK-8 kit (Beyotime, Shanghai, China). The transfected cells were seeded into 96-well plates (3000 cells/well), and the fresh medium was replaced every day. The proliferation of cells was assessed with the CCK-8 kit every 24 h for 5 consecutive days: 10 μ L of CCK-8 solution was added to cells/well for 2 h of incubation at 37°C. The absorbance (optical density, OD) was assessed by SpectraMax M5 microplate reader (Molecular Devices) at 450 nm wavelength.

Cell Scratch Assay

Cells in the Control group and experimental group were aspirated into a 12-well plate (1×10^5 cells). Following reached 100% confluence, cells were scratched by using a 10 μ L pipette tip. Dulbecco's phosphate-buffered saline (DPBS, 14190250, Gibco, New York, USA) was used to wash the cells thrice to remove the scratched cells, and then cells were cultured in fresh DMEM covering 2% FBS. Photographs of cells in the same field of vision were captured under an inverted microscope (Olympus) at time 0 and 24 h post-cell scratch, and the change of scratch width was observed. The migrate rate = (scratch distance at 0 h – scratch distance at 24 h)/scratch distance at 0 h. The test was done in triplicate.

Transwell Assay

The invasion ability of cells was displayed by using a transwell chamber (Corning, New York, USA). Following 2 h of Matrigel coating at 37°C, the membrane was inserted in the middle of the two-chamber. Cells ($3 \times$

10^4) suspended in culture medium with 1% FBS were pipetted into an apical chamber, and 0.8 mL medium with 10% FBS was added into the basolateral chamber. After 24 h of incubation, non-invaded cells on the upper compartment of the membrane were removed by using a cotton swab. The invaded cells on the bottom surface of the membrane were fixed in methyl alcohol for 30 min, followed by staining with 10% Giemsa and three times of PBS wash. Cell counting of invaded cells was done by using an inverted microscope, and three independent experiments were conducted to confirm the reproducibility.

Luciferase Reporter Gene Assay

The binding site of OIP5-AS1 and *miR-143-3p* was predicted by online prediction software TargetScan. The mutated type sequences and wild-type sequences (MT-OIP5-AS1, WT-OIP5-AS1) were accordingly designed, cloned and combined with pGL3-Basic (Promega, USA). After cotransfection of GBC-SD cells with MT-OIP5-AS1 /WT-OIP5-AS1 and *miR-143-3p* agomir/*miR-143-3p* antagomir for 48 h, the luciferase activity was evaluated by the luciferase kit (RG051S, Beyotime).

RNA Pull-Down Assay

The RNA pull-down assay was conducted with the instruction of an RNA pull-down kit (20164, Thermo Fisher). In brief, OIP5-AS1 purified in vitro was transcribed by Takara kit (Takara, Dalian, China). Then, OIP5-AS1 was labeled with biotin using Pierce RNA 3'Desthiobiotinylation kit (20163, Thermo Fisher). To ensure the formation of the secondary structure of RNA, the synthesized RNA was heated at 95°C for 2 min, placed on ice for 2 min and then put at room temperature for 20 min. The streptavidin magnetic beads were incubated with biotin-labeled OIP5-AS1 to obtain molecules that interacted with OIP5-AS1. Then, the RNA-binding protein was harvested after folded magnetic bead-biotin labeled-OIP5-AS1 was stirred or rotated with cell lysate in RIP buffer at 4°C for 2 h. The beads were washed six times with RIP wash buffer, and the collected RNA was detected by qRT-PCR.

Statistical Analysis

Data were analyzed by employing SPSS 18.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 6.0 (GraphPad Software Inc.). Data were displayed as mean \pm standard deviation (SD). *T* test was applied to the comparison between

Table 1 Primer Sequence Information

Name of the Primer	Sequences (5'-3')
OIP5-AS1-F	GGTCGTGAAACACCGTCG
OIP5-AS1-R	GTGGGGCATCCAGGGT
<i>miR-143-3p</i> -F	TGAGATGAAGCACTG
<i>miR-143-3p</i> -R	GTGCAGGGTCCGAGGT
GAPDH-F	GGAGCGAGATCCCTCCAAAAT
GAPDH-R	GGCTGTTGTCATACTTCTCATGG

Abbreviations: F, forward primer; R, reverse primer.

two groups, whereas comparisons between multiple groups were conducted utilizing one-way analysis of variance (ANOVA). Significance was set at $P < 0.05$. All experiments were repeated three times unless otherwise specified.

Results

High Expression of OIP5-AS1 and Low Expression of miR-143-3p in GBC Cell Lines

Result of qRT-PCR presented that compared with HGBE cells, the expression of OIP5-AS1 in GBC cell lines (GBC-SD cells, NOZ cells, SGC996 cells) was significantly increased (Figure 1A, $P < 0.01$), and *miR-143-3p* level was remarkably reduced in GBC cell lines (Figure 1B, $P < 0.01$). The maximum expression gap between OIP5-AS1 and *miR-143-3p* was found in GBC-SD cells, rather than the other two cell lines and therefore GBC-SD cells were most suitable for subsequent experiments.

OIP5-AS1 Promotes Invasion, Proliferation and Migration of GBC Cells

To assess the role of lncRNA OIP5-AS1 in GBC-SD cells, GBC-SD cells were transfected with pcDNA3.1-OIP5-AS1 or si-OIP5-AS1. qRT-PCR results displayed good transfection efficiencies of pcDNA3.1-OIP5-AS1 and si-OIP5-AS1 in GBC-SD cells, as performed by elevated expression of OIP5-AS1 in pcDNA3.1-OIP5-AS1 group and decreased level of OIP5-AS1 in the si-OIP5-AS1 group (Figure 2A, $P < 0.01$). No obvious change was noticed in the si-NC, pcDNA3.1-NC group and Blank groups with regard to mRNA expression of OIP5-AS1 (Figure 2A, $P > 0.05$).

CCK-8 manifested that transfection with pcDNA3.1-OIP5-AS1 heightened GBC cell viability (Figure 2B, $P < 0.01$, vs the pcDNA3.1-NC group), while transfection with si-OIP5-AS1 repressed GBC cell viability (Figure 2B, $P < 0.01$, vs the si-NC group), suggesting that OIP5-AS1 can accelerate the growth of GBC-SD cells.

Results obtained from cell scratch assay and transwell revealed that overexpression of OIP5-AS1 enhanced the abilities of cell invasion (Figure 2D, $P < 0.01$) and migration (Figure 2C, $P < 0.01$), while knockdown of OIP5-AS1 diminished these abilities (Figure 2C and D, $P < 0.01$). These data illustrated that the biological functions of GBC cells can be enhanced by OIP5-AS1.

OIP5-AS1 Negatively Targets and Modulates miR-143-3p

The StarBase database was employed to predict the relationship between miRNAs and OIP5-AS1, and we found that *miR-143-3p* may be a potential target of OIP5-AS1. The correlation analysis by the online software StarBase2.0 showed the negative correlation between OIP5-AS1 and *miR-143-3p* in mesothelioma and adrenocortical cancer (Figure 3A). Based on this analysis, OIP5-AS1 was speculated to mediate the biological function of GBC-SD cells through *miR-143-3p* regulation.

Subsequently, qRT-PCR was applied to measure the expression level of *miR-143-3p*. The measurement showed increased mRNA level of *miR-143-3p* in the si-OIP5-AS1 group and decreased *miR-143-3p* expression in the pcDNA3.1-OIP5-AS1 group (Figure 3B, $P < 0.01$).

To verify the interaction between *miR-143-3p* and OIP5-AS1, the binding site of OIP5-AS1 and *miR-143-*

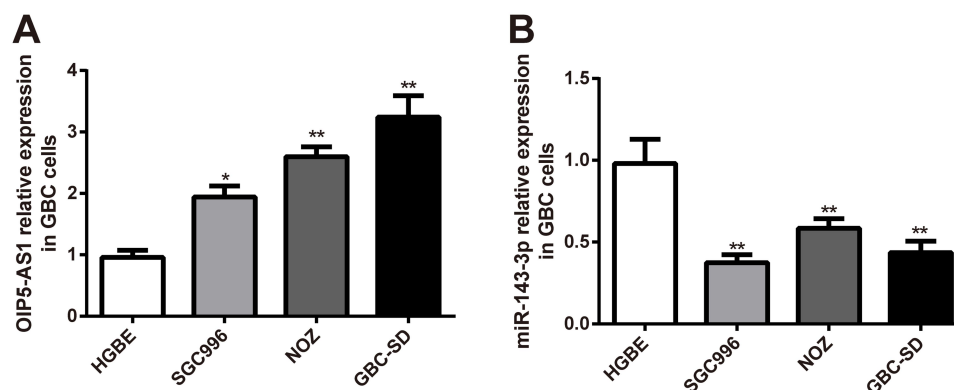


Figure 1 The suppressed OIP5-AS1 and elevated *miR-143-3p* in GBC cell lines.

Notes: The mRNA expressions of OIP5-AS1 (A) and *miR-143-3p* (B) in gallbladder epithelial cells (HGBE) and GBC cell lines were determined by qRT-PCR; * $P < 0.05$, ** $P < 0.01$ vs the HGBE cell group.

Abbreviation: GBC, gallbladder cancer.

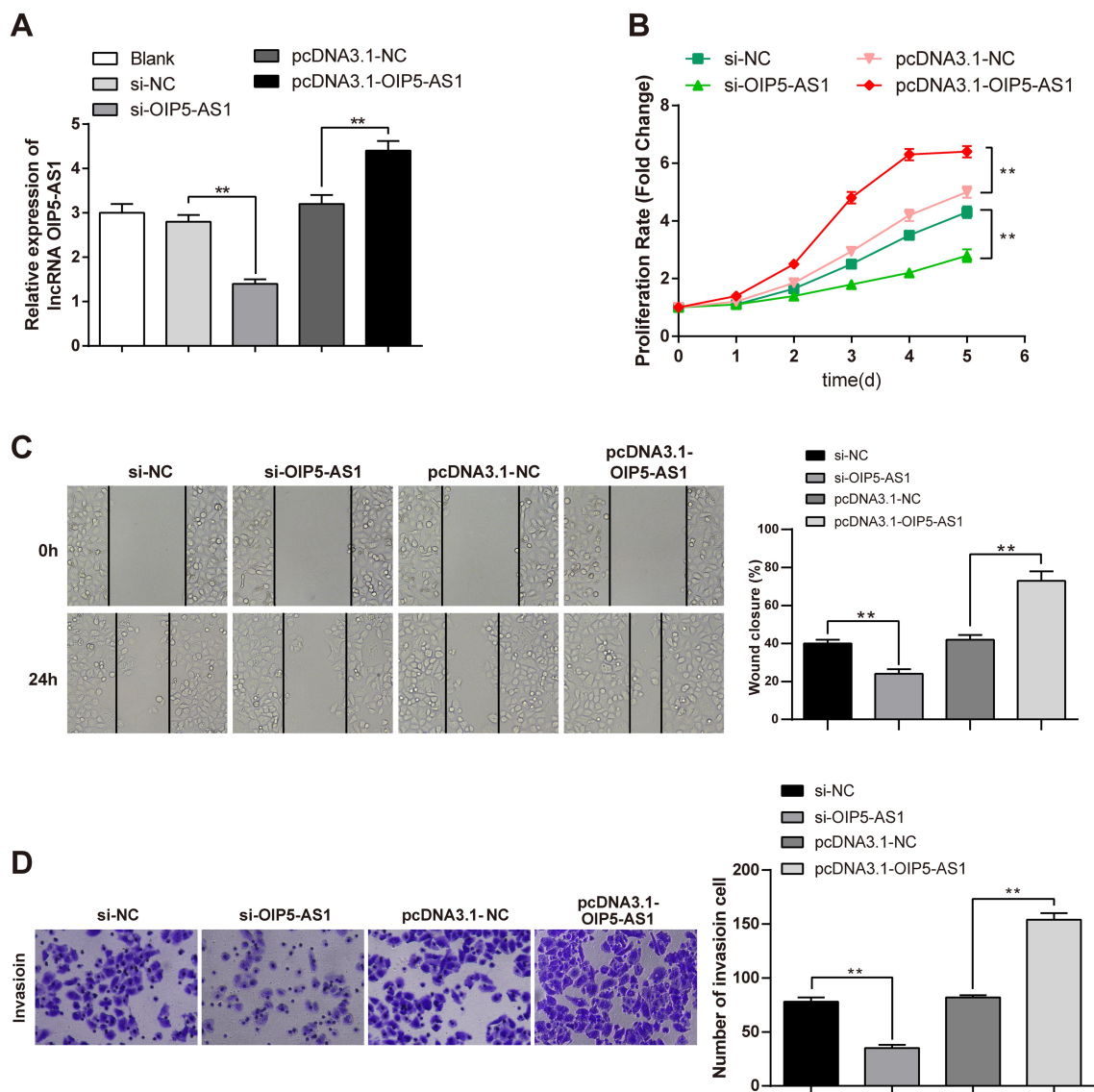


Figure 2 Overexpression of OIP5-AS1 affects the migration, invasion and proliferation of GBC-SD cells.

Notes: After GBC-SD cells transfected with si-NC, si-OIP5-AS1, pcDNA3.1-NC or pcDNA3.1-OIP5-AS1, qRT-PCR was adopted to detect the mRNA expression of OIP5-AS1 (A), CCK-8 to assess cell viability (B), cell scratch assay to measure the cell migration rate (C) and transwell chamber to inspect cell invasion rate (D); ** $P < 0.01$. **Abbreviation:** GBC, gallbladder cancer.

3p was predicted, and the mutant and wide types of OIP5-AS1 and *miR-143-3p* were accordingly designed, which are depicted in Figure 3C. To ascertain targeting relationship between OIP5-AS1 and *miR-143-3p*, wild-type OIP5-AS1 luciferase promoter plasmid (WT-OIP5-AS1) and a mutant OIP5-AS1 luciferase promoter plasmid (MT-OIP5-AS1) were constructed. Result of luciferase reporter gene assay of the OIP5-AS1 promoter demonstrated that exposure to *miR-143-3p* agomir obviously reduced the luciferase activity of WT-OIP5-AS1 (Figure 3D, $P < 0.01$) while did not affect the luciferase activity of MT-OIP5-AS1. Additionally, after the RNA pull-down experiment and qRT-PCR detection, a large number of OIP5-

AS1 were found in the bio-*miR-143-3p*-WT group (Figure 3E, $P < 0.01$), but there was no expression of OIP5-AS1 in the bio-*miR-143-3p*-MT group or bio-NC group (Figure 3E), indicating that *miR-143-3p* and OIP5-AS1 directly bound to each other. Together, there was a negative correlation of OIP5-AS1 and *miR-143-3p* in GBC cells.

MiR-143-3p Inhibits Biological Functions of GBC Cells

The *miR-143-3p* was overexpressed or suppressed in GBC-SD cells to investigate the impact of *miR-143-3p* on GBC

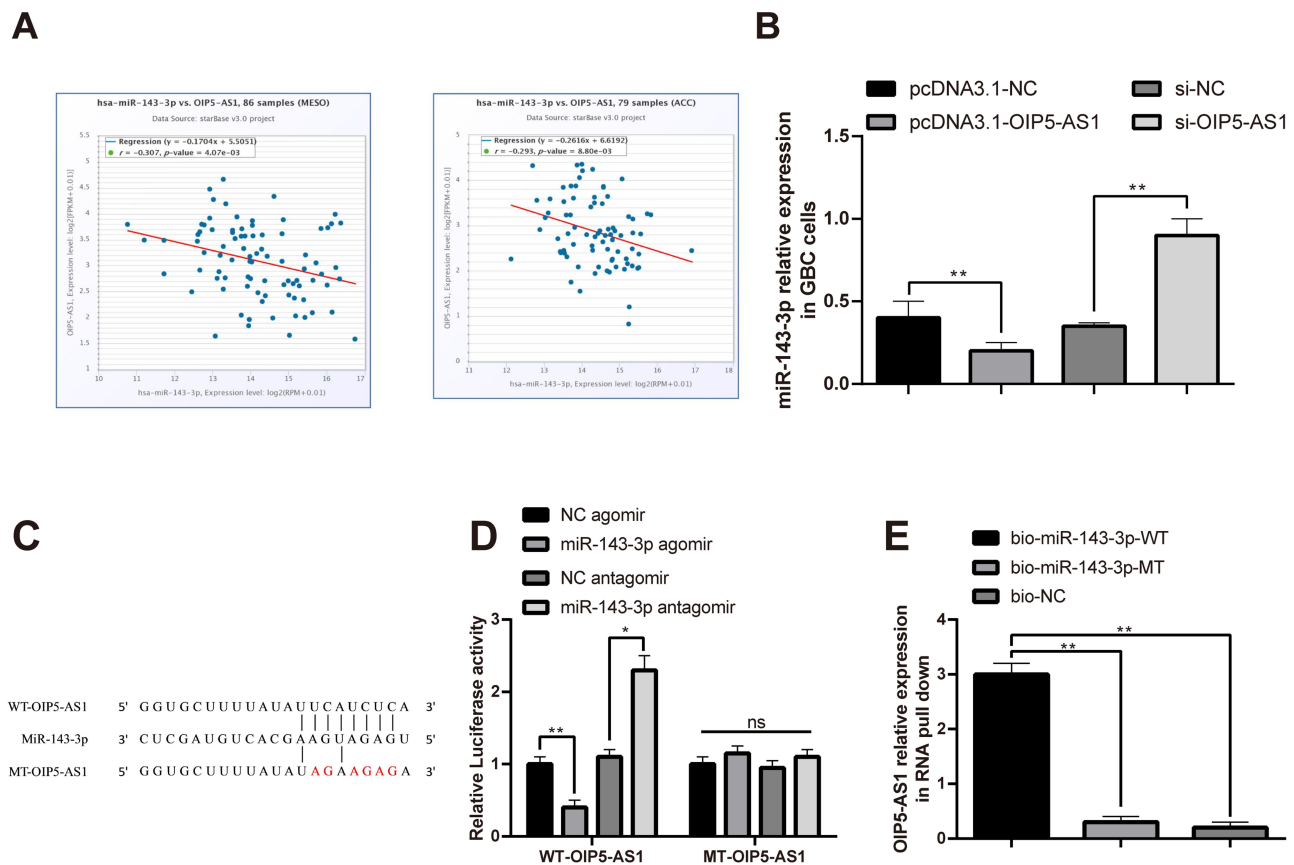


Figure 3 The negative correlation between OIP5-AS1 and *miR-143-3p*.

Notes: The correlation analysis of OIP5-AS1 and *miR-143-3p* in mesothelioma and adrenocortical cancer was displayed by the online software StarBase2.0 (A). After transfection pcDNA3.1-OIP5-AS1 or si-OIP5-AS1 with GBC-SD cells, qRT-PCR was used to detect the expression level of *miR-143-3p* (B). The online software StarBase predicted the binding site and designed the mutation site of OIP5-AS1 and *miR-143-3p* (C). The luciferase reporter gene assay verified the targeting relationship between OIP5-AS1 and *miR-143-3p* (D). qRT-PCR was utilized to perform the mRNA level of OIP5-AS1 in RNA pull-down experiment (E); ns $P > 0.05$, * $P < 0.05$, ** $P < 0.01$.

Abbreviation: GBC, gallbladder cancer.

cells. Analysis of qRT-PCR displayed that the level of *miR-143-3p* was increased in the *miR-143-3p* agomir group (Figure 4A, $P < 0.05$, vs the NC agomir group) and suppressed in the *miR-143-3p* antagomir group (Figure 4A, $P < 0.01$, vs the NC antagomir group). No marked difference among the NC antagomir, NC agomir and Blank groups was noticed (Figure 4A, $P > 0.05$). These data manifested that *miR-143-3p* was notably repressed and overexpressed in GBC-SD cells, illustrating that *miR-143-3p* agomir and *miR-143-3p* antagomir with good transfection efficiencies can be applied to the subsequent experiments.

Then, the effect of *miR-143-3p* on the viability of GBC-SD cells was measured by the CCK-8 kit. We found that treatment with *miR-143-3p* antagomir increased cell viability (Figure 4B, $P < 0.01$, vs the NC antagomir group), while subjection to *miR-143-3p* agomir inhibited the ability of GBC cell proliferation (Figure 4B, $P < 0.01$, vs the NC agomir group).

The roles of *miR-143-3p* upregulation and *miR-143-3p* downregulation in the capacities of GBC-SD cell migration

and invasion were assessed by cell scratch and transwell chamber experiments, respectively. The *miR-143-3p* antagomir group had enhanced abilities of cell invasion (Figure 4D, $P < 0.01$) and migration (Figure 4C, $P < 0.01$) in comparison to the NC antagomir group, while comparing with the NC agomir group, lower migration and invasion abilities were discovered in the *miR-143-3p* agomir group (Figure 4C and D, $P < 0.01$). Collectively, *miR-143-3p* can repress migration, invasion and growth of GBC cells.

OIP5-AS1 Governs Biological Functions of GBC Cells by *miR-143-3p* Downregulation

To investigate whether OIP5-AS1 contributes to the biological function of GBC-SD cells by inhibiting *miR-143-3p*, si-OIP5-AS1 and *miR-143-3p* antagomir were transfected or cotransfected into GBC-SD cells. Results of CCK-8 presented a pronounce increase in cell viability in the si-NC

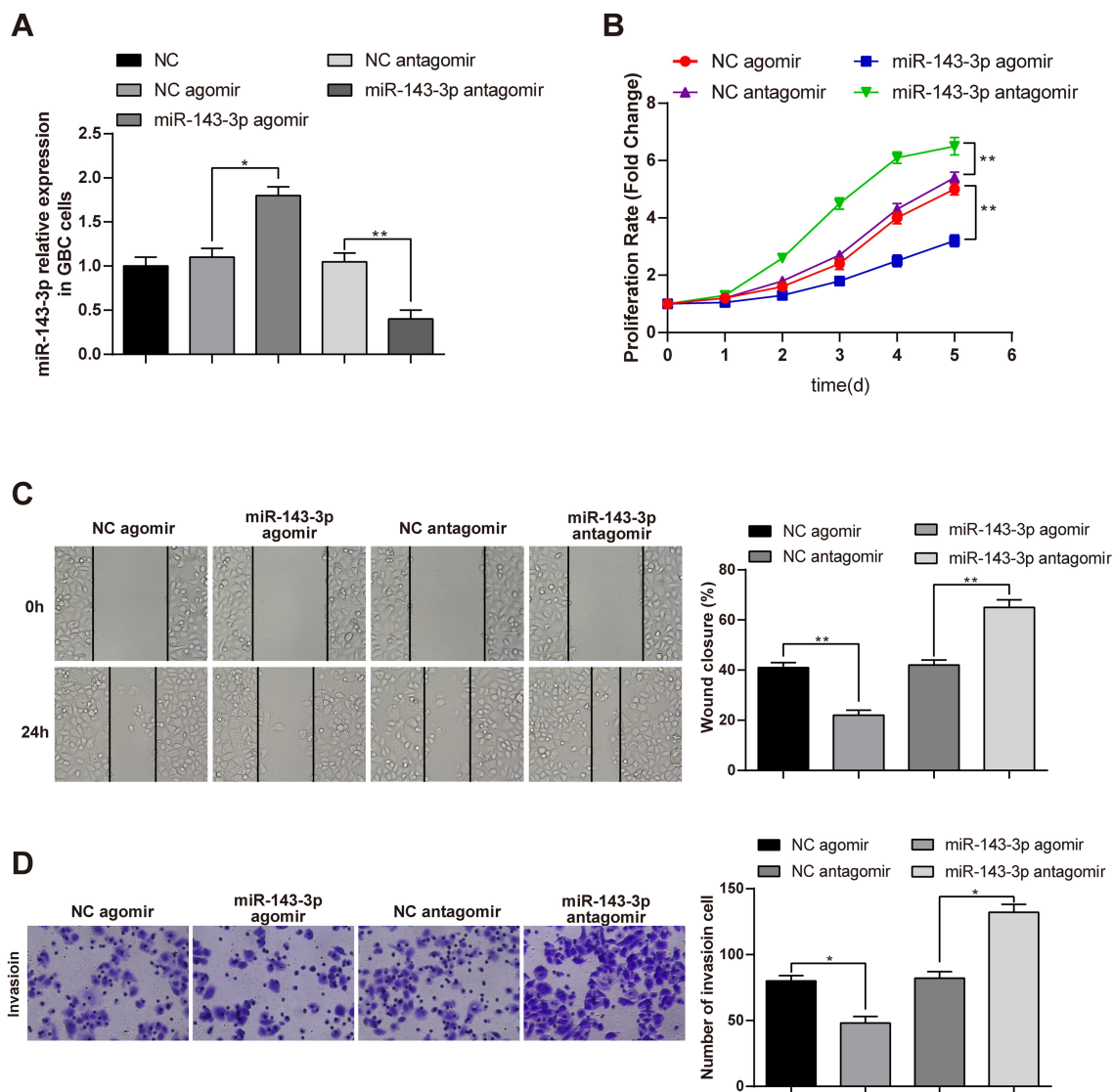


Figure 4 The migration, invasion and proliferation of GBC cells can be inhibited by *miR-143-3p*.

Notes: After transfection with NC antagonist, *miR-143-3p* antagonist, NC agomir or *miR-143-3p* agomir plasmids in GBC-SD cells, qRT-PCR was used to examine the *miR-143-3p* expression in GBC-SD cells (**A**), CCK-8 to detect the cell proliferation ability (**B**), cell scratch assay to assess cell migration ability and transwell to inspect cell invasion ability (**C** and **D**); * $P < 0.05$, ** $P < 0.01$.

Abbreviations: GBC, gallbladder cancer; NC, negative control.

+*miR-143-3p* antagonist group, and a prominent decreased in the si-OIP5-AS1+NC antagonist group (Figure 5A, $P < 0.01$, vs the si-NC+NC antagonist group). Additionally, cell scratch assay showed that the si-NC+*miR-143-3p* antagonist group had elevated cell migration ability, while the si-OIP5-AS1+NC antagonist group possessed suppressed cell migratory property (Figure 5B, $P < 0.01$, vs the si-NC+NC antagonist group). The findings of the transwell assay revealed that there was higher cell invasion ability in the si-NC+*miR-143-3p* antagonist group, and lower cell invasive property in the si-OIP5-AS1+NC antagonist group (Figure 5C, $P < 0.01$, vs the si-NC+NC antagonist group). Compared with the si-NC+*miR-143-3p* antagonist group, the si-OIP5-AS1+*miR-143-*

3p antagonist group possessed markedly weakened abilities of cell proliferation, migration and invasion (Figure 5A–C, $P < 0.01$). No notable difference was observed in the Blank, si-NC+NC antagonist and si-OIP5-AS1+*miR-143-3p* antagonist groups (Figure 5A–C, $P > 0.05$). These results revealed that *miR-143-3p* antagonist can eliminate the inhibitory effect of OIP5-AS1 knockdown on the GBC-SD cell activities. Taken these data together, OIP5-AS1 mediates *miR-143-3p* to regulate progression of GBC.

Discussion

GBC is a frequently diagnosed biliary tract with high incidence and mortality.^{14,15} Here, GBC-SD cells were

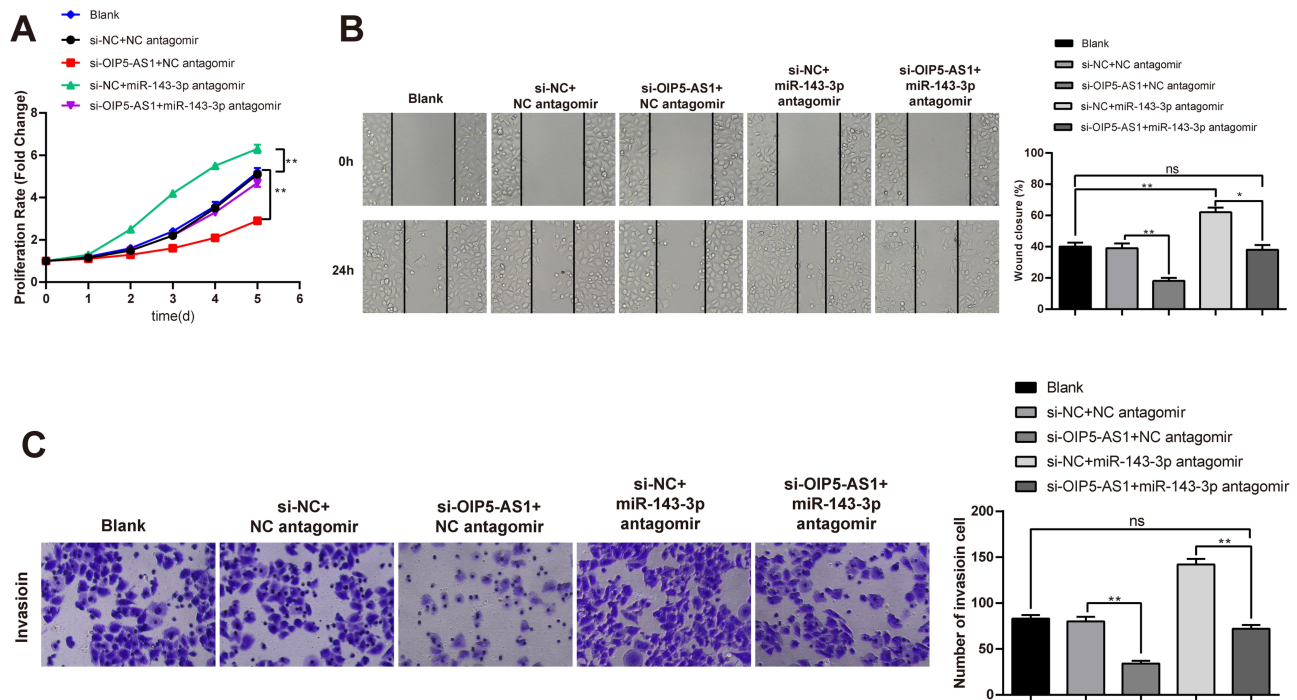


Figure 5 OIP5-AS1 facilitates the invasion, migration and proliferation of GBC-SD cells through *miR-143-3p*.

Notes: After cotransfection of si-NC+NC antagonist, si-OIP5-AS1+NC antagonist, si-NC+miR-143-3p antagonist or si-OIP5-AS1+miR-143-3p antagonist with GBC-SD cells, the proliferation ability of GBC-SD cells was determined by CCK-8 assay (A), and the cell migration and invasion abilities were measured by cell scratch assay (B) and transwell (C); ns $P > 0.05$, * $P < 0.05$, ** $P < 0.01$.

Abbreviations: GBC, gallbladder cancer; NC, negative control.

selected from GBC cell lines and used for the subsequent experiments. We put the spotlight on the function of lncRNA OIP5-AS1 or *miR-143-3p* in GBC progression after identified the differentially expressed OIP5-AS1 and *miR-143-3p* in GBC-SD cells. Herein, we proposed that the invasion, migration and proliferation of GBC cells can be accelerated by the OIP5-AS1/*miR-143-3p* axis.

LncRNAs inactivate tumor suppressors or activate oncogenes to trigger the growth or metastasis phenotype of cancer cells by interacting with specific RNA-binding proteins.¹⁶ Herein, we characterized OIP5-AS1, which is transcribed in the antisense direction from the same gene that encodes OIP5.¹⁷ LncRNA OIP5-AS1 is reported to be upregulated and promotes cell proliferation in lung cancer.¹⁸ An important implication of our findings was that the high expression of OIP5-AS1 was identified in GBC-SD cells (Figure 1A, $P < 0.01$). Then, the loss- and gain-of-function experiments for lncRNA OIP5-AS1 were performed to explore whether overexpressed or silenced OIP5-AS1 affects the activities of GBC cells. Functionally, CCK-8, cell scratch and transwell assays showed that amplifying OIP5-AS1 in GBC cells heightened capacities of cell migration, invasion and proliferation, whereas

knockdown of OIP5-AS1 had opposite results (Figure 2B–D, $P < 0.01$). Growing evidence proposed the oncogenic role of OIP5-AS1 in kinds of cancers. For instance, the growth, metastasis and EMT progress of hepatoblastoma are elicited by OIP5-AS1 through the *miR-186a-5p/ZEB1* axis.¹⁹ OIP5-AS1 enhances CDK14 expression to induce osteosarcoma tumorigenesis by targeting *miR-223*.²⁰ LncRNA OIP5-AS1 drives hemangioma endothelial cell invasion, proliferation and migration through the *miR-195-5p/NOB1* axis modulation.²¹ These data proved that OIP5-AS1 is an oncogene to participate in GBC, and we speculated that the implication of OIP5-AS1 in GBC cells may be in a ceRNA manner.

The former study suggested that OIP5-AS1 performs itself in cervical cancer, microglioma, hemangioma and other tumors, and expressed in the cytoplasm through a ceRNA manner.^{22,23} *miR-143-3p* is reported to be abnormally expressed and directly correlated with the prognosis and progression of various cancers.^{24,25} Of note, *miR-143-5p* dramatically represses the invasion, proliferation and migration of GBC cells through targeting the HIF-1 α /EMT-related signaling pathway.²⁶ Toward this end, the

relationship between OIP5-AS1 and *miR-143-3p* in GBC cells needs to be clarified. Subsequently, the negative correlation between OIP5-AS1 and *miR-143-3p* in mesothelioma and adrenocortical cancer was exhibited by the online software StarBase2.0 (Figure 3A). Considering these findings, we speculated that lncRNA OIP5-AS1 interferes with the development of GBC through *miR-143-3p* regulation. Toward this end, RNA pull-down assay and luciferase reporter assay corroborated the binding relationship between *miR-143-3p* and OIP5-AS1 in GBC cells. In brief, OIP5-AS1 bound to *miR-143-3p*, and OIP5-AS1 negatively mediated *miR-143-3p* (Figure 3D and E). Subsequently, the results of CCK-8, cell scratch and transwell assays further confirmed that OIP5-AS1 may be responsible for cell migration, invasion and proliferation by regulating *miR-143-3p* in GBC (Figure 5A–C). This is supported by the recent work of Chen et al, who reported that the OIP5-AS1/*miR-143-3p* axis contributes to metastasis of cervical cancer.²⁷ Additionally, these results are similar to those reported by Yang et al, which showed that lncRNA OIP5-AS1 accelerates proliferation and invasion through elevating integrin $\alpha 6$ expression by targeting *miR-143-3p* in cervical cancer.²⁸

In summary, our findings identify the novel lncRNA OIP5-AS1 as an oncogene in GBC. The upregulation of OIP5-AS1 is associated with deterioration of GBC. Molecular mechanism exploration manifests that OIP5-AS1 enhances the biological activities of GBC cells regarding cell invasion, migration and proliferation by targeting *miR-143-3p* to accelerate GBC tumorigenesis. This study provides novel insights into the pathogenesis of GBC and potential therapeutic targets for this malignancy. Future targeted therapies likely offer the best hope for improving survival in GBC patients. However, there are some limitations in this study. First, the experimental data is limited. Second, no animal experiments and clinical experiments are done to further support our findings.

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Disclosure

The authors declare they have no conflicts of interest.

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