InlRNA GCln1 may contribute to the progression of ovarian cancer by regulating p53 signaling pathway

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Ovarian cancer (OC) is one of the most prevalent and deadly types of gynecological malignancy. Since current treatments are not effective against OC, it is imperative to develop novel potential therapeutic targets for managing OC. In this study, we aimed to uncover the underlying molecular mechanism of long non-coding RNA (lncRNA)GCln1 related to p53 signaling pathway in OC. The expression of lncRNA H19 GCln1 was markedly higher in OC samples than the related normal tissues. Next, we found that lncRNA GCln1 inhibited p53. In addition, the lncRNA GCln1 overexpression promoted the cell proliferation and migration in vitro. Subsequently, p53 silencing obligated the effect of lncRNA GCln1 knock down on cell proliferation and migration. To sum up, lncRNA GCln1 contributes to the progression of OC by regulating p53 signaling pathway. Meanwhile, our findings also suggested that lncRNA GCln1 may serve as a novel therapeutic target for OC patients.

**Key words:** lncRNA GCln1; p53; ovarian cancer; target therapy.

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Patient consent for publication: All subjects gave their informed consent for inclusion before they participated in the study.
**Introduction**

Ovarian cancer (OC) is one of the common tumors of the female reproductive system and leading causes of death among females. Current treatments are not effective against OC, the overall survival of OC patients is still unsatisfactory. The five year survival rate of around 30% with advances in surgery and chemotherapy. Thus, it is imperative to develop novel potential therapeutic targets for managing OC.

Long non-coding RNAs (lncRNAs) belongs to the non-coding RNAs family with more than 200 nucleotides in length. Although lncRNAs do not template protein synthesis, they can regulate gene expression at transcriptional or post-transcriptional level. lncRNAs have been shown to interact with p53 to play regulating roles in various cancers. However, the interaction of lncRNA GGlnc1 and p53 on the progression of OC remains largely unclear.

In this study, we aim to uncover whether lncRNA GGlnc1 could regulate the progression of OC via p53 signaling pathway.

**Materials and Methods**

**Clinical samples collection**

Human OC tissue samples as well as related non-tumorous tissues were obtained from 42 patients, and all the specimens were reviewed and verified by pathologists and immediately frozen in liquid nitrogen. All subjects gave their informed consent for inclusion before they participated in the study. All experimental protocols were approved by the Ethics Committee.

**RNA in situ hybridization**

The RNA in situ hybridization (ISH) was performed as previously described. The in situ detection of GGlnc1 was performed on 6-μm formalin-fixed, paraffin-embedded sections using DIG-labeled miRCURYTM Detection probe (Exiqon, Woburn, MA, USA). Nikon 80i microscope with Nikon NIS-Elements F 2.3 software (Nikon, Shanghai, China) were used to analyze.

**Cell culture**

The human OC cell line, ES-2, was purchased from ATCC (Rockville, MD, USA). Cells were maintained in RPMI 1640 (Gibco, Grand Island, NY, USA). The normal immortalized human ovarian surface epithelial cell line T1074 was obtained from Abcam (Shanghai, China) and cultured in Prigrow 1 medium (Abcam, Shanghai, China). Medium was plus 10% fetal bovine serum (FBS, HyClone, Logan, UT, USA), and 1% streptomycin and penicillin (Sigma-Aldrich, St. Louis, MO, USA), and the cells were grown at 37°C with 5% CO₂.

**Overexpression and suppression constructs**

The si-GClnc1, si-p53, pcDNA-GGlnc1 and their related negative controls (si-NC and pcDNA-NC) were purchased from Suzhou Hongxin Biotechnologies (Suzhou, China). These constructs were transfected when cell density reached 60% according to the manufacturer’s instructions of Lipofectamine 2000 (Invitrogen). The culture medium was replaced after 6 h.

**Cell counting kit-8 (CCK-8) assay**

CCK-8 assay kit was purchased from Dojindo (Shanghai, China) to monitor the cell viability. Cells were seeded in the 96-well plate (BD Biosciences, Shanghai, China) at a density of 5000 cells per well. After incubation for indicated time (0, 6, 24, 48, 72 and 96 h), we added 10 µL CCK-8 reagent into each well at indicated time point followed by incubation for 1 h at 37°C. Subsequently, the optical density (OD) was measured at 450 nm on a microplate reader.

**Transwell assay**

Cell migration was assayed by Boyden chamber assay with 24 well transwell permeable supports with 8 μm pores (Corning Coaster, Lowell, MA, USA). 200 μL of serum-free medium containing 0.1M cells for the migration assay were added to the filter. The bottom chamber was prepared with 750 μL complete cell culture medium in which the FBS as a chemottractant. After incubated for indicated time, the non-invasive cells were cleaned by scrubbing with a cotton swab. The cells that adhered to the outside of the membrane were fixed and dyed with crystal violet solution. The stained cells were dissolved in extraction buffer and solutions were transferred to a 96-well culture plates for colorimetric reading at OD 560 nm. The OD values reflected the cell ability of migration or invasion.

**Western blotting**

The protein expression levels of p53, p21 and BAX after different treatments were measured by Western blotting. The protein expression levels of p53 and p21 were quantified using ImageJ software (National Institute of Health, USA).

**Table 1. List of the primers used.**

| Forward primer   | Reverse primer       |
|------------------|----------------------|
| lnRCG GClnc1     | GGCAAGGCATGTCACTCTCTGAAGCCACAC |
| p53             | ATGAGGGTGCTGTCTTTGTAGG |
| p21             | GGGATTAGaGGCTTCCTCT |
| GAPDH           | GGGAAGGATCTGTCTCTGACC |
rent treatment was detected by Western blotting as described previously.11 The primary antibodies: anti-p53 (1:500, Millipore, Bedford, MA, USA), anti-p21 (1:500, Millipore), and GAPDH (Millipore). The secondary antibody was the goat anti-rabbit horseradish peroxidase (HRP)-conjugated antibody (1:2,000; Bio-Rad, Philadelphia, PA, USA). All the results are from separate blots.

Dual luciferase reporter assay

Cells were inoculated into the 24-well plate (3 x 10^5 cells / well). Then, the cells were co-transfected with wild-type or mutant psiCHECK-2 p53 vector (Generay, Shanghai, China) and pcDNA-GClnc1 or pcDNA-NC with Lipofectamine 2000 (Thermo Fisher Scientific, Beijing, China) according with the manufacturer’s instruction. The luciferase activity of cells was measured by a Dual-Luciferase Reporter assay kit (Promega, Shanghai, China) after 24 h.

RNA-binding protein immunoprecipitation analysis

RNA immunoprecipitation (RIP) assay was used to detect the association of p53 and lncRNA GClnc1 according to the manufacturer’s instructions of the Magna RIP RNA Binding Protein Immunoprecipitation Kit (Sigma-Aldrich). Finally, RNA was dissolved in 10 µL of diethyl pyrocarbonate (DEPC) water (Beyotime, Shanghai, China), and stored at -80°C. Subsequently, qRT-PCR was used to determine the expression of GClnc1 in co-p53 protein and IgG protein precipitate.

Statistical analysis

Statistical Product and Service Solutions (SPSS, Chicago, IL, USA) 16.0 statistical software was used for all statistical analysis. Results are shown as means ± SE (standard errors). The unpaired student’s t-test were used to compare the significance of differences between the mean of different groups. A value of p<0.05 indicated the statistical significance.

Results

lncRNA GClncl is enriched in OC tissues

lncRNA GClncl has been found to be upregulated and play important roles in bladder cancer and colorectal cancer.12 ISH (Figure 1A) was used to establish the pathologic and clinical significance of GClncl expression in OC, which was carried out by the pathology department. To explore the relationship between the expression of GClncl and the development of OC, we also measured the expression levels of lncRNA GClncl in 18 OC tissue samples and related non-tumorous tissues using qRT-PCR. As shown in Figure 1B, the expression levels of lncRNA GClncl in OC cancer tissues were conspicuously higher than the related normal tissues. It indicated that GClncl was involved in the progression of OC.

lncRNA GClncl expression level alters cell proliferation and migration in vitro

To elucidate the underlying molecular mechanism of lncRNA GClncl regulating the progress of OC, we subsequently measured the expression of GClncl in ES-2 and T1074 cells. As shown in Figure 2A, GClncl expression in ES-2 cells was markedly higher than that of T1074 cells by qRT-PCR. In order to investigate the impact of GClncl on cell proliferation of OC cells, we knocked down GClncl by si-GClncl. The expression level of GClncl was obviously decreased in ES-2 cells compared with si-NC, the negative control (Figure 2B). The cell proliferation and migration were significantly decreased after knockdown of GClncl expression by CCK-8 and transwell assays (Figure 2 C,D). Next, we overexpressed GClncl in T1074 cells. The expression level of GClncl was markedly increased in T1074 cells compared with pcDNA-NC (Figure 2E). Similarly, the cell proliferation and migration were significantly increased after overexpression of GClncl by CCK-8 and transwell assays (Figure 2 F,G).

lncRNA GClncl inhibits p53 activity

To illustrate the underlying mechanism of GClncl regulating the progression of OC, we further investigate the interaction between GClncl and p53. The level of GClncl in the p53 antibody

Figure 1. The expression of lncRNA GClncl in 18 OC patients was significantly higher than that of non-tumorous tissues. A) Representative images of GClncl expression in OC tissue and non-tumorous tissues were evaluated by ISH; scale bar: 100 µm. B) GClncl expression in OC tissue and non-tumorous tissues was measured by qRT-PCR. *p<0.05 vs non-tumorous tissues.
Figure 2. lncRNA GCINc1 expression altered the cell proliferation and migration in vitro. A) GCINc1 expression in ES-2 cells was significantly higher compared with T1074 cell by qRT-PCR. B) GCINc1 expression was remarkably decreased in ES-2 cells after knock down. C) GCINc1 knockdown significantly suppressed the proliferation of ES-2 cells. D) GCINc1 knockdown significantly decreased the migration of ES-2 cells; scale bar: 10 μm. E) GCINc1 expression was markedly increased in ES-2 cells after overexpression. F) GCINc1 overexpression significantly promoted the proliferation of T1074 cells. G) GCINc1 overexpression significantly increased the migration of T1074 cells; scale bar: 10 μm. *p<0.05 vs si-NC or pcDNA-NC; ^p<0.05 vs ES-2 or T1074 (n=3).
precipitation complex was found to be significantly higher than that of the IgG control group via RIP experiment in ES-2 cells (Figure 3A). Next, the luciferase reporter gene assay proved that overexpression of GClnc1 inhibited the luciferase activity of p53 in T1074 cells (Figure 3B). Furthermore, the mRNA and protein expression of p53, as well as p21, were markedly decreased after GClnc1 overexpression (Figure 3 C,D). Those data suggested that GClnc1 inhibited the activity of p53.

**IncRNA GClnc1 promotes cell proliferation and migration via p53**

To verify that GClnc1 promoted the proliferation of OC cells by altering p53 activity, silenced GClnc1 was knocked down in ES-2 cells. At the same time, p53 was simultaneously silenced in cells as well (Figure 4A). The CCK-8 and transwell experiments showed that p53 silencing markedely reversed the inhibition in cell proliferation and migration caused by GClnc1 knockdown (Figure 4 B,C).

**Discussion**

Molecular mechanisms underlying the progression of OC still remain complex and largely unknown. In this study, we mainly investigated the biological function of IncRNA GClnc1 and p53 interaction in the progression of OC.

Numerous studies have reported that IncRNAs are regulators in a wide range of biological functions and play complex and extensive roles in cancer development and progression. Among those widely studied IncRNAs, IncRNA GClnc1 has attracted a lot of attention with targeting multiple genes, such as MYC. IncRNA GClnc1 has been reported to promote proliferation and invasion of bladder cancer through activation of MYC. Meanwhile, IncRNA GClnc1 has been identified to promote gastric carcinogenesis and may act as a modular scaffold of WDR5 and KAT2A complexes to specify the histone modification pattern. In our work, GClnc1 was found to be remarkably upregulated in OC tissue samples compared with related normal tissues. What’s more, the overex-

![Figure 3](image-url)
pression of GClnc1 was also confirmed to promote the cell proliferation and migration, while the knockdown of GClnc1 was verified to suppress the cell proliferation and migration in vitro.

Recently, accumulated studies have shown that p53 signaling pathway interacted with lncRNA GClnc1 to play important roles in various cancers. lncRNA GClnc1 has been confirmed to promote the progression of colorectal cancer by inhibiting p53 signaling pathway.9 In addition, lncRNA GClnc1 has also been reported to promote tumorigenesis in osteosarcoma by inhibiting p53 signaling.16 In our work, we found that lncRNA GCln1 inhibit p53, as well as p21, activity in OC. Moreover, the silencing of p53 reversed the effect of GClnc1 knockdown on the proliferation of the human OC cell line, ES-2. These results allow suggesting that lncRNA GClnc1 may contribute to the progression of OC by regulating p53 signaling pathway. Our findings suggested that GClnc1 may serve as a novel therapeutic target for OC patients.

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