IncRNA GCInc1 may contribute to the progression of ovarian cancer by regulating p53 signaling pathway

Hu Li,1,2 Zheng Zeng,3 Xiang Yang,1 Ye Chen,1 Lei He,4 Ting Wan5
1Department of Gynecology, Panyu Central Hospital, Guangzhou
2Cancer Institute of Panyu Central Hospital, Guangzhou
3Department of Gynecology, Third Affiliated Hospital of Sun Yat-sen University, Guangzhou
4Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou
5Sun Yat-Sen University Cancer Center, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Guangzhou, China

Abstract

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 (IncRNA) GCInc1 related to p53 signaling pathway in OC. The expression of IncRNA H19 GCInc1 was markedly higher in OC samples than the related normal tissues. Next, we found that IncRNA GCInc1 inhibited p53. In addition, the IncRNA GCInc1 overexpression promoted the cell proliferation and migration in vitro. Subsequently, p53 silencing obligated the effect of IncRNA GCInc1 knock down on cell proliferation and migration. To sum up, IncRNA GCInc1 contributes to the progression of OC by regulating p53 signaling pathway. Meanwhile, our findings also suggested that IncRNA GCInc1 may serve as a novel therapeutic target for OC patients.

Key words: IncRNA GCInc1; p53; ovarian cancer; target therapy.

Correspondence: Dr. Lei He, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, China.
E-mail: helei33@mail.sysu.edu.cn

Contributions: HL, ZZ, contributed equally to this study. LH, TW, study conception; HL, ZZ, experiments design and completion, manuscript drafting; XY, YC, sample collection, contribution to data analysis. All the authors have read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Funding: This research was funded by startup project of clinical medicine in Guangzhou (20171A010334) and by Science and Technology Planning Project of Panyu in Guangzhou (2017-Z04-20).

Conflict of interest: The authors declare that they have no competing interests, and all authors confirm accuracy.

Availability of data and materials: The data used to support the findings of this study are available from the corresponding author upon request.

Ethical Approval: All procedures with human subjects in this study were conducted in accordance with the Human Ethics Committee of the Sun Yat-sen Memorial Hospital of Sun Yat-sen University, China.

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 reported to play vital roles in regulating cellular processes, such as signaling transduction, in various cancers. Currently, it has been found that lncRNAs plays important roles in the progression of OC. However, the specific influence of lncRNA GGln1 on the progression of OC remains largely unclear.

The transcription factor p53 is ~16-20 kb in length, is a pair of alleles and acts as a tumor suppressor gene localized on human chromosome 17. P53 functions as a suppressor of cell growth, and alterations in p53 lead to loss of this negative growth regulation and more rapid cell proliferation. The dysfunction of p53 contributes to the development of most human cancers. Recently, lncRNAs have been shown to interact with p53 to play regulating roles in various cancers. However, the interaction of lncRNA GGln1 and p53 on the progression of OC still remains unknown.

In this study, we aim to uncover whether lncRNA GGln1 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 GGln1 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) was used to analyze.

Cell culture

The human OC cell line, ES-2, was purchases 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.

qRT-PCR

TRiZol Plus RNA purification system was used to extract total RNA from specimens (Thermo Fisher Scientific, Beijing, China). Residual DNA was removed using DNA-free DNase (Ambion, Austin, TX). The cDNA was generated by reverse transcription using Moloney murine leukemia virus reverse transcriptase (Invitrogen, Beijing, China). The SYBR Green method was performed to detect the expression of lncRNA GGln1, p21, p53 and GAPDH. The primer sequences were listed in Table 1.

Overexpression and suppression constructs

The si-GGln1, si-p53, pcDNA-GGln1 and their related negative controls (si-NC and pcDNA-NC) were purchased from Suzhou Hongxun 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 chemoattractant. 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 the OD values reflected the cell ability of migration or invasion.

Western blotting

The protein expression levels of p53, p21 and BAX after differ-

Table 1. List of the primers used.

<table>
<thead>
<tr>
<th></th>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>lncRNA GGln1</td>
<td>TGGGGTAACCTAGCAGCTTCAT</td>
<td>GGCGAAGCAGTATCTTACATGACC</td>
</tr>
<tr>
<td>p53</td>
<td>CCCAAGCATGGTGAATTTGGA</td>
<td>ATGAGGGTGCTGTCTTTGTAGG</td>
</tr>
<tr>
<td>p21</td>
<td>GGCGACACGACGATGACGATT</td>
<td>GGGATTAGGGGCTCTCTCTCT</td>
</tr>
<tr>
<td>GAPDH</td>
<td>CGGAGTCCAGGGATTTGCTGTGCT</td>
<td>GGGAAGGATCTGTCTCTGACC</td>
</tr>
</tbody>
</table>

[page 324] [European Journal of Histochemistry 2020; 64:3166]
rent treatment was detected by Western blotting as described previously.\textsuperscript{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\textsuperscript{5} cells / well). Then, the cells were co-transfected with wild-type or mutant psiCHECK-2 p53 vector (Generay, Shanghai, China) and pcDNA-GCln1 or pcDNA-NC with Lipofectamine 2000 (Thermo Fisher Scientific, Beijing, China) according with the manufacture’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 IncRNA GCln1 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 GCln1 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**

**IncRNA GCln1 is enriched in OC tissues**

IncRNA GCln1 has been found to be upregulated and play important roles in bladder cancer and colorectal cancer.\textsuperscript{12} ISH (Figure 1A) was used to establish the pathologic and clinical significance of GCln1 expression in OC, which was carried out by the pathology department. To explore the relationship between the expression of GCln1 and the development of OC, we also measured the expression levels of IncRNA GCln1 in 18 OC tissue samples and related non-tumorous tissues using qRT-PCR. As shown in Figure 1B, the expression levels of IncRNA GCln1 in OC cancer tissues were conspicuously higher than the related normal tissues. It indicated that GCln1 was involved in the progression of OC.

**IncRNA GCln1 expression level alters cell proliferation and migration in vitro**

To elucidate the underlying molecular mechanism of IncRNA GCln1 regulating the progress of OC, we subsequently measured the expression of GCln1 in ES-2 and T1074 cells. As shown in Figure 2A, GCln1 expression in ES-2 cells was markedly higher than that of T1074 cells by qRT-PCR. In order to investigate the impact of GCln1 on cell proliferation of OC cells, we knocked down GCln1 by si-GCln1. The expression level of GCln1 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 GCln1 expression by CCK-8 and transwell assays (Figure 2C,D). Next, we overexpressed GCln1 in T1074 cells. The expression level of GCln1 was markedly increased in T1074 cells compared with pcDNA-NC (Figure 2E). Similarly, the cell proliferation and migration were significantly increased after overexpression of GCln1 by CCK-8 and transwell assays (Figure 2F,G).

**IncRNA GCln1 inhibits p53 activity**

To illustrate the underlying mechanism of GCln1 regulating the progression of OC, we further investigate the interaction between GCln1 and p53. The level of GCln1 in the p53 antibody
Figure 2. IncRNA 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 GCInc1 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 GCInc1 overexpression (Figure 3 C,D). Those data suggested that GCInc1 inhibited the activity of p53.

**IncRNA GCInc1 promotes cell proliferation and migration via p53**

To verify that GCInc1 promoted the proliferation of OC cells by altering p53 activity, silenced GCInc1 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 markedly reversed the inhibition in cell proliferation and migration caused by GCInc1 knockingdown (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 GCInc1 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 GCInc1 has attracted a lot of attention with targeting multiple genes, such as MYC. IncRNA GCInc1 has been reported to promote proliferation and invasion of bladder cancer through activation of MYC. Meanwhile, IncRNA GCInc1 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, GCInc1 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.

Based on our study results, we conclude the lncRNA GClnc1 contributes 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.

References