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LncRNA GIHCG promotes development of ovarian cancer by regulating microRNA-429

N. YAO, L. YU, B. ZHU, H.-Y. GAN, B.-Q. GUO

Department of Pathology, The First Affiliated of Bengbu Medical College, Bengbu, China

Abstract. – OBJECTIVE: To explore whether lncRNA GIHCG participates in the pathogenic progression of ovarian cancer (OC) and its underlying mechanism.

PATIENTS AND METHODS: Expression levels of GIHCG and microRNA-429 in 30 OC tissues and normal ovarian tissues were detected by quantitative Real time-polymerase chain reaction (qRT-PCR). Subsequently, 15 pairs of OC tissues and paracancerous tissues were selected for correlation analyses of GIHCG, microRNA-429 and the overall survival (OS) of OC patients using Kaplan-Meier method. Pearson correlation analyses were conducted for investigating the correlation between GIHCG and microRNA-429. GIHCG expression in OC cell lines (HEY, A2780 and HO8910) and normal epithelial OC cell line (IOSE-386) was detected by qRT-PCR. After transfection of GIHCG overexpression plasmid in HEY cells, cell cycle, proliferation and colony formation ability were detected by flow cytometry, cell counting kit-8 (CCK-8) and colony formation assay. MicroRNA-429 expression in HEY cells overexpressing GIHCG was detected by qRT-PCR. Rescue experiments were conducted by co-transfection of GIHCG overexpression plasmid and microRNA-429 mimics, followed by cell cycle and colony formation detection.

RESULTS: GIHCG was highly expressed, whereas microRNA-429 was lowly expressed in OC tissues than that of paracancerous tissues. OC patients with higher expression of GIHCG showed shorter OS than those with lower expression. However, OC patients with higher expression of microRNA-429 had longer OS than those with lower expression. GIHCG expression was positively correlated to microRNA-429. In vitro experiments showed that GIHCG was highly expressed in HEY, A2780 and HO8910 cells than that of IOSE-386 cells. GIHCG overexpression in HEY cells promoted cell cycle and colony formation abilities, which were reversed by microRNA-429 overexpression.

CONCLUSIONS: GIHCG is highly expressed in OC, which promotes OC development by stimulating cell cycle progression and cell proliferation by regulating microRNA-429.

Key Words:
OC, GIHCG, Cell cycle, Proliferation.

Introduction

The incidence, development and recurrence of ovarian cancer (OC) are a complex processes involving multiple factors. OC is one of the three common malignancies in the gynecological reproductive system. Its incidence has significantly increased in recent years, ranking the third only after cervical cancer and endometrial cancer. More seriously, the mortality rate of OC ranks the first in gynecological malignancies, which seriously threatens female health[1]. Since the occult symptoms and lacked early diagnostic approaches, micrometastases have generally occurred when clinically diagnosed as OC. The treatment effect and prognosis are extremely poor, and the five-year survival rate of OC is low[2]. The incidence, development and recurrence of OC involve multiple processes[3]. It is important to screen out specific regulators in OC, so as to improve therapeutic efficacy of OC. LncRNA consists with a transcript length for more than 200 nt. Although lncRNA itself does not encode proteins, it can regulate gene expression at transcriptional, post-transcriptional and epigenetic levels[4]. With the further study of lncRNA, it has been found to be involved in many biological processes, such as cell cycle, immune function, and tumor development[5,6]. Scholars[7,8] have found some certain lncRNAs could regulate OC development. For example, HOTAIR is highly expressed in epithelial OC. Downregulation of HOTAIR expression can decrease invasion and migration of OC cells. As a ceRNA, HOTAIR regulates malignancy through regulating miR-214 and miR-217 to target PIK3R3 and MAPK1, respectively[9,10]. LncRNAs could also be served as diagnostic and prognostic biomarkers, which are remarkably associated with drug resistance of OC.

Corresponding Author: Bingqin Guo, MM; e-mail: Gbq22@qq.com

ZFAS1 promotes SP1 expression by interacting with miRNA-150-5p, further inducing drug resistance of OC cells to cisplatin and paclitaxel[11]. Recent investigations have shown that GIHCG is significantly overexpressed in a variety of malignant tumors, such as renal cell carcinoma, hepatocellular carcinoma, and tongue squamous cell carcinoma. In hepatocellular carcinoma, GIHCG overexpression enhances proliferation, migration, and invasion of hepatocellular carcinoma cells. GIHCG expression[12] is positively correlated with survival rate, tumor progression, microvascular invasion, tumor size, etc. Overexpression of GIHCG in renal cell carcinoma is associated with poor prognosis, Fuhrman classification and tumor nodule metastasis (TNM) stage[13]. However, the role of GIHCG in OC has not been well elucidated.

Patients and Methods

Sample Collection

30 pairs of OC tissues and paracancerous tissues were harvested from patients undergoing surgical resection in the First Affiliated of Bengbu Medical College from July 2016 to September 2017. Enrolled patients did not receive preoperative chemotherapy, radiotherapy and hormone therapy. They signed informed consent before the study. All experimental procedures were approved by The First Affiliated of Bengbu Medical College Ethic Committee. OC tissues were immediately preserved in liquid nitrogen.

RNA Extraction and Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Total RNA in treated cells was extracted using TRIzol method (Invitrogen, Carlsbad, CA, USA) for reverse transcription according to the instructions of PrimeScript RT reagent Kit (TaKaRa, Otsu, Shiga, Japan). RNA concentration was detected using spectrometer and those samples with A260/A280 ratio of 1.8-2.0 were selected for the following qRT-PCR reaction. QRT-PCR was then performed based on the instructions of SYBR Premix Ex Taq TM (TaKaRa, Otsu, Shiga, Japan). The relative gene expression was calculated using $2^{-\Delta Ct}$ method. Primers used in the study were as follows: GAPDH, F: 5′-CACCCACTCCTCCACCTTTG-3′, R: 5′-GCTCATTCAACGGATAAGTC-3′; GIHCG, F: 5′-CTTTCAAGAAGTTTGGCTGTC-3′, R: 5′-GTCTCCCTCCTTCTGCCTTT-3′; microRNA-429, F: 5′-TTATCGCGGCTCCACATGTT-3′, R: 5′-TCTCTATGGCCACTTGCTCC-3′.

Cell Culture and Transfection

Ovarian epithelium cell line (IOSE-386) and OC cell lines (HEY, A2780 and HO8910) were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). HCT116 cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) containing 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA). One day prior to cell transfection, cells were seeded in the 6-well plates at a density of 4×10^4 cells per well. Cells were transfected with corresponding plasmids when the confluence was up to 50-60%, following the instructions of Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Culture medium was replaced 6 hours later.

Cell Counting Kit-8 (CCK-8) Assay

OC cells were seeded into 96-well plates with 2×10^3 cells per well. 10 μL of CCK-8 solution (cell counting kit-8, Dojindo, Kumamoto, Japan) were added in each well. 4 hours later, fresh medium was replaced for 1-h incubation. The absorbance at 450 nm of each sample was measured by a microplate reader (Bio-Rad, Hercules, CA, USA).

Cell Cycle Detection

Cells were washed with Hanks buffer for three times, digested with trypsin and centrifuged at 800 rpm/min for 5 min. Subsequently, cells were stained with 70% ethanol at -20°C for 8 h or longer. Before cell cycle detection, fixed cells were centrifuged and washed, followed by incubation with 10 μL of Propidium Iodide (PI) (1 mg/mL) in dark for 30 min, and cell cycle was detected using flow cytometry.

Colony Formation Assay

Cells were digested for preparing cell suspension at a dose of 1×10^5/mL. 200 μL of suspension were added in the culture dish for 2-week incubation. Colonies visible to the naked eye were fixed with hematoxylin for 30 min, observed and captured using a microplate.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 (IBM, Armonk, NY, USA) was used for data analysis. GraphPad (La Jolla, CA, USA) was utilized for figure editing. Data were expressed as mean ± standard deviation ($\bar{x}$ ±

s). Measurement data were compared using the *t*-test. Survival analyses were conducted by Kaplan-Meier and Log-Rank test. Correlation between gene expression and prognosis of OC patients was conducted by Pearson correlation analysis. $p < 0.05$ considered the difference was statistically significant.

Results

GIHCG Was Highly Expressed in OC

To explore the role of GIHCG in the pathogenic progression of OC, we detected GIHCG expression in 15 OC tissues and 15 normal ovarian tissues by qRT-PCR. The data showed higher GIHCG expression in OC tissues than that of normal ovarian tissues (Figure 1A). We further detected GIHCG expression in 30 pairs of OC tissues and matched paracancerous tissues. Similarly, GIHCG was highly expressed in OC tissues than that of paracancerous tissues (Figure 1B). Survival analyses were conducted and found that GIHCG expression is negatively correlated to OS in OC patients (Figure 1C). OC patients with higher GIHCG expression presented shorter OS than those with lower expression.

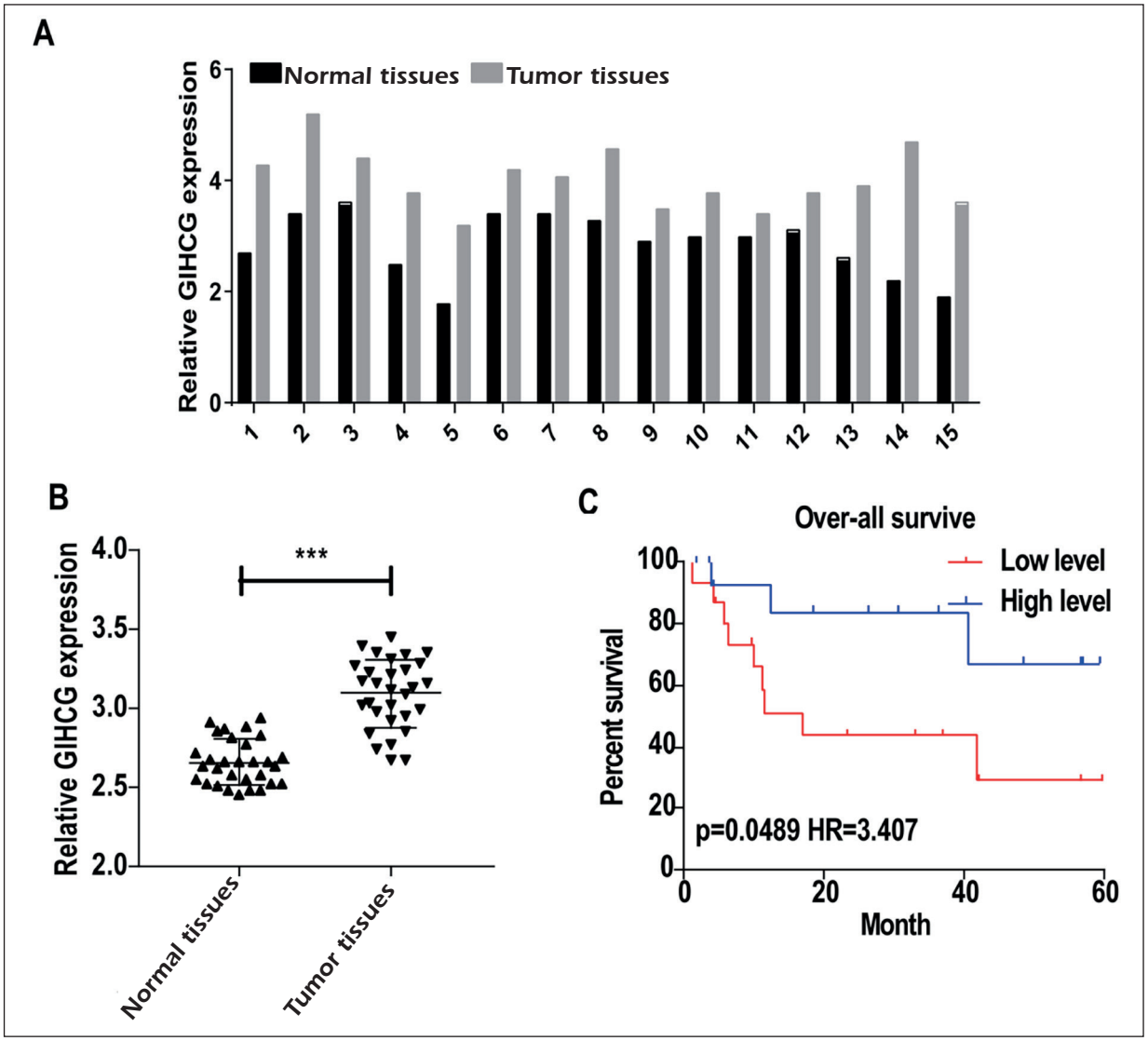

Figure 1. GIHCG was highly expressed in OC. ***A,*** Higher GIHCG expression was observed in OC tissues than that of normal ovarian tissues. ***B,*** GIHCG was highly expressed in OC tissues than that of paracancerous tissues. ***C,*** GIHCG expression is negatively correlated to OS in OC patients.

MicroRNA-429 was Lowly Expressed in OC

Previous researches[14,15] have demonstrated the regulatory effects of GIHCG on liver cancer and kidney cancer *via* targeting microRNA-429. To explore the role of microRNA-429 in OC development, qRT-PCR was performed to detect microRNA-429 expression in 15 OC tissues and 15 normal ovarian tissues. MicroRNA-429 was lowly expressed in OC tissues than that of normal ovarian tissues (Figure 2A). Similarly, microRNA-429 was lowly expressed in 30 pairs of OC tissues than that of matched paracancerous tissues as well (Figure 2B). In addition, OC patients with higher expression of microRNA-429 presented longer OS than those with lower expression (Figure 2C). Furthermore, we analyzed whether GIHCG is correlated to microRNA-429. We found that GIHCG expression is negatively correlated to microRNA-429 expression in OC (Figure 2D).

GIHCG Promoted Cell Cycle of OC

To explore the regulatory effect of GIHCG on OC cells, we first detected GIHCG expression in ovarian epithelium cell line (IOSE-386) and OC

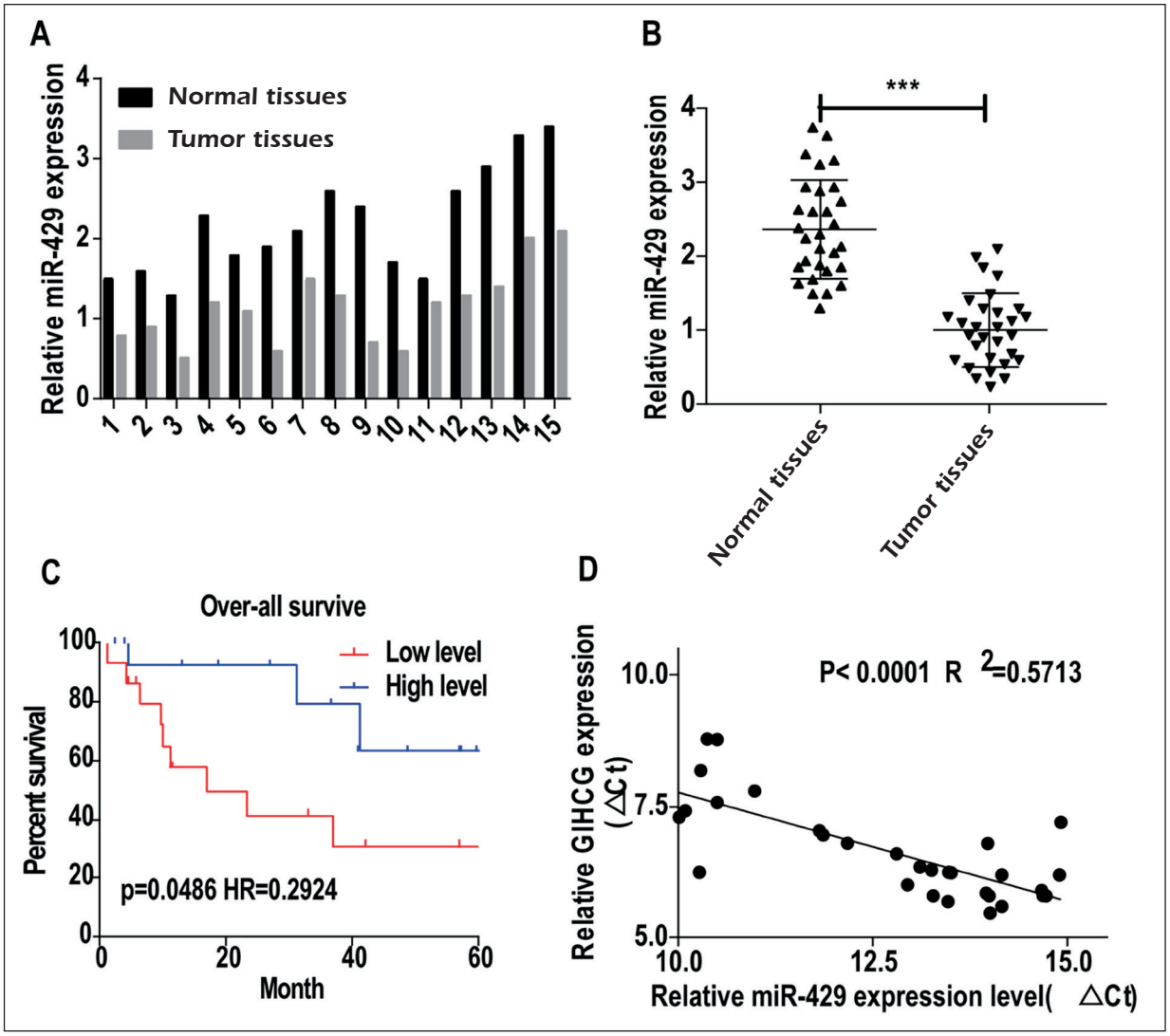

Figure 2. MicroRNA-429 was lowly expressed in OC. ***A,*** MicroRNA-429 was lowly expressed in OC tissues than that of normal ovarian tissues. ***B,*** MicroRNA-429 was lowly expressed in 30 OC tissues than that of matched paracancerous tissues. ***C,*** OC patients with higher expression of microRNA-429 presented longer OS than those with lower expression. ***D,*** GIHCG expression was negatively correlated to microRNA-429 expression in OC.

cell lines (HEY, A2780 and HO8910). GIHCG was highly expressed in OC cells compared with that of ovarian epithelium cells (Figure 3A). HEY cells were selected for the following experiments. Transfection of GIHCG overexpression plasmid into HEY cells remarkably upregulated GIHCG expression (Figure 3B). Subsequently, we detected cell cycle in HEY cells by flow cytometry. It is indicated that GIHCG overexpression decreased the duration of G0/G1 phase, but increased the duration of S phase and G2/M phase (Figure 3C). Meanwhile, CCK-8 assay was performed to detect proliferative ability at 0, 24, 48 and 72 h after transfection, respectively. GIHCG overexpression remarkably elevated proliferative ability in HEY cells (Figure 3D). Colony formation ability of HEY cells also increased by GIHCG overexpression (Figure 3E). The above data elucidated that GIHCG overexpression promoted proliferation and cell cycle of OC cells.

GIHCG Promoted Cell Cycle of OC by Regulating microRNA-429

We observed that microRNA-429 may be involved in OC development by GIHCG regulation. First, we found that GIHCG overexpression in HEY cells downregulated microRNA-429 expression (Figure 4A). Furthermore, HEY cells co-transfected with GIHCG overexpression plasmid and microRNA-429 mimics. Cell cycle was promoted by GIHCG overexpression, whereas it was arrested by microRNA-429 knockdown (Figure 4B). The promoted colony formation ability by GIHCG overexpression decreased after transfection of microRNA-429 mimics in

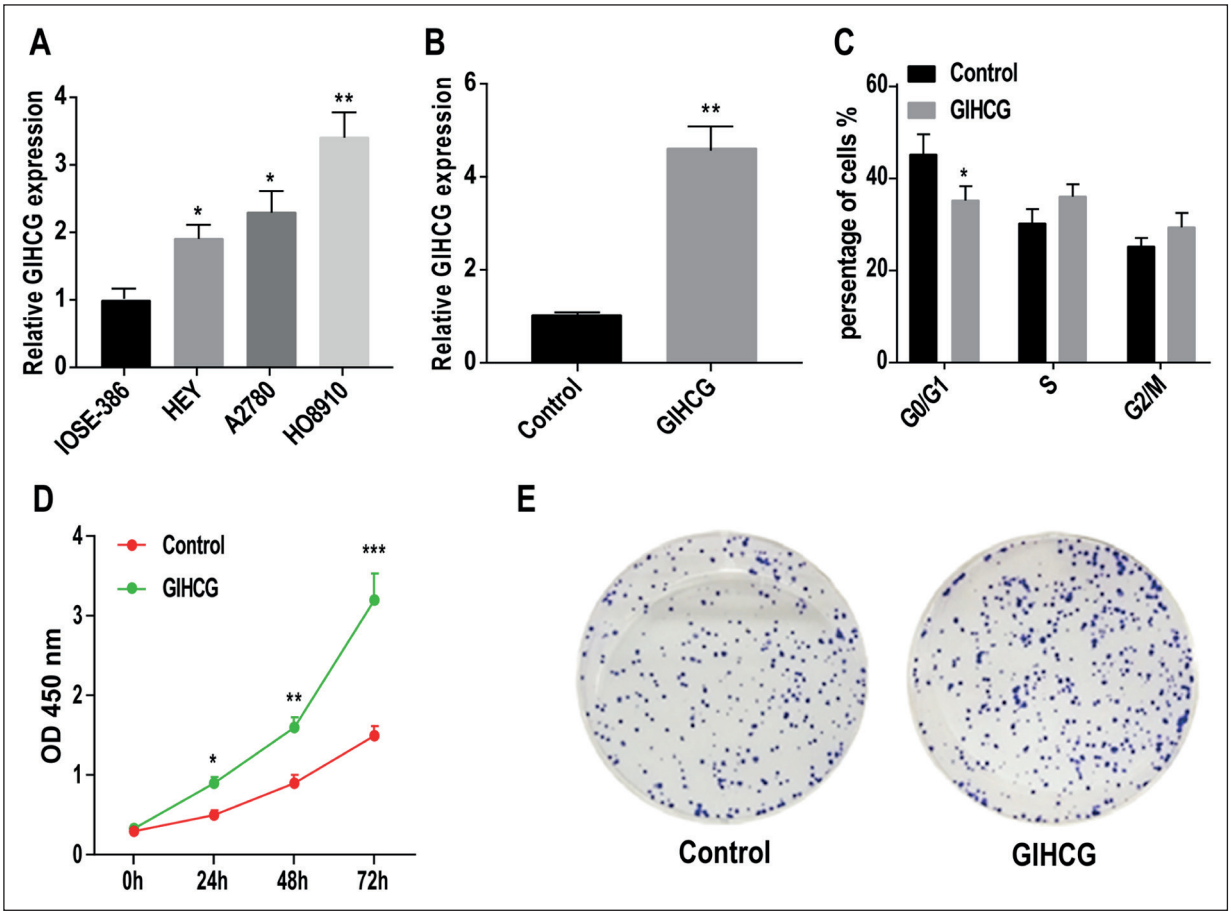

Figure 3. GIHCG promoted cell cycle of OC. ***A,*** GIHCG was highly expressed in OC cells compared with that of ovarian epithelium cells. ***B,*** After GIHCG overexpression plasmid was transfected into HEY cells, GIHCG expression was remarkably upregulated. ***C,*** GIHCG overexpression decreased the duration of G0/G1 phase, but increased the duration of S phase and G2/M phase. ***D,*** GIHCG overexpression remarkably elevated proliferative ability was observed in HEY cells. ***E,*** Colony formation ability of HEY cells increased by GIHCG overexpression.

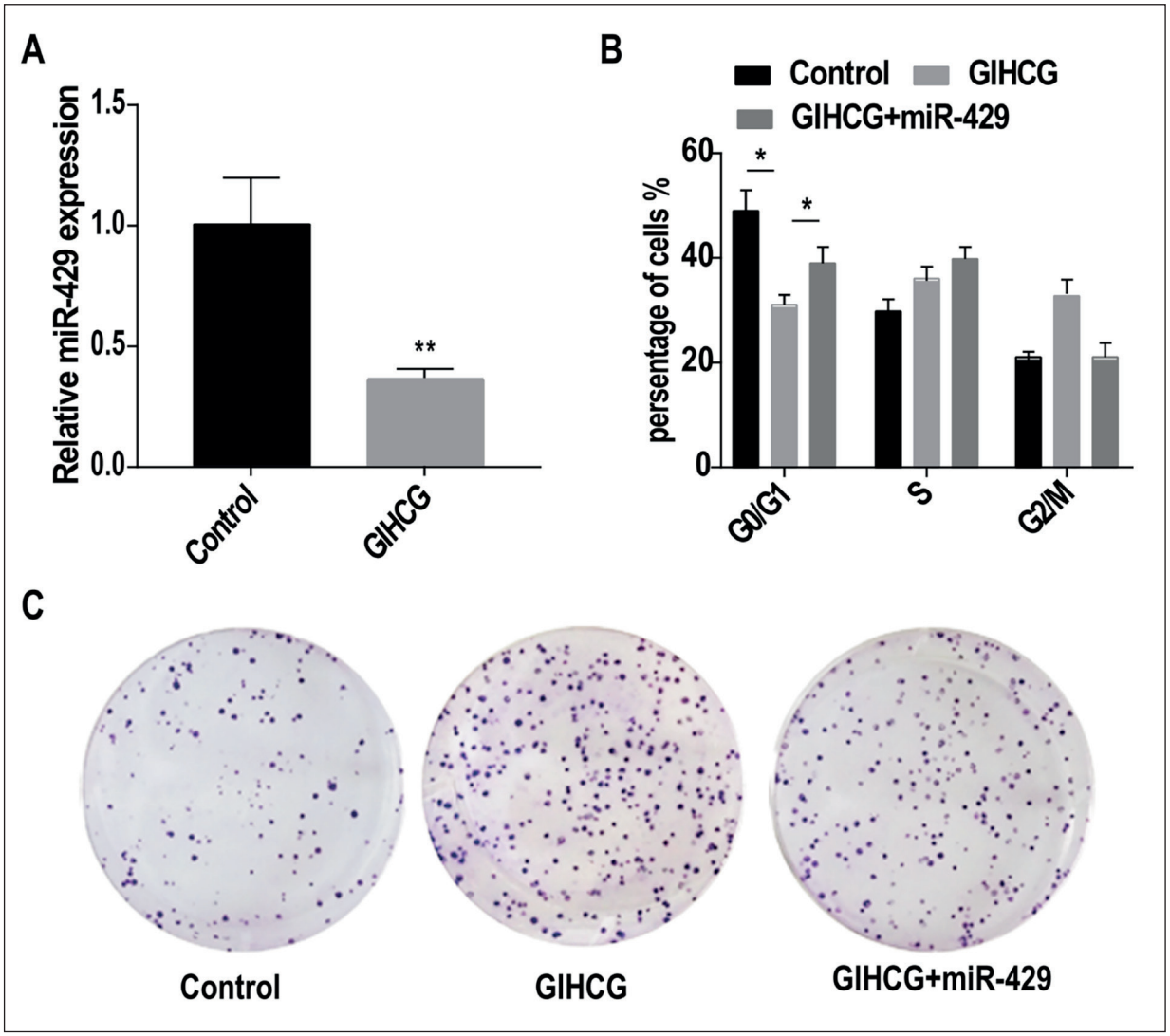

Figure 4. GIHCG promoted cell cycle of OC by regulating microRNA-429. ***A,*** GIHCG overexpression in HEY cells downregulated microRNA-429 expression. ***B,*** Cell cycle was promoted by GIHCG overexpression, whereas it was arrested by microRNA-429 knockdown. ***C,*** The promoted colony formation ability by GIHCG overexpression decreased after transfection of microRNA-429 mimics.

HEY cells (Figure 4C). We considered that GIHCG promotes cell cycle by regulating microRNA-429.

Discussion

OC is one of the three major malignant tumors of gynecology. Because the anatomical location of the ovary is relatively concealed and typical symptoms of OC in the early stage are occult, most OC patients have progressed to advanced stage at the time of diagnosis. In addition, OC manifests as strong invasion and metastasis, high recurrence rate and susceptibility to resistance. OC patients present poor prognosis, and the mortality rate of OC is the highest in female reproductive system malignant tumors[16]. Recent works[17,18] have confirmed the specific role of lncRNAs in OC. Analyzing the biological function of lncRNAs in OC may provide new directions in improving early diagnosis and target therapy of OC. Some certain lncRNAs have shown their regulatory effects on OC development, including MALAT1, PVT1 and PCGEM1[19]. It is reported[20] that MALAT1 promotes metastasis, invasion and proliferation of epithelial OC cells through inhibiting Wnt/β-catenin pathway. PVT1 is capable of

promoting OC development by regulating miR-133a[21]. PCGEM1 promotes OC development by upregulating RhoA, further increasing expressions of YAP, P70S6K, MMP2 and Bcl-xL[22]. In the present study, GIHCG was highly expressed in OC tissues and cell lines, promoting proliferation and cell cycle of OC cells. Multiple studies showed that miRNAs may serve as oncogenes and participate in the pathogenic progression of tumors. MicroRNA-429 is a member of the miR-200 family and located on chromosome 1. The miR-200 family has been reported[23] to be involved in tumor development and prognosis. Studies have shown that microRNA-429 can induce MDR by regulating BCL2 and XIAP in lung cancer and gastric cancer[24]. Hence, microRNA-429 is considered to be a new therapeutic target that could promote tumor sensitivity or induce tumor cell death. MicroRNA-429 is also closely related to the survival rate of OC and can be served as a potential biomarker of OC[25]. This study found that microRNA-429 is lowly expressed in OC, which is positively correlated to overall survival of OC patients. Moreover, overexpression of microRNA-429 can partially reverse the regulatory effects of GIHCG on cell cycle and colony formation ability of OC cells. It is indicated that GIHCG promotes cell cycle and proliferation of OC by regulating microRNA-429, thereafter promoting OC development.

Conclusions

We demonstrated that GIHCG was highly expressed in OC promoting OC development by stimulating cell cycle progression and cell proliferation by regulating microRNA-429. GIHCG may serve as a potential hallmark in diagnosing and treating OC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

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