European Review for Medical and Pharmacological Sciences 2018; 22: 46-54

Long noncoding RNA GIHCG is a potential diagnostic and prognostic biomarker and therapeutic target for renal cell carcinoma

Z.-H. HE[1], X.-H. QIN[2], X.-L. ZHANG[3], J.-W. YI[1], J.-Y. HAN[4]

[1]Department of Nephrology, The People's Hospital of Yichun City, Yichun, Jiangxi, China
[2]Department of Nephrology, The Second Affiliated Hospital of Nanchang University, Nanchang, Jiangxi, China
[3]Department of Laboratory Diagnostics, The People's Hospital of Yichun City, Yichun, Jiangxi, China
[4]Institute of Immunology, Fujian Provincial Key Laboratory of Medical Test, Fujian Academy of Medical Sciences, Fuzhou, Fujian, China

Zhihong He and Xiaohua Qin contributed equally

Abstract. – OBJECTIVE: Long noncoding RNA (lncRNA) GIHCG has been reported as an oncogene in hepatocellular carcinoma. However, the expression, roles, and clinical values of GIHCG in renal cell carcinoma (RCC) remain unclear. The aim of this study was to investigate the expression, roles, diagnostic and prognostic values of GIHCG in RCC.

PATIENTS AND METHODS: The expression of GIHCG in 46 pairs of RCC tissues and adjacent normal renal tissues was measured by quantitative real-time polymerase chain reaction (qRT-PCR). GIHCG serum level in 46 RCC patients, 46 age- and sex-matched healthy controls, 20 pre- and post-surgery RCC patients was measured by qRT-PCR. The diagnostic values of serum GIHCG were evaluated by receiver operating characteristic (ROC) curves analysis. The effect of GIHCG on RCC cell proliferation was evaluated using Cell Count Kit-8 assay, and the effect of GIHCG on RCC cell migration was evaluated using transwell migration assay.

RESULTS: GIHCG is upregulated in RCC tissues compared with adjacent normal renal tissues. Increased expression of GIHCG is positively correlated with advanced TNM stages, Fuhrman grades, and poor prognosis. Serum GIHCG level is also significantly upregulated in RCC patients and correlated with advanced TNM stages. Serum GIHCG could accurately discriminate RCC patients from healthy controls, and also early stage RCC patients from healthy controls. Furthermore, serum GIHCG level is positively correlated with GIHCG expression in RCC tissues. Serum GIHCG level is significantly reduced after radical resection of RCC. Functional assays showed that knockdown of GIHCG significantly represses proliferation and migration of RCC cells.

CONCLUSIONS: Long noncoding RNA GIHCG would sever as a novel diagnostic and prognostic biomarker and therapeutic target for RCC.

Key Words:
Long noncoding RNA, Renal cell carcinoma, Biomarker, Diagnosis, Prognosis, Therapy.

Introduction

Deriving from renal tubular epithelial cells, renal cell carcinoma (RCC) is the third most common genitourinary malignant neoplasms, with approximately 338,000 new cases and 143,000 deaths in 2012 worldwide[1, 2]. Unfortunately, the incidences of RCC is still rising throughout the world[3]. Most RCCs are asymptomatic and nonpalpable in early stages. Approximately 30% of RCC patients are diagnosed at advanced stages with metastasis[4]. Until now, surgical resection remains the unique effective treatment for early-stage RCCs[5]. However, for advanced stages RCC, there is still no effective treatment and the prognosis is still very poor[6]. Therefore, accurate identification of RCC patients in early stages is critical for successful therapy of RCC and improving the prognosis of RCC patients[7].

Approximately 20% of patients with localized RCC will develop recurrence and metastasis after surgery[8]. Unfortunately, most advanced stage RCCs and recurrent RCCs are refractory to radiation therapy, chemotherapy, and immunotherapy[9]. Therefore, it is necessary to further revealing the

Corresponding Author: Zhihong He, MD; e-mail: hezhihongyichun@163.com
Junyong Han, MD; e-mail: junyonghanfams@yeah.net

molecular mechanisms underlying the initiation and progression of RCC[10], which will promote the identification of effective prognostic biomarkers and therapeutic targets for RCCs[11].

Increasing evidence revealed that circulating cell-free nucleic acids could serve as interesting non-invasive biomarkers for early diagnosis of several cancers[12]. Among these circulating nucleic acids, long noncoding RNAs (lncRNAs) have been frequently identified in peripheral blood of various cancers, such as hepatocellular carcinoma (HCC), lung cancer, gastric cancer, and et al[13-19]. LncRNAs are a novel class of RNA transcripts with no protein-coding potential and over 200 nucleotides in length[20-22]. Furthermore, lncRNAs have been reported to be dysregulated in many cancer tissues and to serve as prognostic biomarkers for several cancers[23-25]. Many lncRNAs have been revealed to have important roles in many pathophysiological processes[20]. In cancers, lncRNAs have been reported to regulate cell proliferation, cell cycle, apoptosis, senescence, migration, invasion, drug resistance, and et al[26-29].

lncRNA-GIHCG is first reported to be upregulated in HCC tissues and associated with poor prognosis of HCC patients[30]. GIHCG is revealed to promote HCC progression via epigenetically inhibiting miR-200b/a/429 expression. However, the expression, roles, and clinical significances of GIHCG in RCC are still unknown. In this work, we measured GIHCG expression in peripheral blood of RCC patients and also in RCC tissues. We detected the presence of GIHCG in the serum of RCC patients. We also found that serum GIHCG level is significantly unregulated in RCC patients compared with that in healthy controls. Therefore, we further analyzed the clinical values of GIHCG in RCC.

Patients and Methods

Patient Specimens

A total of 46 pairs of RCC tissues and adjacent normal renal tissues were obtained from primary RCC patients who received radical resection at Fujian Provincial Hospital (Fuzhou, Fujian, China). All the tissues were diagnosed by pathological examination. None of the patients received pre-surgery anti-cancer treatments. These tissues were immediately flash frozen in liquid nitrogen after surgery and stored at -80°C until use. Venus blood was collected from these 46 RCC patients before surgery and 46 age- and sex-matched healthy controls. Veins blood was also collected from 20 patients of these 46 RCC patients one week after surgery. All these veins blood was collected from fasting participants and centrifuged at 3000 rpm for 15 min at 4°C. The supernatant was immediately collected and stored at -80°C until use. This study was approved by the Ethics Committee of Fujian Provincial Hospital (Fuzhou, Fujian, China). All the patients and healthy controls signed informed consent.

Cell Culture

Human RCC cell lines 786-O and Caki-1 were obtained from American Type Culture Collection (ATCC, Rockville, MD, USA). The 786-O cell line was cultured in RPMI-1640 Medium (Gibco, Thermo Fisher Scientific, Waltham, MA USA). The Caki-1 cell line was cultured in McCoy's 5A (Modified) Medium (Gibco). All the cells were cultured in the above-described medium supplemented with 10% fetal bovine serum (Gibco) at 37°C in an atmosphere containing 5% CO_2.

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

Total RNA was extracted from tissues, serum, and cells using the TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's protocol. After being treated with DNase I to remove genomic DNA, the RNA was reverse transcribed into complementary DNA (cDNA) using the PrimeScript™ II 1st Strand cDNA Synthesis Kit (TaKaRa, Dalian, Liaoning, China) in accordance with the manufacturer's protocol. Then, the RNA expression level was quantified using the qRT-PCR assays. The qRT-PCR assays were carried out using the SYBR® Premix Ex Taq™ II (TaKaRa) on StepOnePlus™ Real-Time PCR Systems (Applied Biosystems, Foster City, CA, USA) in accordance with the manufacturer's protocols. β-actin was used as an endogenous control for quantification of GIHCG expression. GIHCG expression was calculated using the comparative cycle threshold (CT; $2^{-\Delta\Delta CT}$) method. The primer sequences used were as follows: for GIHCG, 5'-CTTTCAAGAAGTTTGGCTGTC-3' (forward) and 5'-GCTCATTCAACGGATAAGTC-3' (reverse); for β-actin, 5'-TCCTCTCCCAAGTCCACACA-3' (forward) and 5'-GCACGAAGGCTCATCATTCA-3' (reverse).

Small Interfering RNA (siRNA) Synthesis and Transfection

The siRNAs specifically targeting GIHCG and control siRNA were purchased from GenePharma

(Shanghai, China). The siRNAs sequences were as follows: for siRNA-GIHCG-1, 5'-GCGGGAGAGGCAGAGAUAUTT-3'; for siRNA-GIHCG-2, 5'-GCCACCUUUGCAACCACAUTT-3'; for siRNA-negative control (NC), 5'-UUCUCCGAACGUGUCACGUTT-3'. When cells grew to 50% confluence, transfections were carried out using Lipofectamine 3000 (Invitrogen) in accordance with the manufacturer's protocol.

Cell Proliferation Assay

Cell proliferation was measured using the Cell Counting Kit-8 (CCK-8, Dojindo Laboratories, Kumamoto, Japan) in accordance with the manufacturer's protocol. Briefly, 2×10^3 indicated RCC cells/well were seeded into 96-well plates. 2 hours after the adding of 10 µl CCK-8 regent to each well, the absorbance values at 450 nm were measured to calculate the cell number.

Transwell Cell Migration Assay

The migration of RCC cells was measured using the transwell cell migration assay. Briefly, 1×10^5 indicated RCC cells suspended in serum-free medium were seeded in the upper chamber of a 24-well transwell insert (Millipore, Bedford, MA, USA). Medium supplemented with 10% fetal bovine serum was added to the lower chamber. After incubation for 24 hours, cells remaining in the upper chamber were carefully wiped out with cotton wool. The cells that had migrated to the lower surface of the inserts were fixed with 4% paraformaldehyde, stained with 1% crystal violet, and counted.

Table I. Correlation between GIHCG expression level and clinicopathological features of RCC patients.

Variable	GIHCG expression		*p*-value
	Low (n=23)	High (n=23)	
Gender			0.522
Male	15	17	
Female	8	6	
Age (years)			0.555
< 55	13	11	
≥ 55	10	12	
Tumor location			0.555
Left	12	10	
Right	11	13	
Tumor size (cm)			0.179
≤ 7	19	15	
> 7	4	8	
Lymph node metastasis			0.101
Negative	22	17	
Positive	1	6	
Distant metastasis			0.058
Negative	23	18	
Positive	0	5	
TNM stage			0.028
I	19	12	
II-IV	4	11	
Fuhrman Grade			0.032
G1-G2	12	5	
G3-G4	11	18	

p-value was acquired by Pearson chi-square test.

Statistical Analysis

Statistical analyses were carried out using the GraphPad Prism Software. Wilcoxon signed-rank test, Mann-Whitney U test, Pearson chi-square test, Log-rank test, Receiver operating characteristic (ROC) curves analysis, Pearson correlation analysis, or Student's *t*-test was performed as indicated. A *p*-value of < 0.05 was considered as statistically significant.

Results

GIHCG is Upregulated in RCC Tissues and Associated with Aggressive Clinicopathological Traits and Poor Prognosis

To investigate the expression pattern of GIHCG in RCC, we collected 46 pairs of RCC tissues and adjacent normal renal tissues, and measured GIHCG expression in these tissues using qRT-PCR. The results showed that GIHCG is significantly upregulated in RCC tissues compared with paired adjacent normal renal tissues (Figure 1A). Then, we explored the correlation between GIHCG expression level and clinicopathological traits in these 46 RCC patients. The results showed that GIHCG is markedly increased in RCC tissues with advanced TNM stages (Figure 1B). Furthermore, GIHCG expression is also significantly increased in RCC tissues with Fuhrman III and IV grades compared with that in Fuhrman I and II grades (Figure 1C). Pearson chi-square tests further confirmed the positive correlation between increased GIHCG expression and advanced TNM stages ($p = 0.028$), Fuhrman grades ($p = 0.032$) (Table I). Kaplan-Meier survival analysis in these 46 RCC patients showed that high GIHCG expression in RCC tissues is correlated with a reduction in overall survival ($p = 0.038$) (Figure 1D). Taken together, these results suggested that GIHCG is upregulated in RCC tissues and associated with aggressive clinicopathological traits and poor prognosis.

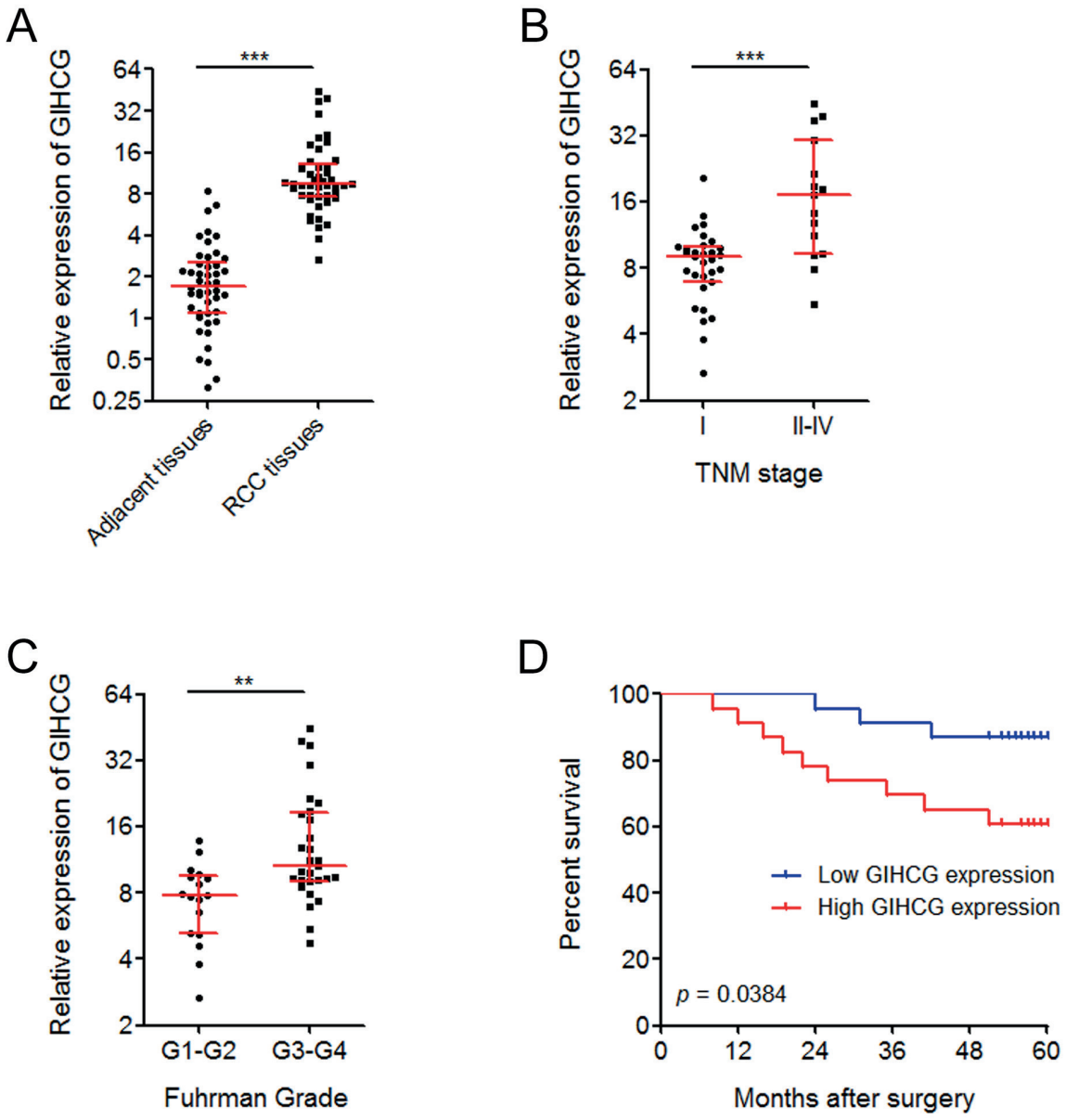

Figure 1. GIHCG is upregulated in RCC tissues and indicates a poor prognosis. ***A,*** GIHCG expression levels in 46 pairs of RCC tissues and adjacent normal renal tissues were measured by qRT-PCR. Results are presented as median with interquartile range. ***$p < 0.001$ by Wilcoxon signed-rank test. ***B,*** GIHCG expression levels in RCC tissues with TNM stage I or TNM stage II-IV. Results are presented as median with interquartile range. ***$p < 0.001$ by Mann-Whitney U test. ***C,*** GIHCG expression levels in RCC tissues with Fuhrman I and II grades or Fuhrman III and IV grades. Results are presented as median with interquartile range. **$p < 0.01$ by Mann-Whitney U test. ***D,*** Kaplan-Meier survival analyses of the correlations between GIHCG expression level in RCC tissues and overall survival of 46 RCC patients. The median expression level of GIHCG was used as the cutoff. $p = 0.038$ by Log-rank test.

Circulating GIHCG is Upregulated in RCC Patients and May Serve as a Novel Non-invasive Diagnostic Biomarker for RCC

Next, we measured GIHCG expression in the serum of the above used 46 RCC patients and 46 age- and sex-matched healthy controls by qRT-PCR. The results showed that serum GIHCG is also significantly upregulated in RCC patients compared with that in healthy controls (Figure 2A). Furthermore, serum GIHCG is markedly increased in RCC patients with advanced TNM stages (Figure 2B). Then, receiver operating characteristic (ROC) curve analyses were carried out

to investigate whether serum GIHCG could act as a diagnostic biomarker for RCC. The results showed that serum GIHCG could accurately discriminate RCC patients from healthy controls with an area under the ROC curve (AUC) of 0.920 (95% CI: 0.866-0.974), a sensitivity of 87.0%, and a specificity of 84.8% (Figure 2C). Moreover, ROC curve analyses also showed that serum GIHCG could accurately discriminate early stage (TNM stage I) RCC patients from healthy controls with an area under the ROC curve (AUC) of 0.886 (95% CI: 0.812-0.959), a sensitivity of 80.7%, and a specificity of 84.8% (Figure 2D). Taken together, these results suggested that serum GIHCG is upregulated in RCC patients and may serve as a non-invasive biomarker for RCC early diagnosis.

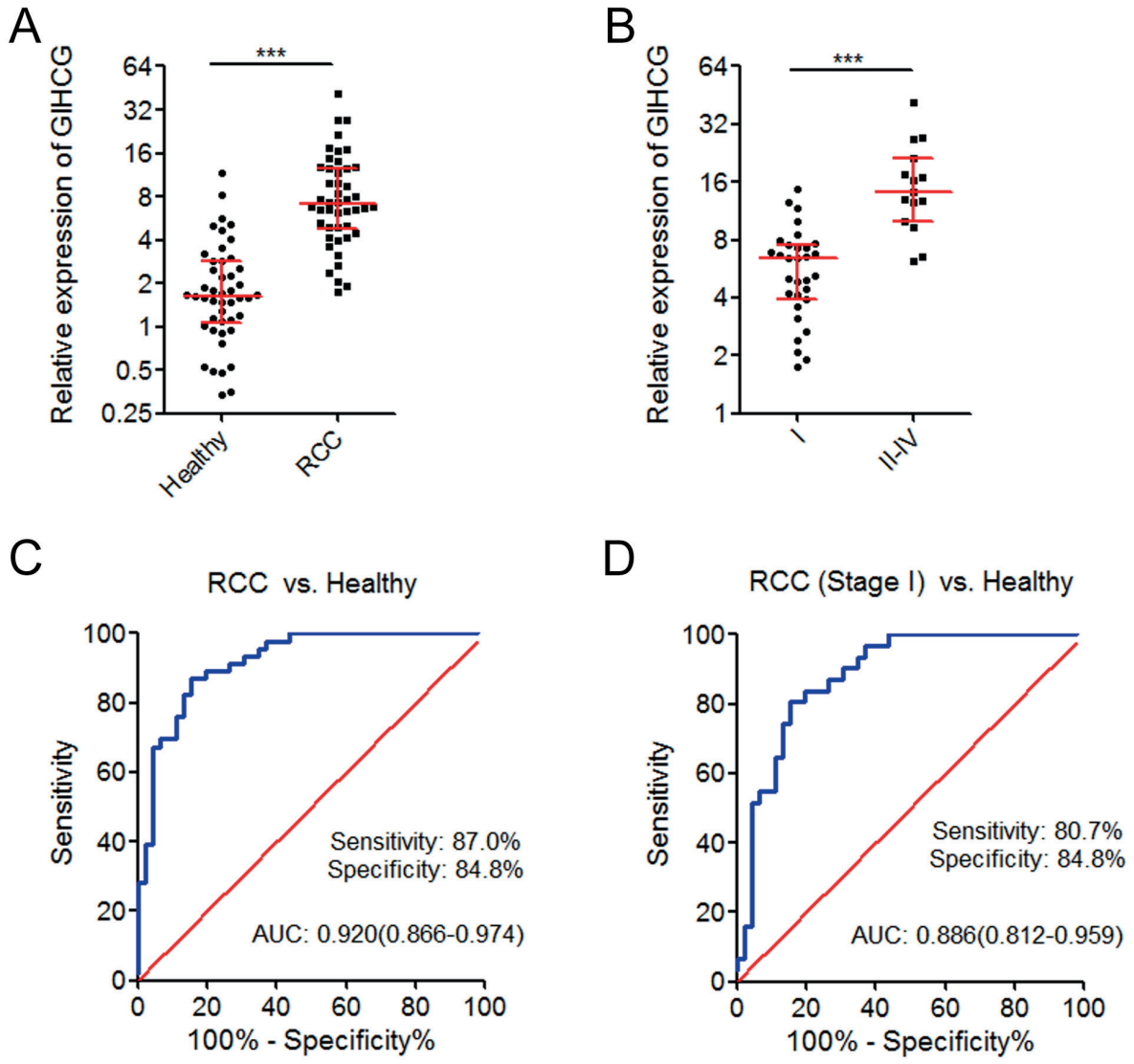

Figure 2. Diagnostic value of serum GIHCG for RCC. ***A,*** Serum GIHCG levels in 46 RCC patients and 46 age- and sex-matched healthy controls were measured by qRT-PCR. Results are presented as median with interquartile range. ***$p < 0.001$ by Mann-Whitney U test. ***B,*** Serum GIHCG levels in RCC patients with TNM stage I or TNM stage II-IV. Results are presented as median with interquartile range. ***$p < 0.001$ by Mann-Whitney U test. ***C,*** ROC curve analysis of serum GIHCG for discrimination between RCC patients and healthy controls (AUC: 0.920, sensitivity: 87.0%, specificity: 84.8%). $p < 0.001$. ***D,*** ROC curve analysis of serum GIHCG for discrimination between TNM stage I RCC patients and healthy controls (AUC: 0.886, sensitivity: 80.7%, specificity: 84.8%). $p < 0.001$.

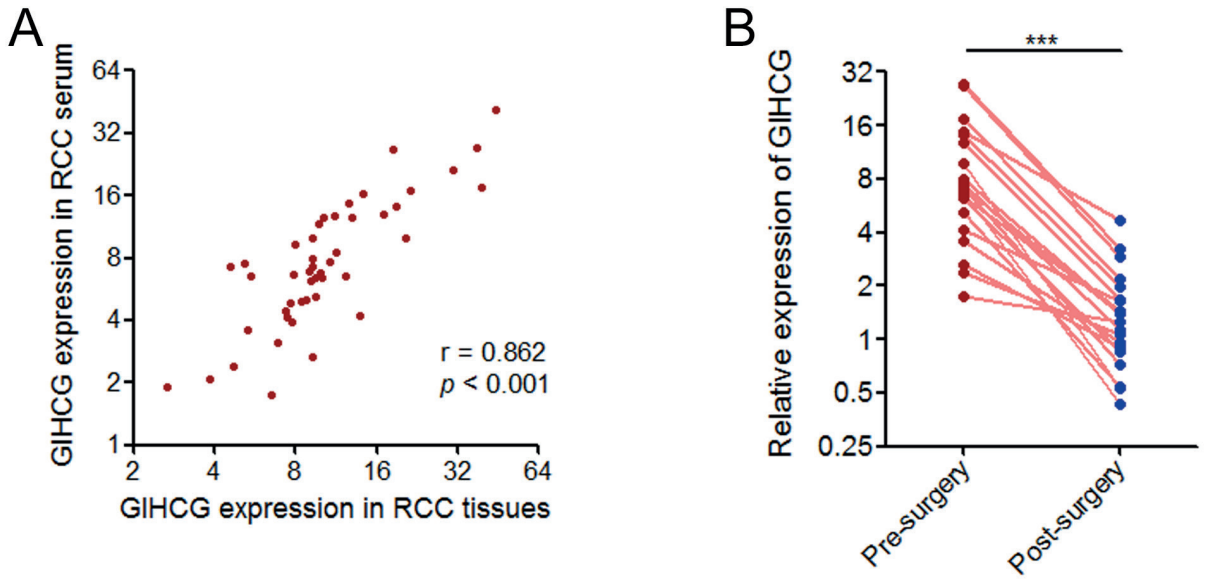

Figure 3. Serum GIHCG levels indicate RCC dynamics. ***A,*** A significant positive correlation was observed between serum GIHCG level and GIHCG expression in RCC tissues. r = 0.862, $p < 0.001$ by Pearson correlation analysis. ***B,*** Serum GIHCG levels in 20 pairs of pre- and post-surgery RCC patients were measured by qRT-PCR. ***$p < 0.001$ by Wilcoxon signed-rank test.

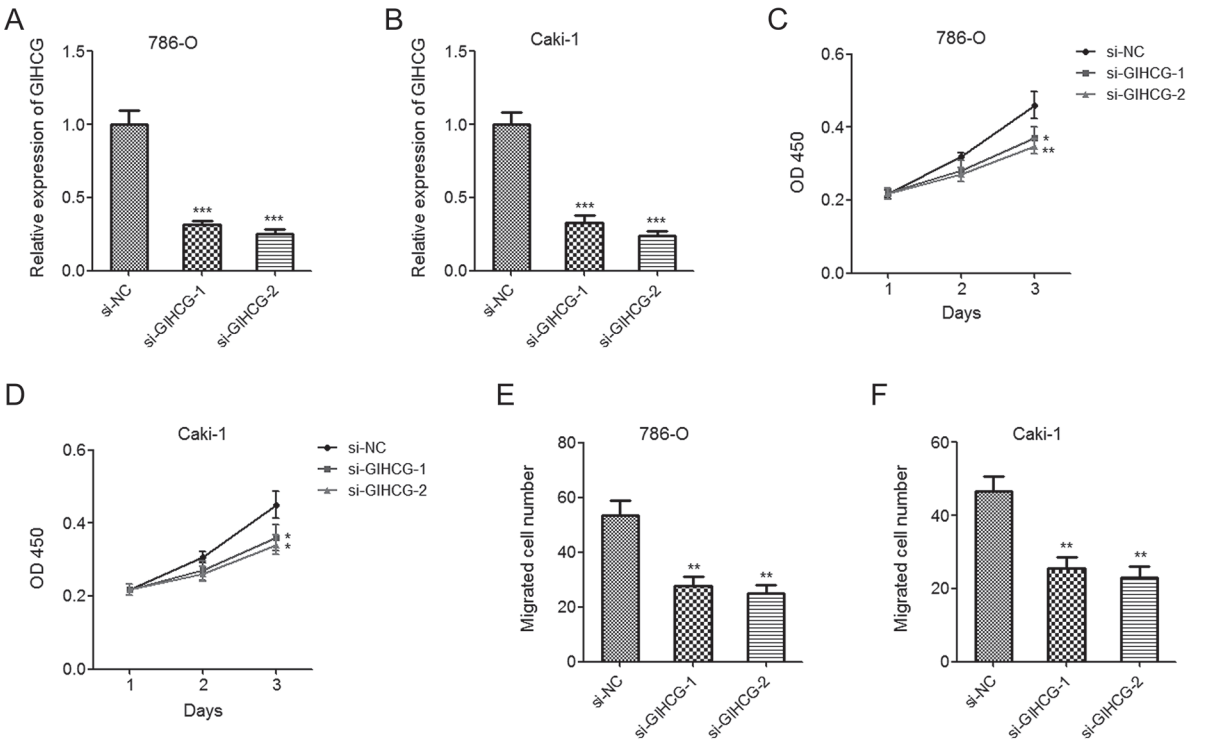

Figure 4. Knockdown of GIHCG inhibits proliferation and migration of RCC cells. ***A,*** GIHCG expression levels in 786-O cells after transfection of GIHCG specific siRNAs or control siRNA were measured by qRT-PCR. ***B,*** GIHCG expression levels in Caki-1 cells after transfection of GIHCG specific siRNAs or control siRNA were measured by qRT-PCR. ***C,*** Cell proliferation of 786-O cells after transfection of GIHCG specific siRNAs or control siRNA was measured by CCK-8 assays. ***D,*** Cell proliferation of Caki-1 cells after transfection of GIHCG specific siRNAs or control siRNA was measured by CCK-8 assays. ***E,*** Cell migration of 786-O cells after transfection of GIHCG specific siRNAs or control siRNA was measured by transwell assays. ***F,*** Cell migration of Caki-1 cells after transfection of GIHCG specific siRNAs or control siRNA was measured by transwell assays. Results are presented as mean ± SD of 3 independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ by Student's *t*-test.

Circulating GIHCG Could Monitor RCC Dynamics

To further explore the clinical significances of serum GIHCG in RCC, we calculated the correlation between GIHCG expression levels in RCC patients' serum and that in RCC tissues of the above used 46 RCC patients. The results showed a significant positive correlation between RCC patients' serum GIHCG level and GIHCG expression in CC tissues ($r = 0.862$, $p < 0.001$) (Figure 3A). In addition, we measured serum GIHCG level in 20 post-surgery RCC patients who received radical resection. The results showed that serum GIHCG level was markedly decreased in post-surgery RCC patients compared with that in pre-surgery RCC patients (Figure 3B). Taken together, these results suggested that serum GIHCG could monitor RCC dynamics.

Knockdown of GIHCG Inhibits Proliferation and Migration of RCC Cells

To investigate the biological roles of GIHCG in RCC cells, we knocked-down GIHCG expression in RCC cells 786-O and Caki-1 by transfecting GIHCG specific siRNAs. As shown in Figure 4A-4B, both siRNAs could knock-down GIHCG expression in both 786-O and Caki-1 cells. CCK-8 assays showed that knockdown of GIHCG by both siRNAs significantly decreased cell proliferation of 786-O and Caki-1 cells (Figure 4C-4D). Transwell migration assays showed that knockdown of GIHCG by both siRNAs significantly decreased cell migration of 786-O and Caki-1 cells (Figure 4E-4F). Taken together, these results suggested that knockdown of GIHCG inhibits proliferation and migration of RCC cells.

Discussion

Compared with other cancers, there are no biomarkers available for RCC screening in routine clinical practice. Exploring novel non-invasive reliable biomarkers for RCC screening is critical for early diagnosis of RCC patients and improving the overall prognosis of RCC patients[31]. In this study, we first identified the presence of GIHCG in the serum of RCC patients. Our results revealed that GIHCG is upregulated in the serum of RCC patients compared with that in healthy controls. Serum GIHCG level is positively correlated with TNM stages of RCC patients. Our data also demonstrated that serum GIHCG level not only discriminates RCC patients from healthy controls, but also discriminates early stage (TNM stage I) RCC patients from healthy controls. Thus, our data suggested that serum GIHCG may serve as a diagnostic biomarker for RCC. Furthermore, our results also revealed that serum GIHCG level is positively associated with GIHCG expression level in RCC tissues. After radical resection of RCC, serum GIHCG level is markedly reduced to a normal level. These results suggested that serum GIHCG could indicate RCC dynamics and be used to monitor RCC recurrence or progression after surgery. Collectively, these data showed the important clinical values of serum GIHCG. Except for this study, other reports have shown the clinical applications of lncRNAs for liquid biopsies in RCC. Wu et al[31] identified a 5-lncRNA signature, including lncRNA-LET, PVT1, PANDAR, PTENP1 and linc00963 that could distinguish RCC patients from healthy controls and benign renal tumors. Qu et al[32] reported that plasma lncARSR indicates sunitinib response of RCC patients. These reports further confirmed the clinical values of lncRNAs as non-invasive biomarkers for RCC. Completely measuring all the dysregulated lncRNAs in RCC patient's serum in enlarged clinical samples would promote the identification of more reliable lncRNAs biomarkers. These studies need further explorations.

We also detected the expression of GIHCG in RCC tissues. Our results revealed that GIHCG is significantly upregulated in RCC tissues compared with that in adjacent normal renal tissues. Increased expression of GIHCG is positively correlated with advanced TNM stages and Fuhrman grades. Furthermore, increased expression of GIHCG indicates poor survival of RCC patients. Several other lncRNAs were also reported to be dysregulated in RCC tissues and associated with prognosis of RCC patients, such as lncRNA-SARCC, TCL6, HEIRCC, lncRNA-ATB, MRCCAT1, and et al[33-37]. These reports demonstrated the importance of lncRNAs in RCC.

Loss-of-function assays showed that deletion of GIHCG significantly represses cell proliferation and migration of RCC cells, which is consistent with the reported oncogenic roles of GIHCG in HCC.

Conclusions

Our study first reports the upregulation of GIHCG in RCC patients' serum and tissues. GIHCG could serve as a potential diagnostic and prognos-

tic biomarker for RCC. Furthermore, GIHCG has oncogenic roles in RCC and could serve as a potential therapeutic target for RCC.

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Bhatt J R, Finelli A. Landmarks in the diagnosis and treatment of renal cell carcinoma. Nat Rev Urol 2014; 11: 517-525.
2) Torre L A, Bray F, Siegel R L, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.
3) Znaor A, Lortet-Tieulent J, Laversanne M, Jemal A, Bray F. International variations and trends in renal cell carcinoma incidence and mortality. Eur Urol 2015; 67: 519-530.
4) Zhai W, Sun Y, Guo C, Hu G, Wang M, Zheng J, Lin W, Huang Q, Li G, Zheng J, Chang C. LncRNA-SARCC suppresses renal cell carcinoma (RCC) progression via altering the androgen receptor(AR)/miRNA-143-3p signals. Cell Death Differ 2017; 24: 1502-1517.
5) Ljungberg B, Bensalah K, Canfield S, Dabestani S, Hofmann F, Hora M, Kuczyk M A, Lam T, Marconi L, Merseburger A S, Mulders P, Powles T, Staehler M, Volpe A, Bex A. EAU guidelines on renal cell carcinoma: 2014 update. Eur Urol 2015; 67: 913-924.
6) Baker H. Sunitinib as adjuvant therapy for renal cell carcinoma. Lancet Oncol 2016; 17: e485.
7) Ellinger J, Gevensleben H, Muller S C, Dietrich D. The emerging role of non-coding circulating RNA as a biomarker in renal cell carcinoma. Expert Rev Mol Diagn 2016; 16: 1059-1065.
8) Rini B I, Campbell S C, Escudier B. Renal cell carcinoma. Lancet 2009; 373: 1119-1132.
9) Cho I C, Chung J. Current status of targeted therapy for advanced renal cell carcinoma. Korean J Urol 2012; 53: 217-228.
10) Kaminski M M, Tosic J, Kresbach C, Engel H, Klockenbusch J, Muller A L, Pichler R, Grahammer F, Kretz O, Huber T B, Walz G, Arnold S J, Lienkamp S S. Direct reprogramming of fibroblasts into renal tubular epithelial cells by defined transcription factors. Nat Cell Biol 2016; 18: 1269-1280.
11) Rini B I. Targeted therapy for patients with renal-cell carcinoma. Lancet Oncol 2011; 12: 1085-1087.
12) Chan K C, Lo Y M. Circulating tumour-derived nucleic acids in cancer patients: potential applications as tumour markers. Br J Cancer 2007; 96: 681-685.
13) Yang J P, Yang X J, Xiao L, Wang Y. Long noncoding RNA PVT1 as a novel serum biomarker for detection of cervical cancer. Eur Rev Med Pharmacol Sci 2016; 20: 3980-3986.
14) Tong Y S, Wang X W, Zhou X L, Liu Z H, Yang T X, Shi W H, Xie H W, Lv J, Wu Q Q, Cao X F. Identification of the long non-coding RNA POU3F3 in plasma as a novel biomarker for diagnosis of esophageal squamous cell carcinoma. Mol Cancer 2015; 14: 3.
15) Wang W, He X, Zheng Z, Ma X, Hu X, Wu D, Wang M. Serum HOTAIR as a novel diagnostic biomarker for esophageal squamous cell carcinoma. Mol Cancer 2017; 16: 75.
16) Gong W, Tian M, Qiu H, Yang Z. Elevated serum level of lncRNA-HIF1A-AS1 as a novel diagnostic predictor for worse prognosis in colorectal carcinoma. Cancer Biomark 2017;
17) Oloomi M, Yardehnavi N, Bouzari S, Moazzezy N. Non-coding CK19 RNA in peripheral blood and tissue of breast cancer patients. Acta Med Iran 2013; 51: 75-86.
18) Jin C, Shi W, Wang F, Shen X, Qi J, Cong H, Yuan J, Shi L, Zhu B, Luo X, Zhang Y, Ju S. Long non-coding RNA HULC as a novel serum biomarker for diagnosis and prognosis prediction of gastric cancer. Oncotarget 2016; 7: 51763-51772.
19) Lu J, Xie F, Geng L, Shen W, Sui C, Yang J. Investigation of serum lncRNA-uc003wbd and lncRNA-AF085935 expression profile in patients with hepatocellular carcinoma and HBV. Tumour Biol 2015; 36: 3231-3236.
20) Schmitt A M, Chang H Y. Long noncoding RNAs in cancer pathways. Cancer Cell 2016; 29: 452-463.
21) Batista P J, Chang H Y. Long noncoding RNAs: cellular address codes in development and disease. Cell 2013; 152: 1298-1307.
22) Wapinski O, Chang H Y. Long noncoding RNAs and human disease. Trends Cell Biol 2011; 21: 354-361.
23) Yuan J H, Yang F, Wang F, Ma J Z, Guo Y J, Tao Q F, Liu F, Pan W, Wang T T, Zhou C C, Wang S B, Wang Y Z, Yang Y, Yang N, Zhou W P, Yang G S, Sun S H. A long noncoding RNA activated by TGF-beta promotes the invasion-metastasis cascade in hepatocellular carcinoma. Cancer Cell 2014; 25: 666-681.
24) Wang Y F, Zhang S, Li X Q, Wang Y. Expression of lncRNA HULC in cervical cancer and its correlation with tumor progression and patient survival. Eur Rev Med Pharmacol Sci 2016; 20: 3987-3991.
25) Yang L, Wei H, Xiao H J. Long non-coding RNA Loc554202 expression as a prognostic factor in patients with colorectal cancer. Eur Rev Med Pharmacol Sci 2016; 20: 4243-4247.
26) Liu X, Xiao Z D, Han L, Zhang J, Lee S W, Wang W, Lee H, Zhuang L, Chen J, Lin H K, Wang J, Liang H, Gan B. LncRNA NBR2 engages a metabolic checkpoint by regulating AMPK under energy stress. Nat Cell Biol 2016; 18: 431-442.
27) Lin A, Li C, Xing Z, Hu Q, Liang K, Han L, Wang C, Hawke D H, Wang S, Zhang Y, Wei Y, Ma G, Park P K, Zhou J, Zhou Y, Hu Z, Zhou Y, Marks J R, Liang H, Hung M C, Lin C, Yang L. The LINK-A lncRNA activates normoxic HIF1alpha signalling in triple-negative breast cancer. Nat Cell Biol 2016; 18: 213-224.

28) Yuan J H, Liu X N, Wang T T, Pan W, Tao Q F, Zhou W P, Wang F, Sun S H. The MBNL3 splicing factor promotes hepatocellular carcinoma by increasing PXN expression through the alternative splicing of lncRNA-PXN-AS1. Nat Cell Biol 2017; 19: 820-832.

29) Zhu X T, Yuan J H, Zhu T T, Li Y Y, Cheng X Y. Long noncoding RNA glypican 3 (GPC3) antisense transcript 1 promotes hepatocellular carcinoma progression via epigenetically activating GPC3. FEBS J 2016; 283: 3739-3754.

30) Sui C J, Zhou Y M, Shen W F, Dai B H, Lu J J, Zhang M F, Yang J M. Long noncoding RNA GIHCG promotes hepatocellular carcinoma progression through epigenetically regulating miR-200b/a/429. J Mol Med (Berl) 2016; 94: 1281-1296.

31) Wu Y, Wang Y Q, Weng W W, Zhang Q Y, Yang X Q, Gan H L, Yang Y S, Zhang P P, Sun M H, Xu M D, Wang C F. A serum-circulating long noncoding RNA signature can discriminate between patients with clear cell renal cell carcinoma and healthy controls. Oncogenesis 2016; 5: e192.

32) Qu L, Ding J, Chen C, Wu Z J, Liu B, Gao Y, Chen W, Liu F, Sun W, Li X F, Wang X, Wang Y, Xu Z Y, Gao L, Yang Q, Xu B, Li Y M, Fang Z Y, Xu Z P, Bao Y, Wu D S, Miao X, Sun H Y, Sun Y H, Wang H Y, Wang L H. Exosome-transmitted lncARSR promotes sunitinib resistance in renal cancer by acting as a competing endogenous RNA. Cancer Cell 2016; 29: 653-668.

33) Qi J J, Liu Y X, Lin L. High expression of long non-coding RNA ATB is associated with poor prognosis in patients with renal cell carcinoma. Eur Rev Med Pharmacol Sci 2017; 21: 2835-2839.

34) Xiong J, Liu Y, Luo S, Jiang L, Zeng Y, Chen Z, Shi X, Lv B, Tang W. High expression of the long non-coding RNA HEIRCC promotes renal cell carcinoma metastasis by inducing epithelial-mesenchymal transition. Oncotarget 2017; 8: 6555-6563.

35) Zhai W, Sun Y, Jiang M, Wang M, Gasiewicz T A, Zheng J, Chang C. Differential regulation of LncRNA-SARCC suppresses VHL-mutant RCC cell proliferation yet promotes VHL-normal RCC cell proliferation via modulating androgen receptor/HIF-2alpha/C-MYC axis under hypoxia. Oncogene 2016; 35: 4866-4880.

36) Li J K, Chen C, Liu J Y, Shi J Z, Liu S P, Liu B, Wu D S, Fang Z Y, Bao Y, Jiang M M, Yuan J H, Qu L, Wang L H. Long noncoding RNA MRCCAT1 promotes metastasis of clear cell renal cell carcinoma via inhibiting NPR3 and activating p38-MAPK signaling. Mol Cancer 2017; 16: 111.

37) Su H, Sun T, Wang H, Shi G, Zhang H, Sun F, Ye D. Decreased TCL6 expression is associated with poor prognosis in patients with clear cell renal cell carcinoma. Oncotarget 2017; 8: 5789-5799.