Histology and Histopathology
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ORIGINAL ARTICLE   **Open Access**

Expression and clinical significance of RHCG in endometrial cancer

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Summary. Endometrial cancer (EC) is the most common gynecological cancer. Rhesus family, C glycoprotein (RHCG) has been evidenced to be involved in the occurrence and development of various tumors. This study aimed to investigate the expression and clinical significance of RHCG in EC. Bioinformatics analysis was based on the RNAseq counts data from TCGA database, and the prognosis analysis was performed using the Kaplan-Meier method; 4 cases of endometrioid adenocarcinomas samples and 4 cases of normal proliferative endometrium were collected for qPCR and western blot; immunohistochemistry analysis was employed to assess the expression of RHCG in a tissue microarray; the correlation between RHCG and clinicopathological factors was analyzed through Mann-Whitney U test. The lentiviral interference vector was further constructed. The results demonstrated that RHCG was highly expressed in EC tissues, and RHCG was an independent factor affecting the overall survival of patients. Additionally, the expression of RHCG was related to FIGO stage and tumor infiltrate. After interfering with shRHCG, the proliferation activity of EC cells decreased, the migration ability of cells decreased, the apoptosis of cells increased, and the tumor outgrowth was arrested. In summary, RHCG promotes the malignant proliferation and migration of EC, and makes the cells have anti-apoptotic activity. Our study provides a theoretical basis for RHCG to become a potential therapeutic target for EC in the future.

Key words: Endometrial cancer, RHCG, Proliferation, Migration, Apoptosis

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Introduction

Endometrial cancer (EC) is the most common malignancy of the inner epithelial lining of the uterus (Makker et al., 2021), the incidence of which is increasing at an alarming rate, with an estimated 200,000 new cases each year (Bell and Ellenson, 2019). EC comprises distinct histological subtypes and molecular phenotypes. EC was historically categorized as Type I or Type II. Type I ECs are primarily composed of grade I or grade II endometrioid adenocarcinomas, whereas Type II ECs include grade III endometrioid adenocarcinomas, serous clear cell, undifferentiated and carcinosarcomas (Yen et al., 2020; Makker et al., 2021). The molecular phenotypes of EC included four categories: POLE (ultra-mutated), microsatellite instability (MSI)/hypermutated, copy number low (CNL)/microsatellite stable, and serous-like/copy number high (CNH) (Cancer Genome Atlas Research et al., 2013). With the development of a molecular classification system, molecular testing to personalize the therapeutic approach is becoming more commonplace. In recent years, a variety of targeted therapies have been assessed for the treatment of EC, with some recently gaining FDA authorization (Yen et al., 2020). Still, many patients remain unable to benefit from targeted therapy due to limited availability of targets. Consequently, a pressing requirement exists to discover more potential therapeutic targets.

Rhesus family, C glycoprotein (RHCG), an ammonia transporter (Weiner and Verlander, 2003; Han et al., 2009), is located on chromosome 15q25 and encodes a 53-kda, 498-amino acid polypeptide (Liu et al., 2000, 2021; Huang and Liu, 2001; Nakhoul and Hamm, 2004). RHCG is widely expressed in the kidney (Liu et al., 2000), lung (Han et al., 2009), liver (Weiner et al., 2003), testis (Liu et al., 2000) and intestinal mucosa (Torre et al., 2017). More importantly, RHCG has been reported to play tumor-promoting and tumor-suppressing functions on different tumors through different mechanisms. For instance, a study from Chen et al. suggested that RHCG promotes the migration and proliferation of gastric cancer by maintaining

intracellular alkalinity (Chen et al., 2020). On the other hand, the tumor suppressor actions of RHCG in prostate cancer are through the activation of hypermethylation of its promoter (Strand et al., 2017). Besides, in esophageal cancer, RHCG exerts a tumor suppressor effect by inhibiting NF-κB signal transduction and the formation of extracellular matrix MMP-1, which is related to the hypermethylation of the promoter (Ming et al., 2018). Moreover, RHCG has also been identified as a tumor suppressor factor in cervical cancer (Wang et al., 2018). However, the relationship between the expression of RHCG and the occurrence, development and prognosis of EC has not been widely discussed.

This study investigated differentially expressed or frequently mutated genes in EC by analyzing the TCGA-UCEC genome database, and selected RHCG as the target. Subsequently, the expression levels and roles of RHCG in EC were verified by clinical specimens and cell function experiments, suggesting that RHCG may serve as an important target for future therapy and clinical diagnosis of EC.

Materials and methods

TCGA database analysis

Bioinformatics analysis in this study was based on the RNAseq counts data of 546 EC and 35 normal endometrial samples from TCGA, and the prognosis analysis was based on the clinical information of TCGA-UCEC samples using the Kaplan-Meier method. The screening criteria of target were $P<0.05$, fold change (FC)>1.5, and the top ten genes were selected as candidate genes.

Tissue collection, tissue microarray and ethics statement

A tissue microarray included 45 cases of tumor samples from FIGO grade I (21 cases), grade II (13 cases) or grade III (11 cases) endometrioid adeno-carcinomas and 10 cases of normal proliferative endometrium from healthy individuals. This study also included 4 cases of clinical endometrioid adeno-carcinomas samples and 4 cases of normal proliferative endometrium from healthy individuals. Detailed clinicopathological data were obtained and summarized. All patients signed informed consent, and this study was approved by the Ethical Committee of the First Hospital of Qinhuangdao (No. 2022A033).

Immunohistochemistry (IHC) staining

The tissue sections were embedded with paraffin, and then baked at 60°C for 30 min. After being dehydrated and rehydrated, citric acid buffer was added for antigen retrieval (120°C for 20 min). The endogenous peroxidase was blocked with 3% H_2O_2 for 10 min, then all slices were incubated with primary antibody RHCG (1:100, Sanyin, #11949-1-AP), E-

Cadherin (1:100, CST, #3195s), Vimentin (1:100, bioss, # bs-8533R), Snail (1:100, Proteintech, #13099-1-AP) as well as Ki67 (1:100, abcam, # ab16667) at 4°C overnight. After that, the secondary antibody IgG H&L (HRP) (1:400, abcam, #ab97080) was added and incubated for 2 h at room temperature. The slides were stained with DAB for 5 min and then dyed again with hematoxylin (Baso DiagnosticsInc., Zhuhai, China) for 10 - 15 s. Finally, the slides were captured with a microscope and viewed with ImageScope and CaseViewer. All slides were randomly assessed by three independent pathologists. Staining scores were divided into: 1 (1%-24%), 2 (25%-49%), 3 (50%-74%) and 4 (75%-100%). The staining intensity was scored from 0 (no signal color) to 3 (light yellow, brown, and dark brown). IHC results were defined based on staining scores and intensity scores, which specifically include negative (0), positive (1-4), ++ positive (5-8) and +++ positive (9-12). Finally, low/high expression depends on the median of all scores.

Cell lines and cell culture

Human endometrial epithelial cells were purchased from Shanghai iCell Biosciencel Inc. Besides, four human EC cell lines ishikawa, HEC-1-A, KLE, and HEC-1-B were purchased from American type culture collection (ATCC). All cells were cultured in RIP1640 or DMEM medium (corning, USA) at 37°C, 5% CO_2 supplemented with 10% FBS (Invitrogen, USA), and the medium was changed every other day.

Lentivirus RNAi construction and infection

The RHCG RNAi target sequences were designed by Shanghai Bioscienceres Co., Ltd. (Shanghai, China). The sequence was then ligated to the linearized vector BR-V-108 through the restriction sites at both ends, and the production was transferred to the prepared DH5α E. coli competent cells. The positive clones were identified by qPCR, and the plasmids were extracted by the Endofree Maxi plasmid kit. The qualified plasmids were transfected into 293T cells, and the cells were harvested after infection for 48-72h. Finally, the cells were cultured for 72h at 37°C, and the infection efficiency was evaluated.

RNA extraction and qRT-PCR

The cells were collected, and the total RNA was extracted with TRIzol reagent (Sigma, St. Louis, MO, USA) according to the manufacturer's instruction. Then, cDNA was obtained by using the Promega M-MLV Kit (Promega Corporation, Madison, Wisconsin, USA). qRT-PCR assay was performed with SYBR Green Mastermixs Kit (Vazyme, Nanjing, Jiangsu, China). GAPDH was chosen as an internal control and the relative expression of mRNA was calculated according to the $2^{-\Delta\Delta Ct}$ method. The sequences of primers used

here as follows: The forward primer of RHCG is TACCGCTGCTGAGATGATGCT, and its reverse primer is CCACGATGCCGCCTATGATG; the forward primer of GAPDH is TGACTTCAACAGCGACAC CCA, and its reverse primer is CACCCTGTTGCTGT AGCCAAA.

Western blot assay

The total proteins were extracted and quantified with BCA protein assay kit (Thermo Fisher Scientific, Cat. # A53227). After that, the proteins were segregated by 10% SDS-PAGE and transferred into PVDF membranes for Western blot assay. Then, the membranes were blocked and incubated with primary antibodies and secondary antibodies at room temperature for 2h. Finally, the ECL+plusTM Western blotting system kit was used for color rendering, and X-ray imaging was carried out. The details of antibodies used in western blot assay were listed as follows: RHCG (1:500, Rabbit, Proteintech, #11949-1-AP), E-Cadherin (1:1000, Rabbit, CST, #3195), β-Catenin (1:1000, Rabbit, CST, #8480), Vimentin (1:1000, Rabbit, CST, #5741), Snail (1:1000, Rabbit, CST, #3879), SIUG (1:1000, Rabbit, CST, #9585), ZO-1 (1:1000, Rabbit, CST, #8193), Claudin-1 (1:1000, Rabbit, CST, #13255), GAPDH (1:30000, Mouse, Proteintech, #60004-1-lg) and Goat Anti-Rabbit (1:3000, Beyotime, A0208).

CCK8 assay

KLE and HEC-1-B cell lines after being infected were resuspended and counted. 100 μL cell suspension was added in a 96-well plate at the density of 3000 cell/well, and three replicates were set for each group. After that, the cells were placed in an incubator. From the second day, 10 μL CCK-8 reagent was added into the well 2~4h before the termination of the culture. After 4h, the 96-well plate was placed on a shaker and oscillated for 2-5 min, and the OD value was measured for 5 days by the microplate reader at 450 nm.

Wound-healing assay

KLE and HEC-1-B cells infected with lentiviruses were inoculated into 96-well plates. Then, images (×50) were obtained after scratches for 0 h, 24h or 48h through Cellomics scanner (cat. no. ArrayScan VT1; Thermo Fisher, USA). Mobility was defined as the ratio of cell migration distance to the width of the "0h" scratch area at different times. The experiment was repeated three times.

Transwell assay

First, the upper chamber was incubated with 100 μL serum-free medium for 1-2h. Then, KLE and HEC-1-B cell lines with indicated lentivirus were diluted and transferred into each chamber. At the same time, 600 μL

medium with 30% FBS was added in the lower chamber. After that, the upper chamber was transferred to the lower chamber and incubated for 40h. 400 μL Giemsa were added for cells staining. Finally, the cells were dissolved in 10% acetic acid and the value of OD570 was detected. The experiment was repeated three times and the migration ability of cells was determined.

Detection of cell apoptosis and cell cycle by fluorescence activated Cells Sorting (FACS)

After infecting indicated lentivirus, KLE and HEC-1-B cell lines were inoculated into a 6-well plate (2 mL/well). When cell confluence reached 85%, the cell suspension was centrifuged, and the supernatants were discarded. Then, the cells were washed with 4°C pre-cooled D-Hanks (pH=7.2~7.4). Next, 10 μL Annexin V-APC (eBioscience, San Diego, CA, USA) was added for staining in the dark. The cell apoptosis level was measured, and the apoptotic rate was analyzed with FACSCalibur (BD Biosciences, San Jose, CA, USA).

The construction of nude mouse tumor formation model

The Ethical Committee of the First Hospital of Qinhuangdao gave their approval to all animal experiments (No. 2022A033). Four-week-old female BALB-c nude mice, from Shanghai Lingchang Animal Research Co., Ltd., were subcutaneously injected with 1×10^7 KLE cells with shRHCG and shCtrl. The tumor volume was tested with the following formula: tumor volume=π/6×L×W×W, where L and W represent tumor length and tumor width, respectively. 0.7% sodium pentobarbital was injected intraperitoneally for several min, and the fluorescence was observed by the *in vivo* imaging system (IVIS Spectrum, Perkin Elmer). After 24 days of cell injection, the mice were sacrificed and the tumors were taken out to weigh and photograph, then frozen in liquid nitrogen and kept at -80°C.

Statistical analysis

All data were analyzed by GraphPad Prism 6 (San Diego, CA, USA) and SPSS version 24.0, and data are presented as the mean ± SD. The association between RHCG expression and patients' clinicopathological characteristics was tested using Mann-Whitney U test. Student's t-test (for comparisons of two groups) was used to analyze the statistical significance, and $P<0.05$ was considered to be statistically significant.

Results

Screening of RHCG

Through analyzing the TCGA database, we investigated differentially expressed genes in EC and selected RHCG with a highest fold change (log FC=5.870, $P<0.001$; Fig. 1A). To verify this, we

subsequently analyzed the patterns of RHCG in 35 normal and 546 cancer samples from the TCGA database and found that RHCG was abundantly expressed in EC (P<0.001, Fig. 1B). Moreover, the relationship between RHCG expression (including 142 high-expression samples and 369 low-expression samples) and the life quality of patients was further analyzed, and it was found that the survival period of patients with high RHCG expression was significantly shorter than that of patients with low RHCG expression (P<0.01, Fig. 1C).

Furthermore, RHCG levels also positively correlate with pathological stages (Fig. 1D), patients' age (Fig. 1E) and histological subtypes (Fig. 1F), implying the clinical importance of studying RHCG. The TCGA project has revealed four prognostic EC subgroups: POLE, MSI, CNL and CNH, and we found that RHCG correlated with MSI (P<0.01, Fig. 1G). In addition, multivariate Cox regression analysis demonstrated that high RHCG level was an independent prognostic factor for poor survival of patients with EC (P<0.05, Table 1).
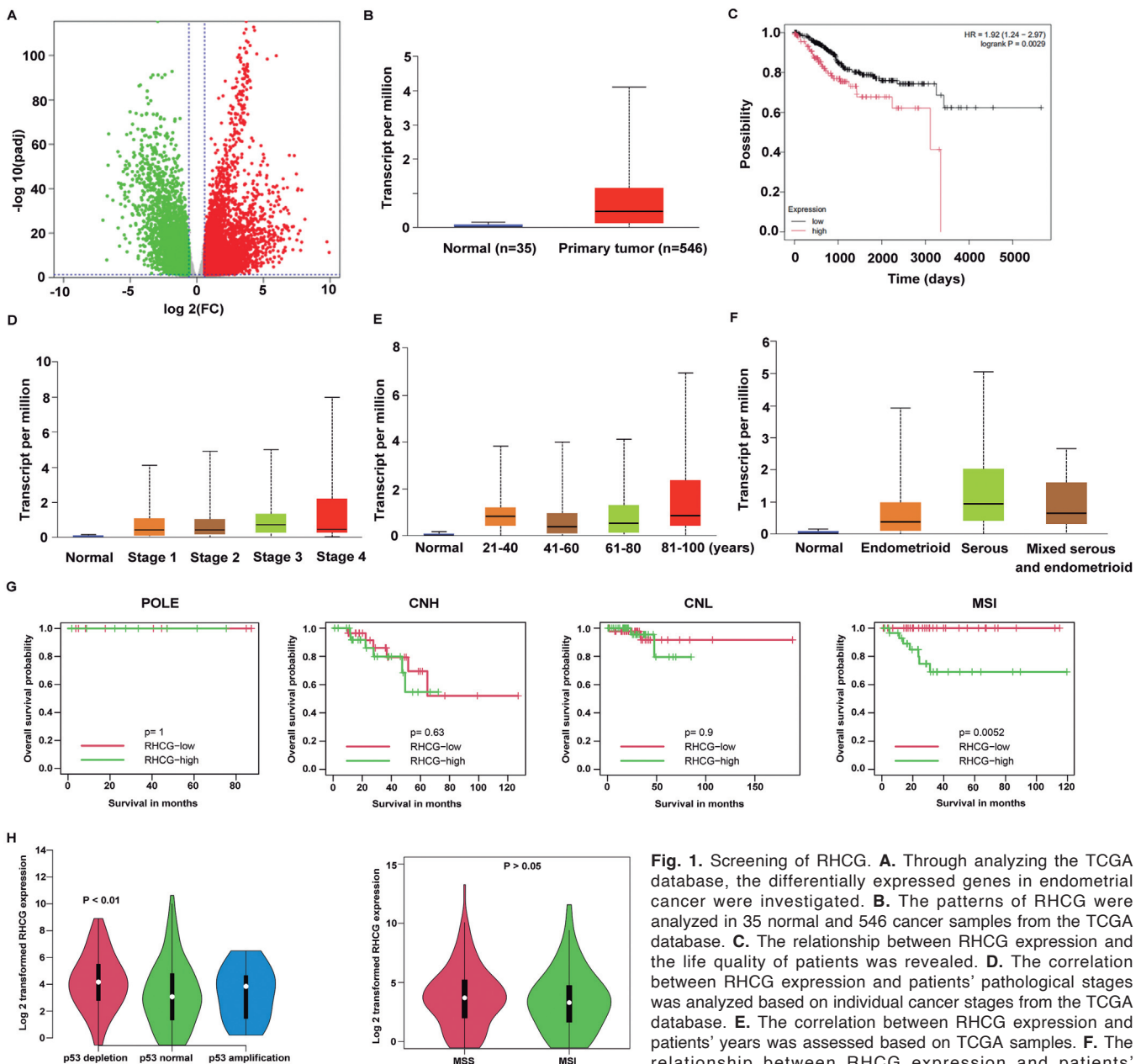

Fig. 1. Screening of RHCG. **A.** Through analyzing the TCGA database, the differentially expressed genes in endometrial cancer were investigated. **B.** The patterns of RHCG were analyzed in 35 normal and 546 cancer samples from the TCGA database. **C.** The relationship between RHCG expression and the life quality of patients was revealed. **D.** The correlation between RHCG expression and patients' pathological stages was analyzed based on individual cancer stages from the TCGA database. **E.** The correlation between RHCG expression and patients' years was assessed based on TCGA samples. **F.** The relationship between RHCG expression and patients' histological subtypes was investigated based on TCGA samples. **G.** The relationship was revealed between RHCG with POLE, MSI, CNL and CNH. **H.** RHCG was associated with p53 depletion but not with MSI (MMR-deficient) and MSS (hypermutated).

Furthermore, there was a link between RHCG and p53-abnormal group (p53 depletion); however, there was no relation between RHCG with MSI (MMR-deficient) and MSS (hypermutated) (Fig. 1H).

RHCG was elevated in endometrial cancer

To explore the clinical significance of RHCG in EC, 4 cases of clinical endometrioid adenocarcinomas samples and 4 cases of normal proliferative endometrium from healthy individuals were collected.

Table 1. Multivariate analysis using the Cox proportional hazards model.

Parameter	Hazard ratio (HR)	P
Age	1.025	0.182068
Stage I	1	-
Stage II	0.5915	0.618228
Stage III	3.302	0.004816
Stage IV	3.128	0.03386
Grade 1	1	-
Grade 2	6.63	0.080933
Grade 3	9.677	0.036092
Subtype CN High	1	-
Subtype CN low	0.9382	0.930422
Subtype MSI	1.258	0.659732
Subtype NSMP	1.034	0.936572
Subtype POLE	7.677E-08	0.996299
RHCG	1.167	0.019357

The data of qPCR experiment showed that RHCG mRNA levels in 75% of endometrioid adenocarcinomas samples (3/4) were significantly higher than that in normal tissues (Fig. 2A). Further western blot analysis revealed that RHCG protein expressed at an abundant level in cancer samples (Fig. 2B). Moreover, we also evaluated RHCG expression in a tissue microarray including 45 cases of endometrioid adenocarcinomas and 10 cases of normal proliferative endometrium tissues through IHC staining. Consistent with the above results, the expression of RHCG in cancer tissues was significantly higher than that in normal tissues ($P<0.001$, Table 2, Fig. 2C). What's more, the Mann-Whitney U analysis of clinicopathological data showed that the expression of RHCG was positively related to myometrial invasion and FIGO stage (Table 3), which was further verified via Spearman rank correlation analysis (Table 4). Besides, IHC analysis showed

Table 2. Expression patterns of RHCG in endometrioid adenocarcinomas tissues and normal proliferative endometrium revealed in immunohistochemistry analysis.

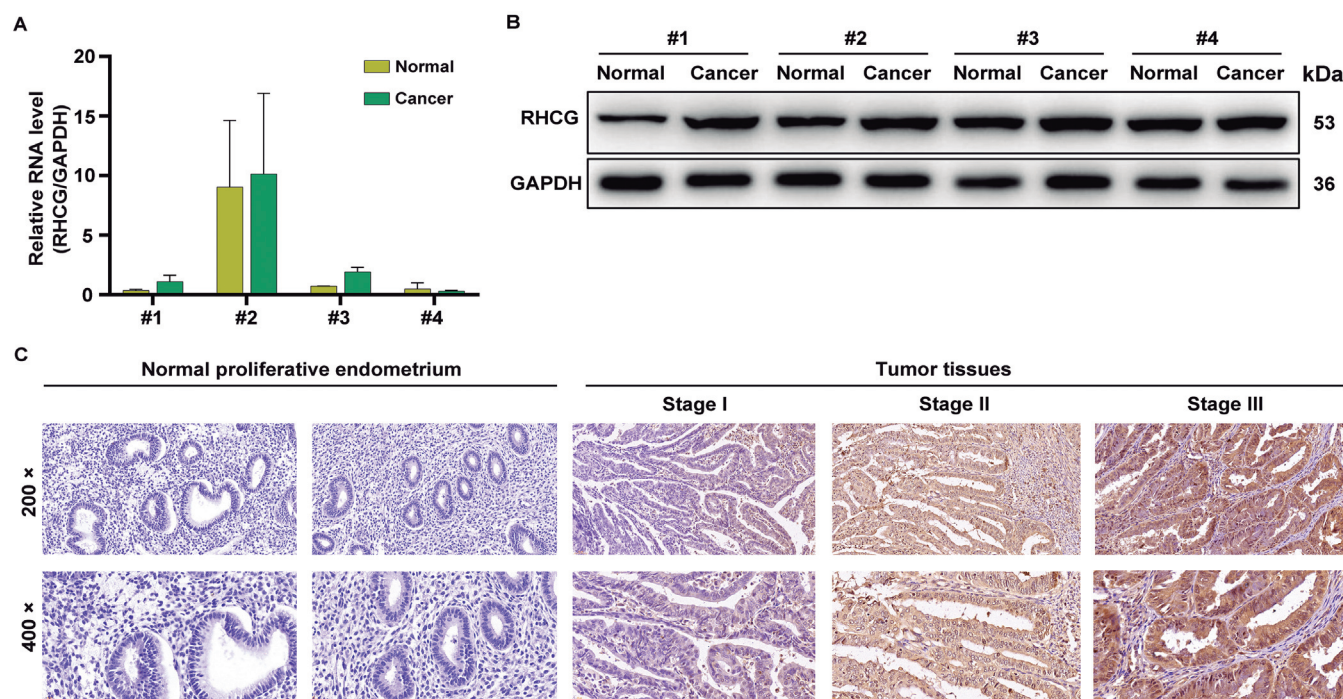| RHCG expression	Tumor tissue		Normal proliferative endometrium		P value
	Cases	Percentage	Cases	Percentage	
Low	23	51.1%	10	100%	<0.001
High	22	18.9%	0	0%	

Fig. 2. RHCG was elevated in endometrial cancer. **A, B.** The levels of RHCG mRNA **(A)** and protein **(B)** were detected in four paired endometrial cancer and normal tissue samples through qRT-PCR and western blot assay. **C.** IHC staining was employed to evaluate RHCG expression in a tissue microarray including 45 tumor tissues and 10 normal tissues.
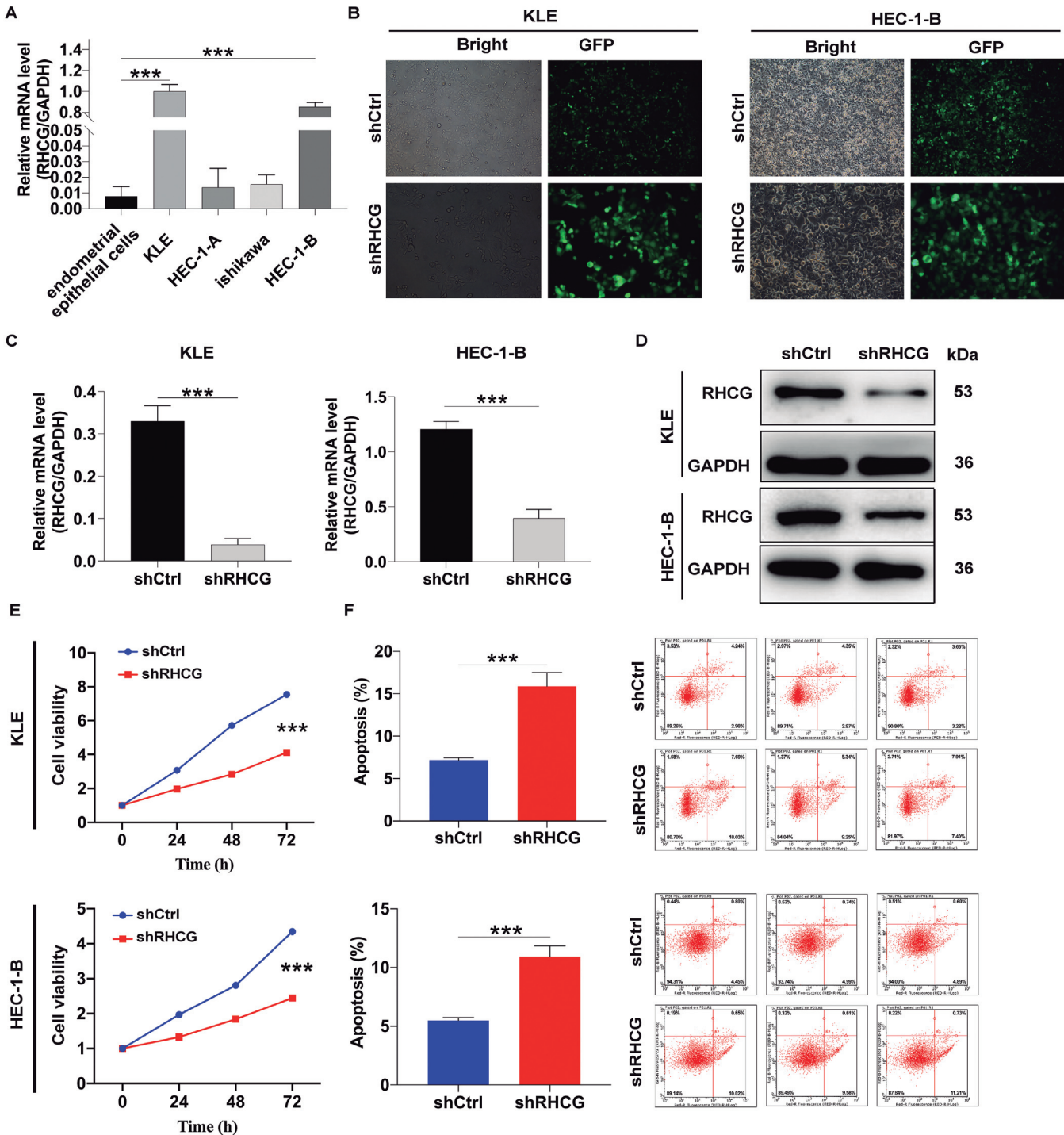
Fig. 3. Silencing RHCG suppresses endometrial cancer cell proliferation while ameliorating apoptosis. **A.** RHCG expression was detected in four human endometrial cancer cell lines, ishikawa, HEC-1-A, KLE and HEC-1-B as well as human endometrial epithelial cells. **B.** The infection efficiency of shRHCG was evaluated in KLE and HEC-1-B cell lines. **C, D.** The levels of RHCG mRNA **(C)** and protein **(D)** in KLE and HEC-1-B cell lines were quantified after shRHCG infection. **E.** The capabilities of KLE and HEC-1-B cell proliferation were evaluated by CCK8 assay after knocking down RHCG. **F.** Flow cytometry experiment was operated to visualize the changes in cell apoptosis upon RHCG depletion. ***$P<0.001$. × 200.

positive expression of p53 and MMR in most endometrioid adenocarcinoma with low RHCG (Table 3), which were negatively correlated with RHCG expression, but not statistically significant (Table 4), possibly due to limited samples. Based on the above findings, we proposed that RHCG might participate in the occurrence and development of EC, which could be considered as a target in the treatment of EC.

Silencing RHCG suppresses endometrial cancer cell proliferation while ameliorating apoptosis

To explore the functional significance of RHCG in EC development, we detected RHCG expression in four human EC cell lines, ishikawa, HEC-1-A, KLE and HEC-1-B as well as human endometrial epithelial cells. The results demonstrated that compared with human endometrial epithelial cells, RHCG was elevated in EC cell lines, especially in in KLE and HEC-1-B cell lines ($P<0.001$, Fig. 3A). We then designed a lentiviral plasmid targeting RHCG (shRHCG) and infected it into KLE and HEC-1-B cell line. As shown in Fig. 3B, shRHCG achieved the desired effect, and the infection efficiency reached more than 80% via assessing the fluorescence inside cells. On the other hand, the knockdown efficiency of shRHCG was 88.3% ($P<0.001$) and 67.4% ($P<0.001$) in KLE and HEC-1-B cell lines, respectively (Fig. 3C). Furthermore, the expression of RHCG protein was remarkably downregulated after shRHCG infection (Fig. 3D), implying that EC cell
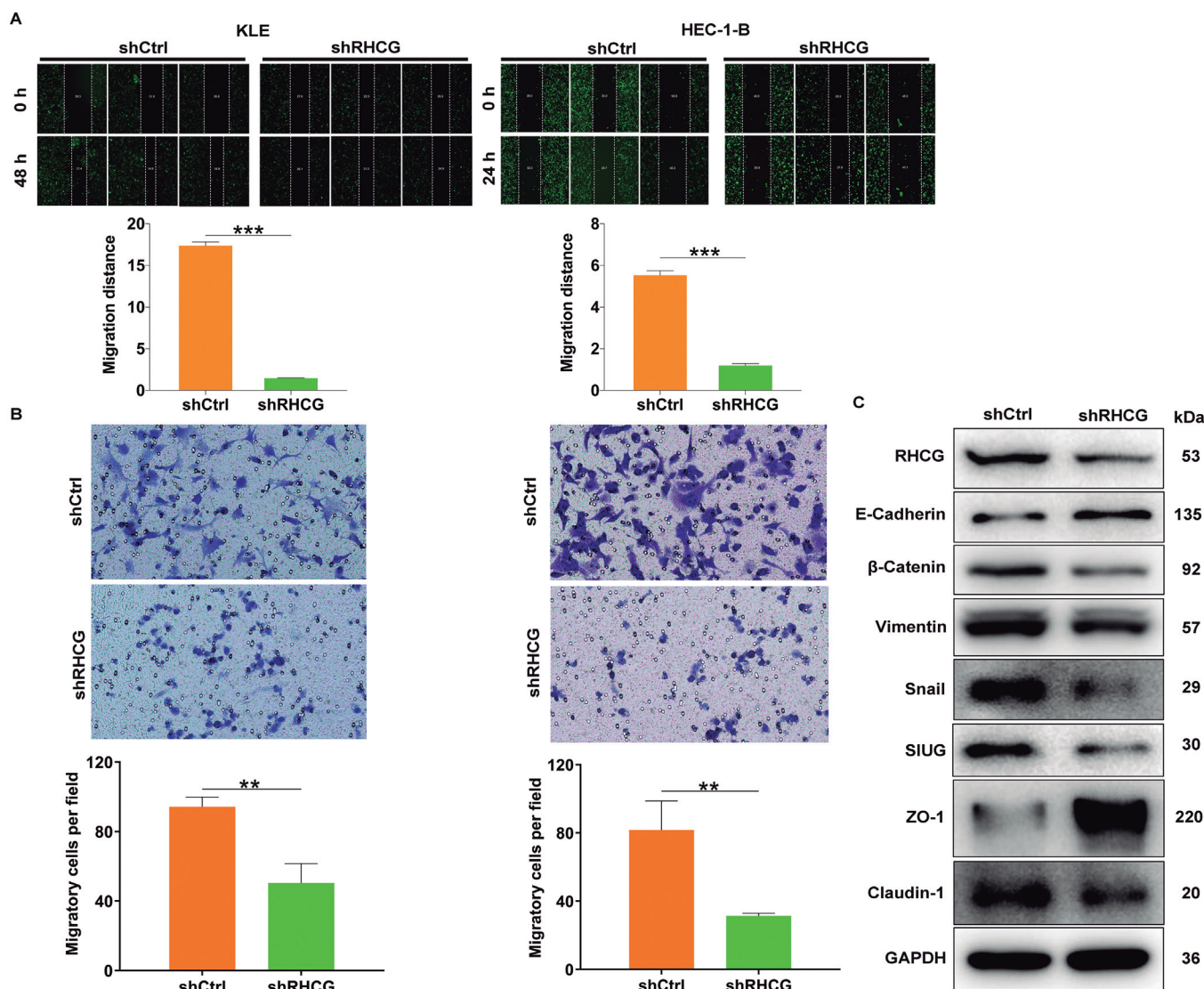

Fig. 4. Silencing RHCG arrests endometrial cancer cell migration. **A, B.** After silencing RHCG in KLE and HEC-1-B cell lines, the alterations in cell migration were observed via wound-healing analysis **(A)** and transwell assay **(B)**. **C.** The protein levels of EMT biomarkers in RHCG-depleted KLE cells were detected by western blotting. × 50 for wound-healing analysis and × 200 for transwell assay. *******P*<0.01, ***P<0.001

models with RHCG depletion were constructed successfully and could be employed in cell function experiments.

Subsequently, we measured the alterations in KLE and HEC-1-B cell phenotypes upon infecting shRHCG. First, the capabilities of cell proliferation were detected by CCK8 assay. The results indicated that after knocking down RHCG, the proliferation levels of KLE and HEC-1-B cells were significantly decreased ($P<0.001$, Fig. 3E). More notably, the data of flow cytometry evidenced that cell apoptosis was ameliorated upon silencing RHCG ($P<0.001$, Fig. 3F). Taken together, these data demonstrated that silencing RHCG suppresses EC cell proliferation while ameliorating apoptosis.

Silencing RHCG arrests endometrial cancer cell migration

In addition to cell proliferation and apoptosis, we also assessed the changes of cell migration in RHCG-depleted KLE and HEC-1-B cell lines. The wound-healing assay suggested that cell migration was arrested in the above cell lines ($P<0.001$, Fig. 4A). Additionally, the transwell assay was also performed, which again evidenced that the abilities of cell migration were impaired upon silencing RHCG ($P<0.01$, Fig. 4B). Several lines of evidence indicate that cancer cells exhibit both epithelial and mesenchymal characteristics, which is known as partial epithelial mesenchymal transition (EMT), during cancer progression, and partial EMT can promote cancer migration and invasion (Saitoh, 2018). At the molecular level, we found E-cadherin and ZO-1 were increased by RHCG shRNA, while β-Catenin, Vimentin, Snail, SIUG and Claudin-1 levels were decreased (Fig. 4C). Our results therefore suggested that silencing RHCG could suppress EMT process of EC.

Silencing RHCG impaired the tumorigenesis of endometrial cancer

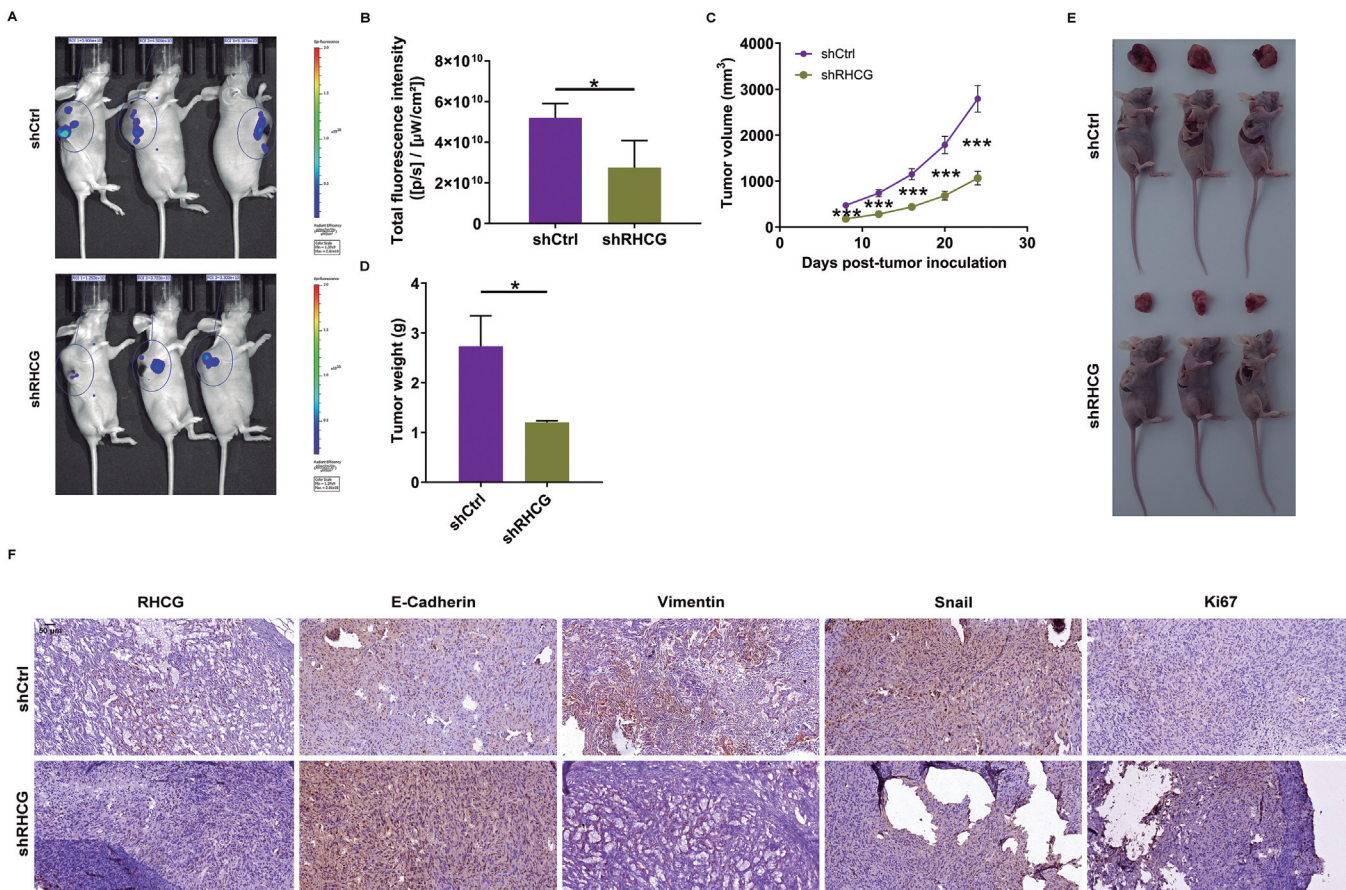To further verify the involvement of RHCG in tumor

Fig. 5. Silencing RHCG impaired the tumorigenesis of endometrial cancer. **A.** Construction of subcutaneous xenograft tumor model. **B.** The fluorescence expression in nude mice was detected through the *in vivo* imaging system. **C, D.** The volume **(C)** and weight **(D)** of tumor derived from shCtrl/shRHCG infected KLE cells was measured. **E.** The photos of mice and their xenografts. **F.** Immunohistochemical staining of RHCG, E-Cadherin, Vimentin, Snail and Ki67 in tumor tissues. *$P<0.05$, ***$P<0.001$

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RHCG promotes endometrial cancer development

progression *in vivo*, we performed a subcutaneous xenograft assay in nude mice using RHCG-depleted KLE cells (Fig. 5A). After 24 days of injection, fluorescence expression was obviously decreased in nude mice with RHCG-depleted KLE cells when compared to the controls (shCtrl) (Fig. 5B). Moreover, a significant reduction in tumor volume and weight was also observed (Fig. 5C-E). Consistent with the results of *in vitro* assays, cell proliferation and migration abilities of KLE cells *in vivo* was significantly attenuated after RHCG was stably knocked down, as indicated by augmented E-Cadherin and diminished Vimentin, Snail as well as Ki67 (Fig. 5F). Taken together, these data suggested that RHCG plays a crucial oncogenic role in promoting EC cell growth and tumorigenicity.

Discussion

EC is the most common gynecologic malignancy. Currently, the incidence of EC is increasing, and the age of onset is younger than in previous years. Although the disease is more common in older women and the mortality rate is on the rise, it is now reported that an increasing number of younger women are also being diagnosed with the disease (Bell and Ellenson, 2019). The preferred treatments for EC are total hysterectomy with bilateral salpingo-oophorectomy. In addition, radiotherapy and chemotherapy also play a key role in the treatment (Brasky et al., 2017; Zhou et al., 2021). However, the efficacy is unsatisfactory, and the recurrence rate is high. Therefore, targeted therapy came into being and has achieved certain results.

RHCG, an ammonia transporter, is a key molecule in uric acid excretion (Donders et al., 2017). In detail, RHCG regulates uric acid by interacting with vacuolar proton-ATPase in renal interstitial cells (Brown et al., 2009; Bourgeois et al., 2018). Previous studies have reported the correlation between RHCG and human cancers. For example, RHCG was positive in primary and metastatic renal cell carcinomas (Skala et al., 2020). Here, we found that RHCG was closely related to the progression of EC.

In this study, through TCGA database, the differential expression of RHCG in EC tissues and normal tissues was found. The data of 581 clinical cases including 546 tumor and 35 normal samples were analyzed, and it was evidenced that RHCG was indeed highly expressed in EC. More interestingly, the expression of RHCG is linked to the prognosis of patients. At the same time, qPCR and western blot experiments were performed to obtain clinical samples, which further confirmed the high expression of RHCG in EC tissues. On the other hand, elevated RHCG was

Table 3. Relationship between RHCG expression and tumor characteristics in patients with endometrioid adenocarcinomas.

Features	No. of patients	RHCG expression low	RHCG expression high	P value
All patients	45	23	22	
Age (years)				0.463
<58	22	10	12	
≥58	23	13	10	
Myometrial invasion				0.037
≤0.5	15	11	4	
>0.5	30	12	18	
Lesion size				0.240
≤1cm	31	14	17	
>1cm	14	9	5	
FIGO stage				0.033
I	21	14	7	
II	13	6	7	
III	11	3	8	
Lymph node metastasis				0.279
-	41	22	19	
+	4	1	3	
Expression of P53				0.468
-	20	9	11	
+	25	14	11	
Expression of MLH1				0.144
-	5	1	4	
+	40	22	18	
Expression of MLH2				0.070
-	3	0	3	
+	42	23	19	
Expression of MLH6				0.322
-	4	3	1	
+	41	20	21	
Expression of PMS2				0.638
-	7	3	4	
+	38	20	18	

-: negative; +: positive.

Table 4. Relationship between RHCG expression and tumor characteristics in patients with endometrioid adenocarcinomas.

		RHCG
FIGO stage	Spearman correlation	0.321
	Signification (double-tailed)	0.031
	N	45
Myometrial invasion	Spearman correlation	0.314
	Signification (double-tailed)	0.035
	N	45
Expression of P53	Spearman correlation	-0.109
	Signification (double-tailed)	0.475
	N	45
Expression of MLH1	Spearman correlation	-0.220
	Signification (double-tailed)	0.146
	N	45
Expression of MLH2	Spearman correlation	-0.273
	Signification (double-tailed)	0.069
	N	45
Expression of MLH6	Spearman correlation	0.149
	Signification (double-tailed)	0.328
	N	45
Expression of PMS2	Spearman correlation	-0.071
	Signification (double-tailed)	0.644
	N	45

also observed in EC cell lines. Then, the EC cell models with RHCG depletion were generated via infecting lentiviral vector targeting RHCG, and the biological activities of the cells were measured. As shown in the above data, after RHCG knockdown, the proliferation and migration levels of KLE and HEC-1-B cell lines were decreased, the apoptosis ability was increased, and the tumorigenesis was impaired, further confirming that RHCG has an important promoting role in the progression of EC and could be used as a new target in clinical diagnosis and targeted therapy in the future. In addition to EC, the involvement of RHGC in other uterine cancers, such as cervical cancer, has been also revealed. For instance, RHCG was decreased in cervical cancer when compared with that in normal cervical tissues, and downregulated in cervical cancer cell lines. At the mechanism level, RHCG blocked cervical cancer progression through inhibiting migration and inducing apoptosis regulated by TGF-β1 (Wang et al., 2018). As for the oncogenic mechanism of RHCG in EC, we will focus on it in subsequent studies.

In conclusion, this current study demonstrated that RHCG was highly expressed in EC. More importantly, it is clear that RHCG plays an important role in promoting malignant proliferation of EC, enhancing the ability of migration, and anti-apoptotic activity. RHCG might be a promising target in the treatment of EC.

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