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Clinical and prognostic significance of CCPG1 in hepatocellular carcinoma

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Abstract. This study aimed to examine the clinical and prognostic significance of cell-cycle progression gene 1 (CCPG1) in hepatocellular carcinoma (HCC). We firstly analyzed CCPG1 expression in various cancers using The Cancer Genome Atlas and the Genotype-Tissue Expression project databases. The relative expression levels of CCPG1 were determined in 164 paired HCC and adjacent tissues using immunohistochemistry. The correlation between CCPG1 and clinicopathological characteristics of HCC was analyzed. Cox proportional models were used to identify the prognostic factors for overall survival (OS) and disease-free survival (DFS). The expression of CCPG1 was lower in HCC tissues than in adjacent non-tumor liver tissues. The expression of CCPG1 was significantly correlated with tumor number ($p = 0.02$) and tumor differentiation ($p = 0.04$) in HCC. Lower expression of CCPG1 in HCC patients was associated with poor OS and DFS ($p < 0.01$). Relative low expression of CCPG1 in HCC is significantly correlated with the poor prognosis of HCC patients after surgical resection, suggesting its possible role as a potential prognostic marker for HCC.

Key words: CCPG1 — Hepatocellular carcinoma — Overall survival — Disease-free survival

Abbreviations: AFP, alpha-fetoprotein; CCPG1, cell-cycle progression gene 1; DFS, disease-free survival; ER, endoplasmic reticulum; GEO, gene expression omnibus; GTEx, the genotype-tissue expression project; HCC, hepatocellular carcinoma; LICH, liver hepatocellular carcinoma; OS, overall survival; PBS, phosphate buffered saline; TCGA, the caner genome atlas; TNM, tumor-node-metastasis.

Introduction

Hepatocellular carcinoma (HCC), ranks as the sixth most commonly diagnosed cancer (Torre et al. 2015), is the fourth leading cause of cancer-related mortality worldwide (Bray et al. 2018). To date, great advances have been made in the treatment of HCC, however, the prognosis in patients with intermediate or advanced HCC is still poor with a 5-year survival rate of less than 18% (Jemal et al. 2017). Uncontrolled cell proliferation and metastasis are the major causes affecting the treatment outcome of HCC (Allemani et al. 2015; Reeves and Aisen 2015). Previously, some HCC biomarkers including alpha-fetoprotein (AFP), glypican-3, osteopontin, and Golgi protein-73 are considered to be beneficial for the diagnosis and prognostic prediction (Tsuchiya et al. 2015; Cao et al. 2019), however, their prognostic efficiencies are still limited. Therefore, it is necessary to develop some biomarkers contributing to the prediction of the prognosis among the HCC patients.

As a single-pass transmembrane protein, cell-cycle progression gene 1 (CCPG1) served as a new reticulophagy car-

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go receptor embedding in the endoplasmic reticulum (ER) (Smith et al. 2018). It involves diverse biological processes by manipulating ER homeostasis (Lahiri and Klianski 2018; Smith et al. 2018; Smith and Wilkinson 2018). Furthermore, increasing evidence indicates that ER stress is closely related to the pathogenesis and progression of cancer, by modulating the protein turnover and autophagy (Li et al. 2019; da Silva et al. 2020). Currently, there are four major mammalian receptors (i.e. FAM134B, SEC62, RTN3 and CCPG1) identified to be associated with the ER autophagy. Among these receptors, FAM134B, SEC62 and RTN3 have been reported to be closely related to the pathogenesis of HCC (Lin et al. 2017; Du et al. 2019; Zhang et al. 2019). These lead us to investigate the possible clinicopathological and prognostic significance of CCPG1 in HCC.

In this study, we investigated the significance of CCPG1 expression and its feasibility in predicting the prognosis of HCC. Firstly, we compared the CCPG1 expression in HCC tissues and adjacent normal liver tissues. Secondly, we evaluated the correlation between CCPG1 and clinicopathological characteristics of HCC. Thirdly, Cox regression analysis was used to identify the prognostic factors for overall survival (OS) and disease-free survival (DFS) in HCC patients.

Materials and Methods

Gene expression data

The expression difference of CCPG1 between tumor and adjacent normal tissues for the different tumors were analyzed using The Caner Genome Atlas (TCGA) data obtained from the Tumor Immune Estimation Resource (TIMER2, Version 2) (http://timer.cistrome.org/). Furthermore, the expression data from The Genotype-Tissue Expression project (GTEx) were combined with TCGA data, in order to extend the analysis to more cancer types. To validate the differential expression of CCPG1 between HCC and normal tissues, we further retrieved three datasets from Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo/) under accession number of GSE84598, GSE39791, GSE36376, respectively. Moreover, Kaplan-Meier plotter (https://kmplot.com/analysis) was utilized to assess the prognostic significance of CCPG1 expression in HCC (Menyhárt et al. 2018).

Patients and samples

In total, 164 paired HCC samples and adjacent tissue samples were prospectively collected from the patients underwent hepatic resection in our hospital between May 2013 and May 2016. Tumor differentiation was assessed using Edmondson's classification. Clinical staging was performed according to the American Joint Committee on Cancer Staging manual (7^{th} edition). Those received radiotherapy, chemotherapy or immunotherapy before hepatic resection, or those lacking of integrated clinicopathological and follow-up data were excluded from this study. The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the Institutional Medical Ethics Committee of Shandong Provincial Qianfoshan Hospital [No. YXLL-KY-2015(009)]. Informed consent has been obtained from all individuals included in this study.

The resected specimens were immediately frozen in liquid nitrogen and stored at −80°C. The clinical and pathological parameters were collected from each patient including age, gender, tumor size, number of tumor nodules, histologic grade, tumor-node-metastasis (TNM) stage, vascular invasion, cirrhosis, hepatitis B virus (HBV) infection, and serum AFP.

Immunohistochemistry

Immunohistochemistry was carried out according to the previous description (Xu et al. 2018). Briefly, the paraffin-embedded HCC sections (4 μm) were placed in an oven at 65°C for 2 h, and then were deparaffinized in xylene and hydrated through a series of graded ethanol. The antigen retrieval was done in sodium citrate buffer (0.01 M, pH 6.0) at 97°C for 15 min in a micro-wave oven. The slides were cooled down for 40 min and rinsed with phosphate buffered saline (PBS, pH 7.4) for 3 min. Subsequently, the sections were immersed in 3% hydrogen peroxide for 10 min to block the endogenous peroxidase activity. Goat normal non-immune serum was used to reduce non-specific binding after incubating for 30 min. Then the sections were incubated with a primary antibody against CCPG1 (1:200, ARP46493-P050, Aviva Systems, USA) at 4°C overnight. Then the slides were incubated with a biotinylated secondary antibody at 37°C for 30 min, followed by staining with 3,3-diaminobenzidine (DAB) after washing with PBS thrice. Finally, the sections were counterstained with hematoxylin, and observed under a microscope (BX53, Tokyo, Japan). Sections incubated with PBS rather than anti-IMPDH2 antibody served as negative control.

Semi-quantitative analysis of CCPG1

Expression of CCPG1 was estimated by semi-quantitative analysis. Staining intensity was recorded as 0 (negative), 1 (weak), 2 (moderate) and 3 (strong), respectively. For the percentage of positive cells, scores were marked as 0 (<5%), 1 (6–25%), 2 (26–50%), 3 (51–75%), and 4 (>76%), respectively. The final immunohistochemistry staining score was

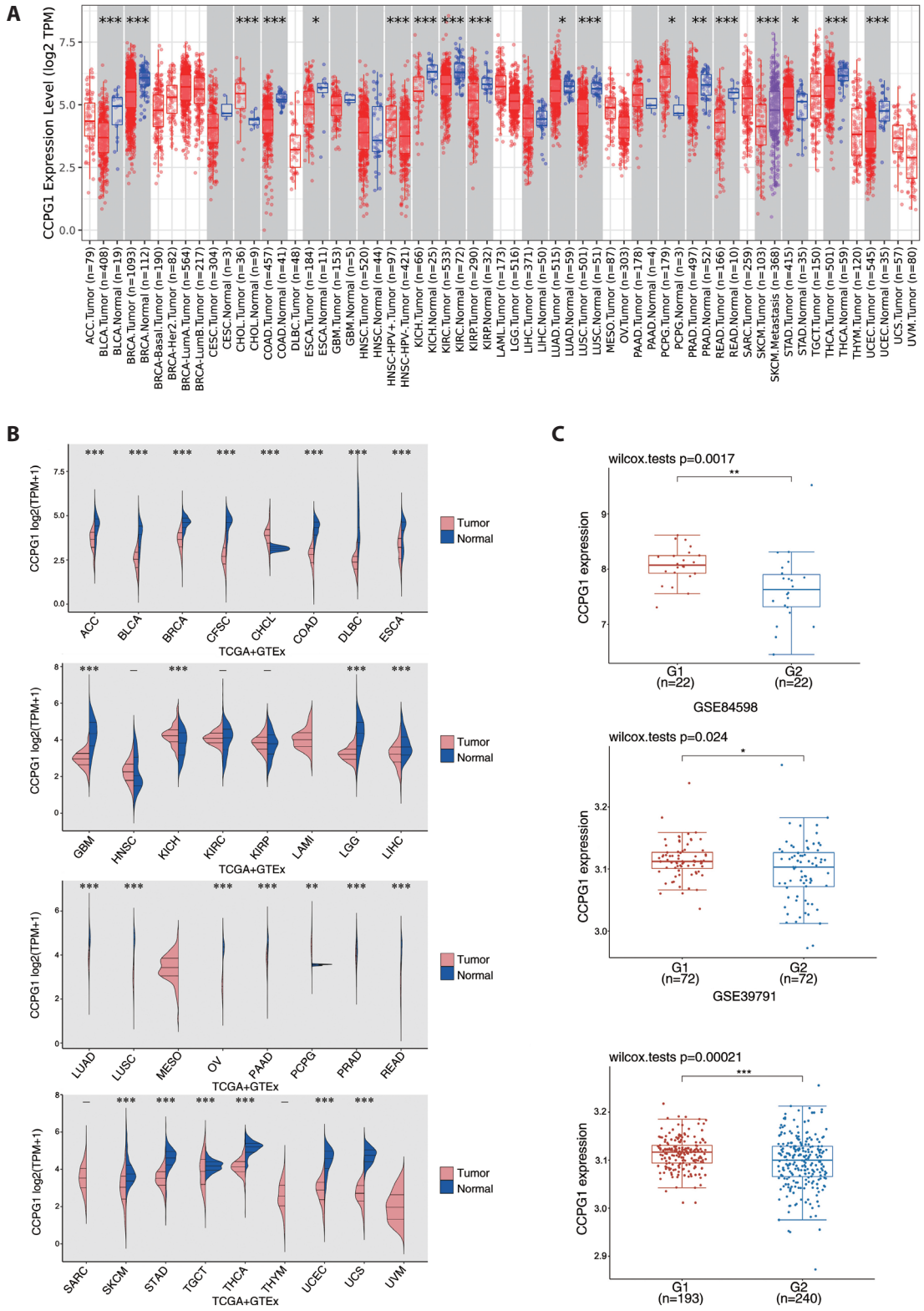

Figure 1. Expression of CCPG1 in different cancers. **A.** Expression of the CCPG1 in different cancers was analyzed through TIMER2. **B.** CCPG1 expression in different cancers from the GTEx database and TCGA database. **C.** Boxplots indicated the expression of CCPG1 in HCC and normal controls from three GEO datasets (i.e. GSE84598, GSE39791, GSE36376). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. G1, peritomoral tissues; G2, HCC tissues.

determined by multiplying the staining intensity score and positive proportion. Finally, the patients were categorized into high CCPG1 group with a score of ≥6 or low CCPG1 group with a score of <6, based on the scores that were independently determined by two professional pathologists blinded to this study. The observation of staining results by microscope and H scores were evaluated as described previously (Liu et al. 2019).

Table 1. Clinicopathological characteristics of 164 HCC patients

Variables	*n* (%)
Sex	
Male	141 (85.98)
Female	23 (14.02)
Age (years)	
≤50	47 (28.66)
>50	117 (71.34)
AFP	
≤200ug/ml	48 (29.27)
>200ug/ml	42 (25.61)
Liver cirrhosis	
No	49 (29.88)
Yes	115 (70.12)
TNM staging	
I–II	94 (57.32)
III–IV	70 (42.68)
Tumor size (cm)	
≤5	80 (48.78)
>5	84 (51.22)
Tumor number	
1	128 (78.05)
>1	36 (21.95)
Lymph node metastasis	
Negative	3 (1.83)
Positive	161 (98.17)
Tumor differentiation	
I–II	122 (74.39)
III–IV	42 (25.61)
Vascular invasion	
Negative	155 (94.51)
Positive	9 (5.49)
Bile duct thrombi	
Negative	146 (89.02
Positive	18 (10.98)
Distant metastasis	
Yes	32 (19.51)
No	132 (80.49)
CCPG1 expression	
High	77 (46.95)
Low	87 (53.05)

Follow-up

Each patient was followed up by telephone or email provided in the medical records. The follow-up for all the patients was completed in May 2019.

Statistical analysis

SPSS version 19.0 software and GraphPad Prism 7.0 software were used for the data analysis. Chi square test was performed to analyze the correlation between CCPG1 expression and clinicopathological characteristics of HCC. Kaplan-Meier analysis was used to calculate the OS and DFS curves. The log-rank test was used to compare the survival rates between the groups with high or low CCPG1 expression. Cox proportional hazards regression model, univariate and multivariate analyses were conducted to identify the prognostic factors including CCPG1 for OS and DFS in HCC patients, which were indicated by hazard ratios (HRs) with 95% confidence intervals (CIs). $p < 0.05$ was considered to be statistically significant.

Results

CCPG1 expression in human pan-cancer

We first analyzed CCPG1expression in 33 kinds of tumors and adjacent normal tumor tissues using TIMER2 approach. As shown in Figure 1A, compared with the corresponding control tissues, the expression of CCPG1 was lower in bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), thyroid carcinoma (THCA), and uterine corpus endometrial carcinoma (UCEC). Moreover, the high expression of CCPG1 was found in cholangio carcinoma (CHOL), pheochromocytoma and paraganglioma (PCPG) and stomach adenocarcinoma (STAD) (all $p < 0.05$). Second, GTEx and TCGA data showed that CCPG1 expression levels were significantly down-regulated in adrenocortical carcinoma (ACC), BLCA, BRCA, CESC, COAD, DLBC, ESCA, glioblastoma multiforme (GBM), brain lower grade glioma (LGG), liver hepatocellular carcinoma (LICH), LUAD, LUSC, ovarian serous cystadenocarcinoma (OV), pancreatic adenocarcinoma (PAAD), PRAD, READ, SKCM (skin cutaneous melanoma), STAD (stomach adenocarcinoma), TGCT (testicular germ cell tumors), THCA, UCEC and UCS (uterine carcinosarcoma) tumor tissues. The expression of CCPG1 was high in CHOL, PCPG and

KICH (all $p < 0.05$) (Fig. 1B). Moreover, we confirmed the remarkable down-regulation of CCPG1 in HCC in three GEO datasets (GSE84598, GSE39791, GSE36376) (Fig. 1C).

Clinicopathological characteristics of HCC cases and CCPG1 expression

The clinicopathologic characteristics of 164 patients (male: 141; female: 23) were presented in Table 1. The median age was 57 years (18–77 years). Among the 164 HCC samples, 87 (53.05%) showed low expression of CCPG1, and 77 (46.95%) showed high CCPG1 expression. Consistent with TCGA network results, immunohistochemistry findings indicated that CCPG1 expression was lower in HCC tissues than that in paired adjacent tissues (Fig. 2A,B). For the semi-quantitative analysis, the expression of CCPG1 was significantly lower in HCC tissues than that in peritumoral tissues based on the score ($p < 0.05$, Fig. 2C).

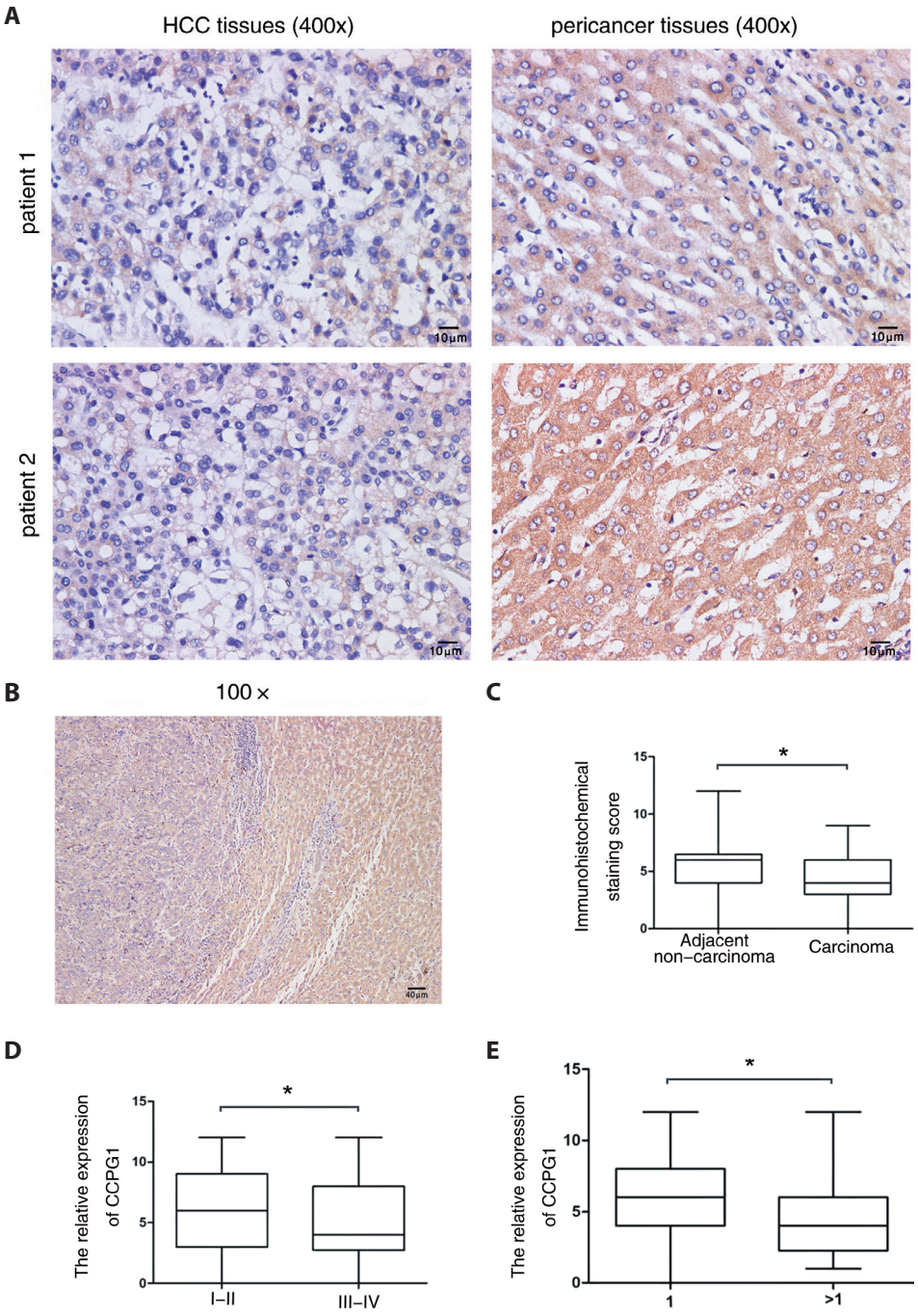

Figure 2. Low expression of CCPG1 in human HCC tissues compared with the levels in adjacent normal liver tissues. **A.** Protein expression of CCPG1 in HCC tissues and adjacent tissues determined by immunohistochemistry, captured under a magnification of 400× (scale bar 10 μm). **B.** CCPG1 expression in the paired adjacent tissues and HCC tumor tissue under a magnification of 100× (scale bar 40 μm). **C.** The statistical analysis of immunohistochemical staining score between HCC and adjacent tissues by Wilcoxon test ($p < 0.05$), * $p < 0.05$. **D.** CCPG1 in HCC tissues with high differentiation (I, II) was significantly higher than that in HCC tissues with low differentiation (III, IV) ($p = 0.04$), * $p < 0.05$. **E.** CCPG1 in HCC tissues with one tumor lesion was significantly higher than that in HCC tissues with at least one tumor lesions ($p = 0.02$), * $p < 0.05$.

Correlation between clinicopathological characteristics and CCPG1 expression

The correlation between characteristics and CCPG1 expression was listed in Table 2. CCPG1 expression was significantly correlated with tumor differentiation (p = 0.04) and number of lesions (p = 0.02). The expression of CCPG1 in HCC tissues was higher in the patients with poor tumor differentiation and less tumor number (p < 0.05, Fig. 2D,E).

Efficiency of CCPG1 in predicting the prognosis of HCC patients

We initially used Kaplan-Meier plotter (https://kmplot.com/analysis) to assess the prognostic significance of CCPG1

Table 2. Correlation between CCPG1 expression and clinicopathologic characteristics in 164 HCC patients

Variables	High CCPG1 expression *n* (%)	Low CCPG1 expression *n* (%)	χ^2	*p*
Sex			0.29	0.59
Male	65 (46.1)	76 (53.9)		
Female	12 (52.2)	11 (47.8)		
Age (years)			3.07	0.08
≤50	17 (36.2)	30 (63.8)		
>50	60 (51.3)	57 (48.7)		
AFP			1.43	0.23
≤200 μg/ml	30 (62.5)	18 (37.5)		
>200 μg/ml	21 (50.0)	21 (50.0)		
Liver cirrhosis			0.47	0.49
No	21 (42.9)	28 (57.1)		
Yes	56 (48.7)	59 (51.3)		
TNM			0.13	0.72
I–II	43 (45.7)	51 (54.3)		
III–IV	34 (48.6)	36 (51.4)		
Tumor size (cm)			0.58	0.44
≤5	40 (50.0)	40 (50.0)		
>5	37 (44.0)	47 (56.0)		
Tumor number			4.98	0.02
1	66 (51.6)	62 (48.4)		
>1	11 (30.6)	25 (69.4)		
Lymph node metastasis			0.23	0.63
Negative	1 (33.3)	2 (66.7)		
Positive	76 (47.2)	85 (52.8)		
Tumor differentiation			4.21	0.04
I–II	63 (51.6)	59 (48.4)		
III–IV	14 (33.3)	28 (66.7)		
Vascular invasion			0.03	0.88
Negative	73 (47.1)	82 (52.9)		
Positive	4 (44.4)	5 (55.6)		
Bile duct thrombi			0.53	0.47
Negative	70 (47.9)	76 (52.1)		
Positive	7 (38.9)	11 (61.1)		
Distant metastasis			0.01	0.99
Yes	15 (46.9)	17 (53.1)		
No	62 (47.0)	70 (53.0)		

High CCPG1 expression: total number of patients 77; Low CCPG1 expression: total number of patients 87.

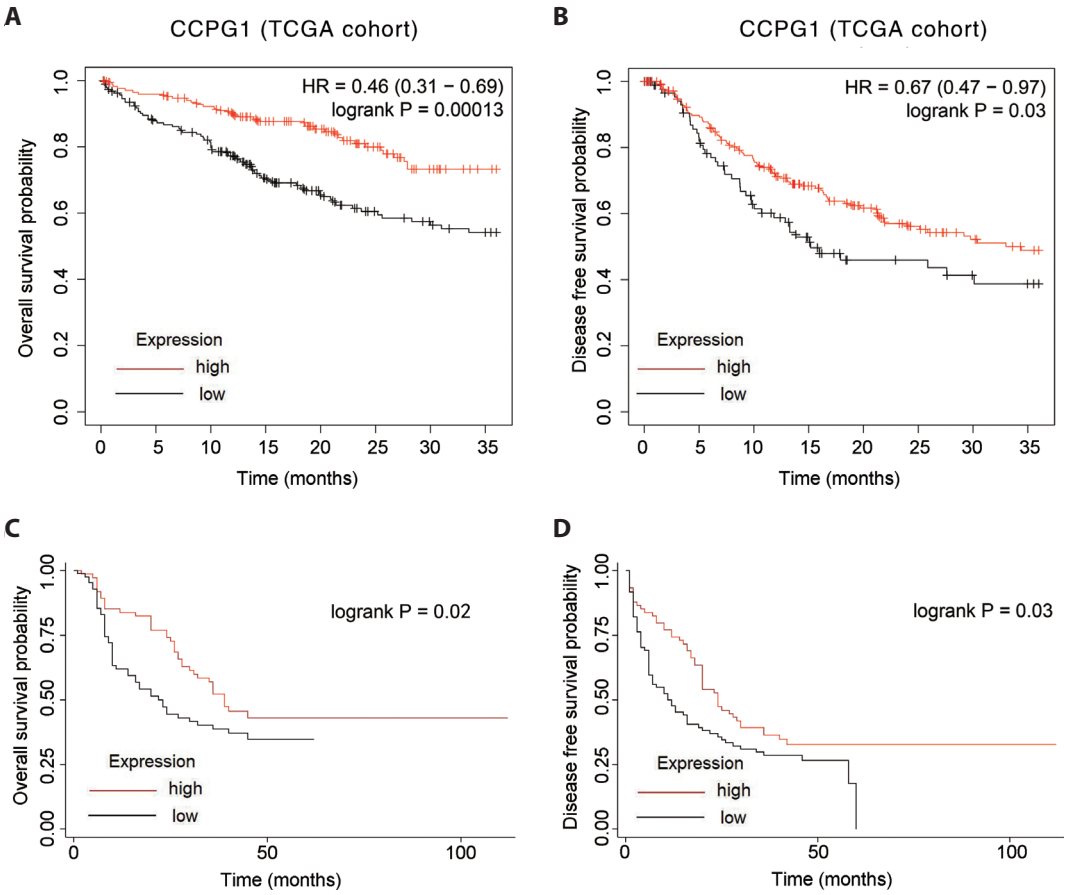

Figure 3. Prognostic value of CCPG1 expression in HCC patients. **A., B.** Kaplan-Meier curves for HCC patients in the TCGA cohort. Low CCPG1 expression was correlated with poor prognosis in HCC. **C.** Kaplan-Meier plots of the OS in 164 HCC patients indicated that low CCPG1 expression had a worse OS than those with high CCPG1 expression. **D.** Patients with low CCPG1 expression had a worse DFS than those with high CCPG1 expression.

expression in HCC. As shown in the Kaplan-Meier curves, CCPG1 expression was significantly associated with the OS and DFS in HCC patients. Moreover, the patients with down-regulation of CCPG1 transcript level had a poor OS and DFS (Fig. 3A,B).

In our clinical data, the 3-year OS rate was 47.6%, and the 3-year DFS rate was 32.0%. Kaplan-Meier analysis by the log-rank test showed that patients with high CCPG1 expression had a longer OS ($p = 0.02$) and DFS ($p = 0.03$) compared with those with low CCPG1 expression (Fig. 3C,D).

Multivariate Cox analysis indicated that low CCPG1 expression (HR = 1.55, 95% CI = 1.01–2.38, $p = 0.04$), larger tumor size (HR = 1.93, 95% CI = 1.24–2.99, $p < 0.01$), advanced TNM stage (HR = 2.35, 95% CI = 1.43–3.88, $p < 0.01$) and poor tumor differentiation (HR = 2.27, 95% CI = 1.44–3.56, $p < 0.01$) were independently associated with poor OS (Table 3). Additionally, low CCPG1 expression (HR= 1.61, 95% CI = 1.07–2.41, $p = 0.02$), larger tumor size (HR = 1.99, 95% CI = 1.34–2.95, $p < 0.01$), advanced TNM stage (HR = 2.41 95% CI = 1.48–3.92, $p < 0.01$) and poor tumor differentiation (HR = 1.72, 95% CI = 1.12–2.62, $p = 0.01$) were independent factors for poor DFS (Table 4).

Discussion

To date, four major mammalian receptors have been identified to be associated with the ER autophagy, including, FAM134B, SEC62, RTN3 and CCPG1. Among these receptors, some have been reported to be closely related to the pathogenesis of HCC. Thus, we aimed to investigate the potential role of CCPG1 in the pathogenesis and prognosis evaluation of HCC. Our pan-cancer analysis indicated that the expression of CCPG1 was remarkably down-regulated in most cancers. CCPG1 expression level in HCC was obviously down-regulated in TCGA and GTEx databases. It is noteworthy that the remarkable down-regulation was also observed in three independent HCC datasets from GEO.

We also collected 164 pairs of clinicopathological data of HCC and its adjacent tissues to analyze the relationship between CCPG1 expression and clinicopathological factors and prognosis. Our data showed that there was low expression of CCPG1 in 53.05% of HCC samples, which is significantly lower than that of the paired adjacent tissue. Meanwhile, CCPG1 expression was correlated with the grade of tumor differentiation and the number of tumor lesions. The expression of CCPG1 was significantly lower in HCC tissues with advanced tumor differentiation compared with those with early tumor differentiation. In addition, the expression of CCPG1 in HCC tissues was

Table 3. Cox regression analysis for overall survival in HCC patients

Variables	*n*	Univariable analysis		Multivariable analysis	
		HR (95% CI)	*p*	HR (95% CI)	*p*
Gender					
Male	141	1			
Female	23	1.13 (0.63–2.03)	0.69		
Age (years)					
≤50	47	1			
>50	117	1.11 (0.70–1.76)	0.66		
Tumor size (cm)					
≤5	80	1		1	
>5	84	2.43 (1.59–3.72)	<0.01	1.93 (1.24–2.99)	<0.01
Tumor number					
1	128	1			
>1	36	1.51 (0.94–2.41)	0.09		
TNM stage					
I–II	94	1		1	
III–IV	70	2.78 (1.82–4.24)	<0.01	2.35 (1.43–3.88)	<0.01
AFP (ng/ml)					
≤200	48	1			
>200	42	1.31 (0.75–2.27)	0.34		
Liver cirrhosis					
Negative	49	1			
Positive	115	0.78 (0.50–1.21)	0.26		
Vascular invasion					
Negative	155	1			
Positive	9	1.98 (0.80–4.88)	0.13		
Bile duct thrombi					
Negative	146	1			
Positive	18	1.26 (0.65–2.44)	0.49		
Lymph node metastasis					
Negative	161	1			
Positive	3	1.46 (0.36–5.93)	0.6		
CCPG1					
High	77	1		1	
Low	87	1.59 (1.05–2.41)	0.03	1.55 (1.01–2.38)	0.04
Distant metastasis					
No	132				
Yes	32	2.28 (1.40–3.71)	<0.01		
Tumor differentiation					
I–II	122			1	
III–IV	42	2.49 (1.60–3.87)	<0.01	2.27 (1.44–3.56)	<0.01

HR, hazard ratios; CI, confidence interval.

higher in the patients with less tumor lesions. We hypothesized that CCPG1 may inhibit the proliferation of HCC, which in turn affected the prognosis of HCC. Regarding the prognostic significance of CCPG1, HCC patients with high CCPG1 expression had prolonged OS and DFS in the Kaplan-Meier analysis. According to Cox regression analysis, low expression of CCPG1 was an independent predictor for poor prognosis of HCC patients, which was consistent with the results from Kaplan-Meier plotter (https://kmplot.com/analysis). Overall, the present study demonstrated that CCPG1 had substantially decreased expression in HCC compared with corresponding normal

Table 4. Cox regression analysis for disease-free survival in HCC patients

Variables	*n*	Univariable analysis		Multivariable analysis	
		HR (95% CI)	*p*	HR (95% CI)	*p*
Gender					
Male	141	1			
Female	23	1.19 (0.72–1.98)	0.49		
Age (years)					
≤50	47	1			
>50	117	1.01 (0.67–1.51)	0.98		
Tumor size (cm)					
≤5	80	1			
>5	84	2.44 (1.67–3.58)	<0.01	1.99 (1.34–2.95)	<0.01
Tumor number					
1	128	1			
>1	36	1.53 (1.01–2.33)	0.05		
TNM stage					
I–II	94	1			
III–IV	70	2.79 (1.90–4.09)	<0.01	2.41 (1.48–3.92)	<0.01
AFP (ng/ml)					
≤200	48	1			
>200	42	1.36 (0.84–2.23)	0.22		
Liver cirrhosis					
Negative	49	1			
Positive	115	0.73 (0.49–1.09)	0.12		
Vascular invasion					
Negative	155	1			
Positive	9	1.86 (0.81–4.23)	0.14		
Bile duct thrombi					
Negative	146	1			
Positive	18	1.64 (0.95–2.83)	0.08		
Lymph node metastasis					
Negative	161	1			
Positive	3	3.09 (0.97–9.88)	0.06		
Distant metastasis					
No	132	1			
Yes	32	2.61 (1.69–4.02)	<0.01		
Tumor differentiation					
I–II	122	1			
III–IV	42	1.93 (1.27–2.92)	<0.01	1.72 (1.12–2.62)	0.01
CCPG1 expression					
High	77	1			
Low	87	1.49 (1.02–2.16)	0.03	1.61 (1.07–2.41)	0.02

HR, hazard ratios; CI, confidence interval.

tissue, and its down-regulation adversely impacted patient outcome.

As a novel scaffold protein, CCPG1 was reported to clearly restrict Dbs exchange toward RhoA by binding to the Dbl homology/pleckstrin homology domain tandem motif (Kostenko et al. 2006). Also, it can interact with additional Rho GEF family members such as Rho, the Rho family member Cdc42, and the regulatory kinase Src (Kostenko et al. 2006). It has been well acknowledged that down-regulation of Rho GEF family (e.g. Dbs) involves in tumorigenic proliferation and invasion (Whitehead et al. 1997). In addition, Rho proteins are directly involved in cancer pathways, especially cellular migration and invasion (Khosravi-Far et al. 1995; Qiu et al. 1995a, 1995b; Qiu et al. 1997). Indeed, overexpression of RhoA was reported in HCC patients, and was associated with poor prognosis in these patients (Li et al. 2006; Xiaorong et al. 2008). According to the previous description, CCPG1 expression in cultured cells was correlated with the activation of endogenous RhoA mediated by Dbs (Kostenko et al. 2006). Therefore, we speculated that CCPG1 may inhibit the RhoA activity, which then participated in suppressing the oncogenic and metastatic potential of tumor cells. Our data showed that CCPG1 expression was significantly correlated with the tumor differentiation and the number of tumor lesions. Also, patients with high CCPG1 level had better OS and DFS compared with those with low CCPG1 expression. Thus, we assumed that CCPG1 may modulate the activity of RhoA by regulating the cycle of Rho GTP-GDP, which subsequently affected the progression of HCC.

Interestingly, CCPG1 was considered as a non-canonical autophagy cargo receptor that was essential for the process of ER-phagy (Smith et al. 2018). ER-phagy referred to the conserved macroautophagic and/or autophagic degradation of ER responding to general nutrient deprivation or ER stress. Among the four main mammalian ER-phagy receptors (i.e. FAM134B, SEC62, RTN3 and CCPG1), FAM134B, SEC62 and RTN3 have been reported to closely involve in the physiology of liver function in normal individuals and the pathogenesis of patients with liver diseases (Weng et al. 2012; Wu et al. 2014; Li et al. 2017; Zhang et al. 2019). As a novel receptor for mammalian ER-phagy, most of the studies on CCPG1 are focusing on its roles in ER stress and ER-phagy. For instance, it could interact with core components involving in autophagy, and regulate the reticulophagy (Smith et al. 2018). In addition, it was closely related to the maintenance of ER homeostasis under physiological and stress conditions. Moreover, it could participate in the ER luminal proteostasis in the exocrine pancreas, which then contributed to the normal function of pancreatic acinar cells (Smith et al. 2018). To our best knowledge, no studies have been conducted to investigate the efficiency of CCPG1 in the evaluation of HCC prognosis. Our data showed that CCPG1 expression in HCC tissues was significantly lower than that of the adjacent tissues. Besides, CCPG1 expression was an independent prognostic factor for postoperative OS and DFS among HCC patients. We speculated that CCPG1 up-regulation may provide sufficient energy to the hepatic cancer cells and improve the viability of cancer cells in the presence of conditions of hypoxia and low nutrient.

There were some limitations for our study. First, this is a retrospective, single-institution study involving a relatively small sample size. In future, a prospective and well-designed study with a large sample size of HCC is needed. Second, we could not elucidate the relationship between down-regulation of CCPG1 and poor prognosis of in HCC patients. Second, more *in vivo* and *in vitro* studies should be performed in the future to elucidate why the down-regulated expression of CCPG1 in HCC is associated with unfavorable prognosis.

Conclusions

To our knowledge, the present study firstly investigated the association between CCPG1 expression and the prognosis of HCC patients. The expression of CCPG1 in HCC tissues was correlated with the number of tumor lesions and the tumor differentiation. Besides, low CCPG1 expression was significantly correlated with poor prognosis of HCC patients after surgery. This suggested its potential roles in the invasion and proliferation of HCC, as well as the feasibility of prognostic marker for HCC.

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Data availability statement. All data generated or analyzed during this study are included in this published article.

Conflict of interest. Authors state no conflict of interest.

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