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Connection Between *CDC20* Expression and Hepatocellular Carcinoma Prognosis

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Background: Hepatocellular carcinoma (HCC) occurs frequently in China, with high morbidity and mortality. Cell division cycle 20 homolog (*CDC20*) is reportedly related to many cancers. In this study, we discuss a potential link of *CDC20* expression to HCC patients' prognoses.

Material/Methods: Quantitative real-time polymerase chain reaction (qRT-PCR) was performed to assess *CDC20* expression in HCC and the paired noncancerous tissues. Chi-square analysis was used to assess potential association of *CDC20* expression with clinicopathologic profiles among HCC patients. The overall survival for HCC patients with different *CDC20* expressions was assessed using the Kaplan-Meier method. To evaluate the prognostic value for HCC patients, Cox regression analyses were performed.

Results: The expression of *CDC20* was elevated among HCC specimens compared with adjacent noncancerous ones ($P<0.05$). The expression of *CDC20* was significantly related to differentiation ($P<0.001$), tumor node metastasis stage ($P<0.001$), and lymphatic metastasis ($P<0.001$). Moreover, HCC patients with high *CDC20* expression had dismal overall survival rates compared with low *CDC20* expression ($P<0.05$). *CDC20* alone could forecast HCC prognoses according to multivariable Cox regression analysis (hazard ratio=2.354, 95% confidence interval=1.177-4.709, $P=0.016$).

Conclusions: Overexpressed *CDC20* may act as a reliable biomarker for dismal prognoses among HCC patients.

Keywords: Carcinoma, Hepatocellular • Cdc20 Proteins • Prognosis

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Background

Hepatocellular carcinoma (HCC) is a primary histologic type of liver malignancy in humans. The incidence of HCC is fifth in the world and it has become the third leading cause of cancer-related death in the world [1,2]. The incidence and mortality for HCC show an annual upward tendency in China. The carcinogenesis of HCC is a complex process regulated by various genes, including suppressor genes and oncogenes. Environmental factors can lead to a high incidence of HCC, too, such as hepatitis B virus/hepatitis C virus infection, cirrhosis, and alcoholic liver disease [3-5]. With surgery and adjuvant treatment technologies, the treatment of HCC has made great progress. However, because of the high recurrence and metastasis rates, the 5-year survival of HCC patients is still very low [6,7]. Thus, there is an urgent need to find novel and effective biomarkers for the prognosis of HCC.

The cell division cycle 20 homolog (*CDC20*) is a cell-cycle checkpoint control factor that can bind with Cdh1 directly to activate the anaphase-promoting complex (APC) [8,9]. *CDC20* assumes crucial functions of cells in anaphase of mitosis [10,11]. Hence, its dysregulation may have considerable impacts on cellular growth and oncogenesis [12]. Reportedly, overexpressed *CDC20* may be correlated with an unsuitably acting spindle assembly checkpoint [13,14]. More and more research has revealed that *CDC20* is a carcinogen that promotes cancer development. Recent reports have unveiled that *CDC20* expression is upregulated in various malignancies such as breast cancer, HCC, cervical cancer, prostate cancer, and gastric cancer [15-19]. Li et al [20] found that *CDC20* was elevated in tumor tissues and positively related to sex, tumor differentiation, and tumor node metastasis (TNM) stage of HCC. *CDC20* could also promote the proliferation of HCC [9,20]. Therefore, we hypothesized that *CDC20* might play an important role in the prognosis of HCC. However, few studies have reported the prognostic role of *CDC20* in HCC until now.

We investigated the potential correlation of clinicopathologic characteristics with *CDC20* expression and assessed its potential significance in HCC prognosis.

Material and Methods

Cases and Sample Collection

A total of 139 HCC and paired noncancerous tissue specimens was collected from HCC patients that were all confirmed by pathologists from Harrison International Peace Hospital. We acquired permission from the Ethics Committee of Harrison International Peace Hospital, and written consents were also signed by eligible patients and their families. No patient had undergone chemotherapy or radiotherapy ahead of surgery. Patients diagnosed by histopathologic biopsy as HCC were included in this study. Individuals with other tumors or a history of tumor, liver or kidney diseases, other lymphatic system disorders, or diseases influencing our results were excluded from this study. All the tissues were immediately placed in liquid nitrogen and afterward maintained at -80°C until ribonucleic acid (RNA) was extracted. The patients were followed for up for 5 years; every 3 months in the first year, then every 6 months in the subsequent 2 years, and then annually. The clinicopathologic features are listed in **Table 1**, including age, sex, tumor size, differentiation, TNM stage, and lymphatic metastasis.

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

Total RNA was isolated with Trizol reagent (Invitrogen) following the manufacturer's instructions. The first-strand complementary deoxyribonucleic acid (cDNA) was synthesized by TransScript and cDNA synthesis kit from TransGen Biotech (Beijing, China). In our study, RT-PCR was completed with Bio-Rad iQ5 multicolor real-time PCR detection system (Bio-Rad, Hercules, CA). Expression of *CDC20* was determined relative to that of β-*actin*. The primers of *CDC20* and β-*actin* were as follow:
CDC20 forward 5'-TCGCATCTGGAATGTGTGCT-3' and
reverse 5'-CCCGGGATGTGTGACCTTTG-3',
β-*actin* forward 5'-TGACGTGGACATCCGCAAAG-3' and
reverse 5'-CTGGAAGGTGGACAGCGAGG-3' [20].
The relative expression for *CDC20* among 139 paired samples was normalized against the β-*actin* internal control and calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical Analysis

Data synthesis was accomplished with SPSS software (SPSS 19.0) and GraphPad Prism 5. Expression level of *CDC20* was given as mean±SD. *CDC20* expression was compared by the *t* test. The association of *CDC20* expression with clinicopathologic traits among HCC patients was determined via the chi-square test. The Kaplan-Meier method with the log-rank test was performed to estimate survival rates and the survival differences of the HCC patients. Cox regression analysis explored the influence of *CDC20* expression and the clinicopathologic variables on survival. $P<0.05$ represented statistical significance.

Results

Upregulation of CDC20 in HCC Tissues

qRT-PCR was performed to examine *CDC20* expression in 139 HCC tissues and matched noncancerous tissues. The HCC cases were categorized into high- and low-expression classes.

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Table 1. Association of *CDC20* gene expression with clinicopathologic features of HCC patients.

Features	No. N=139	*CDC20* expression		*P*-values
		Low (n=65)	High (n=74)	
Age (years)				
<60	69	34	35	0.556
≥60	70	31	39	
Sex				
Men	74	33	41	0.585
Women	65	32	33	
Tumor size				
<5 cm	62	30	32	0.731
≥5 cm	77	35	42	
Differentiation				
High	60	43	17	<0.001
Low-moderate	79	22	57	
Tumor node metastasis stage				
I-II	59	40	19	<0.001
III-IV	80	25	55	
Lymphatic metastasis				
Yes	73	23	50	<0.001
No	66	42	24	

Figure 1 shows that *CDC20* expression exhibited an upward tendency among HCC tissues compared with matched non-cancerous ones (*P*<0.05).

Connection of CDC20 Expression with Clinicopathologic Traits of HCC Patients

In addition, we explored a possible link of *CDC20* expression with clinicopathologic traits among HCC patients. As shown in **Table 1**, the results revealed that *CDC20* expression was obviously related to differentiation (*P*=0.003), TNM stage (*P*=0.000), and lymphatic metastasis (*P*=0.001). However, no significant correlation was found with age, sex, and tumor size of the HCC patients (all *P*>0.05).

Correlation of CDC20 Expression with HCC Patients' Prognoses

The potential connection of *CDC20* expression with survival among HCC patients was evaluated by Kaplan-Meier survival analysis. **Figure 2** shows that HCC patients with high *CDC20* expression faced worse overall survival rates than low-expressing ones (log-rank test *P*=0.004). Hence, *CDC20* is a novel and reliable predictor for HCC prognoses.

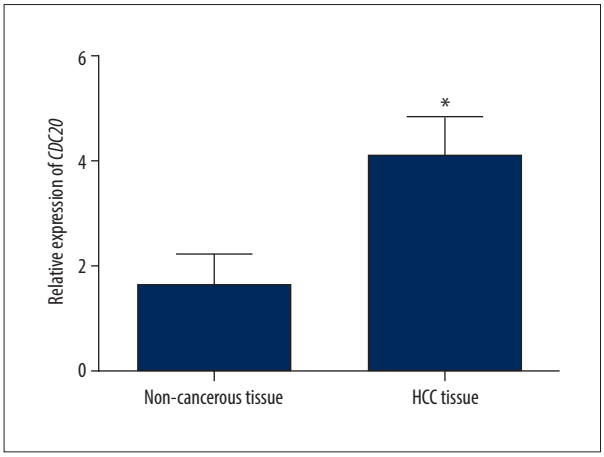

Figure 1. *CDC20* expression level between hepatocellular carcinoma tissues and paired adjacent noncancerous tissues. * *P*<0.05.

Prognosis of CDC20 Expression in HCC Patients

To estimate *CDC20* correlation with HCC patients' prognoses, we performed Cox regression analysis. As listed in **Table 2**, *CDC20* expression alone could signify overall survival among HCC patients (hazard ratio=2.354, 95% confidence interval: 1.177-4.709, *P*=0.016). It indicated that increased expression of *CDC20* had an adverse influence on HCC.

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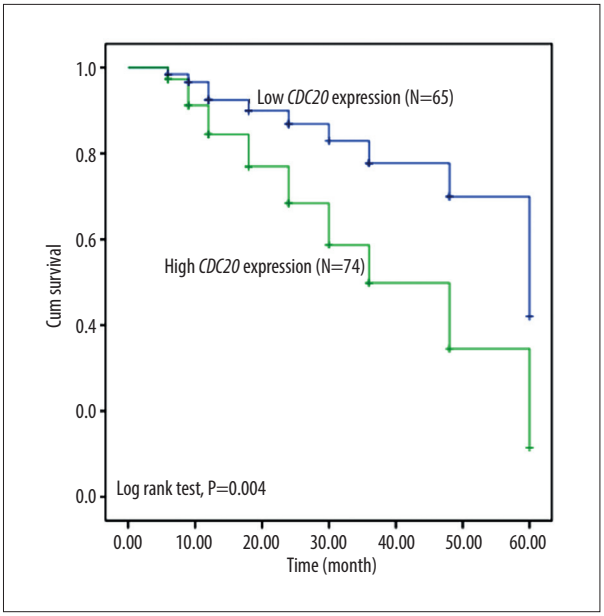

Figure 2. Kaplan-Meier analysis for hepatocellular carcinoma patients based on the expression of *CDC20*.

Discussion

HCC is a type of primary liver cancer and is the fourth most common cancer in men and the fourth leading cause of cancer-related death among men and women in China [21]. Its onset has shown an upward tendency in developed countries in recent years [22]. Because of frequent intrahepatic relapse and frequently associated cirrhosis [23-25], HCC patients have very poor prognoses. It has become a pressing sociomedical problem.

CDC20 influences cell division, and is also a major cofactor for the APC via interaction with A-box, D-Box, or KEN-box, the specific constituents in the substrates [26,27]. In recent years, overexpression of *CDC20* has been found in many human cancers, and is associated with poor prognosis. For example, Kato et al claimed that *CDC20* expression was high among non-small-cell lung cancer tissues and *CDC20* alone could signify disease prognoses [28]. Choi et al demonstrated *CDC20* elevation among urothelial bladder cancer (UBC) cases, and high *CDC20* expression correlated with shorter recurrence-free survival and poorer overall survival in UBC patients [29]. Karra et al reported for the first time a strong connection of high *CDC20* and securin immunoexpression with dismal prognoses among breast cancer patients. The results indicated that *CDC20* might be a promising candidate for clinical application in breast cancer prognostication [30]. This role of *CDC20* has also been found in many other cancers, including pancreatic cancer and colorectal cancer [31,32], as well as in HCC [20]. Li et al investigated the impacts of *CDC20* on HCC progression, and they showed *CDC20* elevation in HCC samples; *CDC20* small interfering RNA transfection in HCC cells could decrease cellular multiplication while raising the cell numbers in G2/M phase [20]. However, few studies have reported the clinical prognostic performance of *CDC20* in HCC cases.

Our results conformed to those from earlier research. We found *CDC20* expression was heightened among HCC tissues compared with matched noncancerous tissues by qRT-PCR. The *CDC20* expression level was significantly related to differentiation, TNM stage, and lymphatic metastasis, but had no significant correlation with age, sex, and tumor size of the patients with HCC. That was in accordance with a previous study. Li et al found that CDC20 was elevated in tumor tissues and positively related to sex, tumor differentiation, and TNM stage of HCC. *CDC20* could promote the proliferation of HCC [9,20]. Kaplan-Meier survival analysis showed that HCC patients with high *CDC20* expression had poorer survival rates than those with low ones. We also explored the prognostic value of *CDC20* expression among HCC patients. Cox regression analysis demonstrated that except for differentiation and lymphatic metastasis, *CDC20* expression alone could signify lower survival

Table 2. Univariable and multivariate Cox regression analyses for *CDC20* in HCC patients.

Characteristics	Univariate analysis		Multivariate analysis	
	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value
CDC20	2.464 (1.245-4.876)	0.010*	2.354 (1.177-4.709)	0.016*
Age	0.670 (0.369-1.219)	0.190	–	–
Sex	0.834 (0.460-1.512)	0.550	–	–
Tumor size	0.863 (0.474-1.573)	0.631	–	–
Differentiation	1.930 (1.007-3.700)	0.048*	–	–
TNM stage	1.304 (0.689-2.469)	0.415	–	–
Lymphatic metastasis	1.944 (1.049-3.602)	0.035*		

HCC – hepatocellular carcinoma; HR – hazard ratio; CI – confidence interval; TNM – tumor node metastasis. * Statistically significant.

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among HCC patients. In other words, overexpression of *CDC20* predicts poor prognoses among HCC patients.

Numerous studies have found *CDC20* acts as an oncoprotein during oncogenesis, and inactivation of *CDC20* might be effective in treating malignancies. Several *CDC20* inhibitors have been discovered, such as toxoid-antitoxoid mixture esterase (TAME), pro-TAME, and 2-[benzyl-(2-nitro-benzenesulfonyl)-amino]-N-hydroxy-3-methyl-N-propyl-butyramide [33,34]. The investigation of *CDC20* inhibitors might help to elucidate the functional mechanism of *CDC20* in human cancers. However, we did not study the related mechanism for *CDC20* affecting HCC, which is a limitation of our study. The effects of *CDC20* in trends in the stages of disease progression were not explored in this study. Therefore, in future research we will pay more attention to the mechanism of *CDC20* and try to find related inhibitors of *CDC20* in HCC.

Conclusions

In summary, *CDC20* expression exhibited a rising tendency among HCC tissues and could act as a prognostic indicator for HCC, but further and more correlational research needs to be conducted.

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