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Down-regulated of *PCDH10* predicts poor prognosis in hepatocellular carcinoma patients

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Abstract

Protocadherin10 (*PCDH10*), a member of the nonclustered protocadherin family, functions as a tumor suppressor in many cancers. The aim of this study was to evaluate the expression level and prognostic value of *PCDH10* in hepatocellular carcinoma (HCC) patients.

Quantitative real-time polymerase chain reaction was used to analyze the expression level of *PCDH10* in HCC tissues and adjacent nontumor tissues. The association of *PCDH10* expression with clinicopathological features of patients was evaluated by chi-squared test. Overall survival was estimated using the Kaplan–Meier method. Besides, the patient prognosis was also evaluated by Cox regression analysis.

PCDH10 expression was significantly lower in HCC tissues than that in adjacent nontumor tissues ($P = .000$). Kaplan–Meier curves showed that patients with lower *PCDH10* expression had a worse overall survival. Moreover, *PCDH10* expression level was associated tumor size ($P = .005$), tumor node metastasis stage ($P = .002$), smoking status ($P = .000$), and drinking status ($P = .005$). Multivariate analysis showed that the expression of *PCDH10* ($P = .000$; hazard ratio = 4.784; 95% confidence interval: 2.550–8.977) was an independently associated with poor overall survival rates, as well as smoking status and drinking status.

Our findings indicated that the decreased expression of *PCDH10* was closely associated with poor prognosis of HCC patients. It might be considered as a valuable biomarker for HCC.

Abbreviations: AFP = alpha fetoprotein, CI = confidence interval, HCC = hepatocellular carcinoma, HR = hazard ratio, PCDH = Protocadherins, *PCDH10* = Protocadherin10, qRT-PCR = quantitative real-time polymerase chain reaction.

Keywords: hepatocellular carcinoma, *PCDH10*, prognosis

1. Introduction

Hepatocellular carcinoma (HCC) is the most common liver neoplasm and accounts for 85% to 90% of primary liver cancers, which is the 3rd-leading cause of cancer-related death worldwide.[1,2] As we know, the carcinogenesis of HCC is a complex process and many genes or regulators including suppressor genes and oncogenes are involved in the progression. Several risk factors have been suggested to be associated with the high incidence of HCC, including hepatitis B and C viruses, aflatoxin exposure, chronic alcohol consumption, cigarette smoking, and elevated endogenous testosterone in serum.[3–5] In the last decades, remarkable improvement has been made in the treatment of HCC as a consequence of combined chemotherapy, radiotherapy, and development in surgical and diagnostic imaging techniques.[6,7] However, because of the high rate of recurrence and metastasis, the 5-year overall survival rate is still low.[8] Therefore, it is imperative to discover valuable diagnostic and prognostic biomarkers for HCC, which may improve patients' survival.

The transmembrane proteins within the cadherin super family of protocadherins (PCDH) are divided into clustered proteins and nonclustered proteins.[8] As a member of the nonclustered *PCDH*, Protocadherin10 (*PCDH10*) has been demonstrated to be a putative tumor suppressor gene in several human cancer types, including prostate cancer, colorectal cancer, gastric cancer, and many other carcinomas.[9–13] It had been reported that *PCDH10* was an important tumor suppression gene in colorectal cancer and the down-regulation of *PCDH10* expression promoted tumor cell proliferation, migration, and invasion.[14] Early studies have demonstrated that *PCDH10* acted as a suppressor in the development of HCC and the deregulated expression of *PCDH10* was found to play an important role in HCC.[15,16] However, the studies about the clinical significance of *PCDH10* in HCC are rare.

In the present study, we analyzed the relative expression level of *PCDH10* in HCC tissues and adjacent nontumor tissues. The correlation between *PCDH10* expression level and clinicopathological factors was then analyzed. We also investigated the prognostic performance of *PCDH10* expression in HCC.

2. Materials and methods

2.1. Patients and specimens

The study was approved by the Ethic Committee of Third Hospital, Peking University. Written consent was obtained from all patients prior to surgery.

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A total of 109 HCC tissues and matched adjacent nontumor tissues were obtained from patients who underwent surgery in the Third Hospital, Peking University. None of the patients had received chemotherapy, radiotherapy, or other anticancer therapy before surgery. The patients were histologically confirmed by experienced pathologists. Paired tissue specimens (tumor and adjacent normal tissues) were collected from the patients, immediately frozen in liquid nitrogen and then stored at −80°C until use. Clinical information and follow-up data were collected and listed in Table 1. The patients who smoked more than 100 cigarettes during their lifetimes were defined as smokers. Nonsmokers referred to the patients who had never smoked, or those smoked <100 cigarettes. The patients who drank at least 1 L of alcohol per week were defined as drinkers, and others were defined as nondrinkers.

2.2. RNA isolation and qRT-PCR

Tissue specimens were used to extract RNA with the Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. RNA was reversely transcribed to cDNA using a Reverse Transcription Kit (TaKaRa, Dalian, China). The expression level of *PCDH10* in HCC was determined by quantitative real-time polymerase chain reaction (qRT-PCR) using the SYBR Green dye (TaKaRa, Dalian, China) on the 7500 Real-Time PCR systems (Applied Biosystems, Carlsbad, CA). *GAPDH* was used as an internal control. The specific primers were as follows: *PCDH10* forward: 5′-ACTGCTATCAGGTATGCCTG-3′; and reverse, 5′-GTCTGTCAACTAGATAGCTG-3′; *GAPDH* forward: 5′-AAACCCATCACCATCTTCCA-3′; and reverse: 5′-GTGGTTCACACCCATCACAA-3′. The relative expression level of *PCDH10* was calculated by the $2^{-\Delta\Delta Ct}$ method, with *GAPDH* as a reference gene. Each test was performed in triplicate.

2.3. Statistical analysis

The SPSS 18.0 software (SPSS Inc, Chicago, IL) and GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA) were applied to complete statistical analyses. All data were presented as the mean ±standard deviation. Student *t* test was used to examine the relationship between groups. Kaplan–Meier curve was used to estimate the impact of *PCDH10* level on the overall survival of HCC cases. Cox regression model was applied to simultaneously adjust all potential prognostic variables. The *P* values < .05 were considered statistically significant.

Table 1

The correlation between *PCDH10* expression and clinicopathological features of HCC patients.

Features	No. of cases (n = 109)	*PCDH10* expression Low (n = 54)	*PCDH10* expression High (n = 55)	*P*
Age, y				
< 60	46	26	20	.213
≥ 60	63	28	35	
Gender				
Female	51	28	23	.294
Male	58	26	32	
Smoking status				
Nonsmoker	50	13	37	.000
Smoker	59	41	18	
Tumor size, cm				
<5	49	17	32	.005
≥5	60	37	23	
Drinking status				
Nondrinker	53	19	34	.005
drinker	56	35	21	
TNM stage				
I–II	53	18	35	.002
III–IV	56	36	20	

HCC = hepatocellular carcinoma, *PCDH10* = protocadherin10, TNM = tumor node metastasis.

3. Results

3.1. The expression level of PCDH10 in HCC

The relative expression of *PCDH10* was detected and analyzed in 109 HCC tissues and matched adjacent nontumor tissues. We found that the expression level of *PCDH10* was significantly decreased in HCC compared with the adjacent normal tissues ($P < .000$; Fig. 1).

3.2. The association between PCDH10 expression and clinicopathological features of HCC patients

One hundred nine HCC patients was divided into low-expressed group (n = 54) and high-expressed group (n = 55) according to the normalized median level of *PCDH10*. The association of *PCDH10* with different pathological factors of 109 HCC patients was shown in Table 1. We found significant correlation between *PCDH10* expression and some clinicopathological features, such as tumor size ($P = .005$), tumor node metastasis (TNM) stage ($P = .002$), smoking status ($P = .000$), and drinking status ($P = .005$). However, the expression of *PCDH10* showed no closely relationship with other clinical parameters including age or gender (all $P > .05$, Table 1).

3.3. The prognostic value of PCDH10 expression in HCC

The correlation between *PCDH10* expression and overall survival of HCC patients was investigated by Kaplan–Meier analysis. The results showed that patients with low expression of *PCDH10* had a shorter overall survival than those with high *PCDH10* expression ($P = .000$) (Fig. 2). Univariate analysis indicated that tumor size ($P = .033$), TNM stage ($P = .003$),

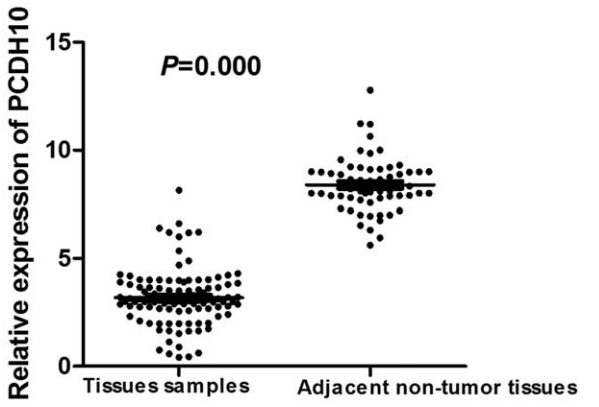

Figure 1. The relative expression of *PCDH10* was detected in HCC tissues compared with adjacent nontumor tissues using quantitative real-time polymerase chain reaction. *PCDH10* expression was significantly lower in hepatocellular carcinoma tissues than that in adjacent nontumor tissues ($P < .05$). *PCDH10* = Protocadherin10.

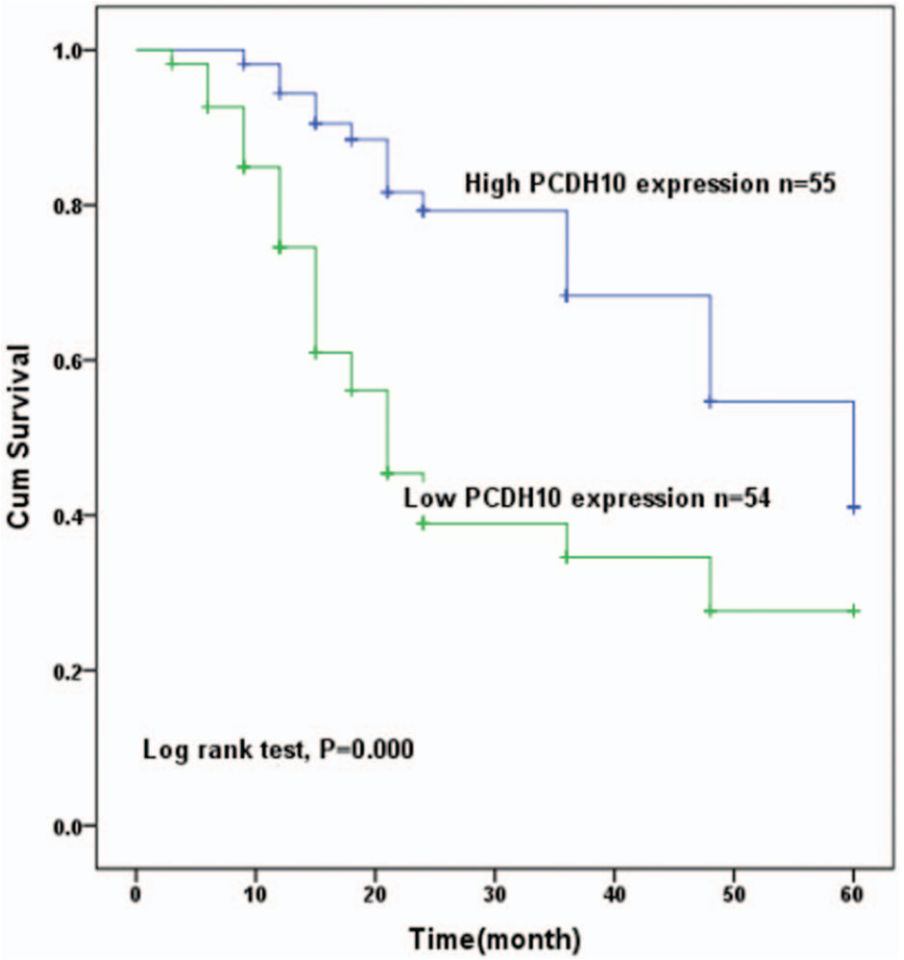

Figure 2. Kaplan–Meier analysis curve of overall survival time of hepatocellular carcinoma patients according to the *PCDH10* relative expression. Patients with low *PCDH10* expression had significantly poorer survival times compared with those with high *PCDH10* expression. *P* values were calculated by Kaplan–Meier analysis (*P* = .001). *PCDH10* = Protocadherin10.

smoking status (*P* = .003), drinking status (*P* = .007), and *PCDH10* expression (*P* = .001) were significantly associated with the survival. Multivariate Cox regression analysis further showed that *PCDH10* expression level (*P* = .000; hazard ratio [HR] = 4.784; 95% confidence interval [CI]: 2.550–8.977) was an independent prognostic indicator for HCC, as well as smoking status (*P* = .014; HR = 2.691; 95% CI: 1.220–5.931) and drinking status (*P* = .036; HR = 2.290; 95% CI: 1.056–4.967) (Table 2).

4. Discussion

HCC, as a highly malignant cancer, is the most prevalent primary malignant tumor of the liver in the world today.[17] There were about 782,000 new HCC patients around the world during 2012, and more than one-half of the patients were in China.[1] Thus, because of the high incidence and mortality of HCC, HCC has already become the current social health burden of our country.[18] In addition, tumor cells are also likely to invade intrahepatic blood vessels resulting in intrahepatic and extrahe-

Table 2

The univariate and multivariate analyses of overall survival for clinicopathological factors in patients with HCC.

	Univariate analysis		Multivariate analysis	
Factors	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
PCDH10 expression	3.318 (1.609–6.843)	.001	4.784 (2.550–8.977)	.000
Age, y	1.271 (0.636–2.539)	.498	—	—
Gender	1.087 (0.554–2.133)	.808	—	—
Smoking status	5.012 (1.762–14.257)	.003	2.691 (1.220–5.931)	.014
Drinking status	3.155 (1.366–7.282)	.007	2.290 (1.056–4.967)	.036
Tumor size, cm	2.484 (1.079–5.721)	.033	—	—
TNM stage	4.747 (1.669–13.500)	.003	—	—

— = no related data, CI = confidence interval, HCC = hepatocellular carcinoma, HR = hazard ratio, *PCDH10* = protocadherin 10, TNM = tumor node metastasis.

patic metastases.[18] Currently the most important treatment of HCC is liver resection and transplantation.[19] However, the long-term survival outcomes are still unsatisfactory due to high incidences of tumor recurrence and metastasis.[20] Over the past several years, the development of novel targeted therapeutics of HCC has led to a dramatic increase in preventive and therapeutic modalities, such as *miR-345*, *miR-138*, *miR-874*.[21–23] Previously, it has been reported that *PCDH10* acted as a tumor suppressor gene in HCC by inhibiting the PI3K/Akt signaling pathway.[15]*PCDH10* might form a potentially useful therapeutic target for HCC. In the present study, the data suggested that *PCDH10* was associated with HCC patient's prognosis.

PCDH10 is cadherin-associated receptor, which is located on human chromosome 4q28.3.[15] Previous studies have revealed that *PCDH10* played an important role in the establishment and function of specific cell–cell connections and in tumor development.[14,24] Recently, growing reports demonstrated that *PCDH10* was involved in the progression of tumors. For example, Pimson et al had demonstrated that *PCDH10* methylations in blood samples could serve as a potential noninvasive diagnostic indicator in blood for gastric cancer.[25] Zhou et al found that *PCDH10* played a critical role in cancer cell growth, by negatively regulating telomerase activity.[26] Harada et al showed that *PCDH10* methylation was a potential biomarker that predicts a poor prognosis after curative resection of pathological stage I non-small-cell lung cancer.[27] However, to our knowledge, no reports have investigated the prognostic significance of *PCDH10* in HCC.

In the present study, qRT-PCR showed that *PCDH10* expression levels were significantly down-regulated in HCC tissues compared with adjacent nontumor tissues. Then we found that the low expression of *PCDH10* was strongly correlated with tumor size, TNM stage, smoking status, and drinking status. Results were consistent with the previous study.[8,28] In order to investigate the prognostic role of *PCDH10* in HCC, we performed Kaplan–Meier analysis. The results showed that low-expression group had obviously shorter overall survival than high-expression group of HCC patients. Furthermore, multivariate analysis with a Cox proportional hazards model showed that low *PCDH10* expression was independently linked to poor survival of patients with HCC, suggesting that *PCDH10* could serve as an independent prognostic factor for overall survival of patients with HCC. *PCDH10* could inhibit HCC cell proliferation and promote apoptosis through multiple signaling pathways, including telomerase activity, PI3K/Akt.[15,26] The expression loss of PCDH10 caused by DNA methylation might lead to excessive growth of HCC cells, thus contributing to malignant disease progression and poor prognosis.[16]

However, there are still limitations in the study. First, the sample size was relatively small that might reduce the statistical power of our results. Second, alpha fetoprotein (AFP) is the recommended biomarker for HCC diagnosis and prognosis evaluation. Whether PCDH10 was superior to AFP in prognosis estimation of HCC remained poorly known. In addition, although we have studied the prognostic role of *PCDH10* in HCC patients, the tumorigenesis mechanism of *PCDH10* was still not elucidated in the present study. According to the previous studies, *PCDH10* promoter methylation could be a common event and may play an important role in tumorigenesis. For example, in the study of Huang et al, they showed that *PCDH10* promoter methylation is an independent prognostic indicator of worse overall survival and methylation status could serve as a valuable biomarker for risk classification for diffuse large B-cell lymphoma,[29] which provides a direction for our future research.

In summary, we have proved that *PCDH10* expression was decreased in HCC and correlated with tumor progression and shorter overall survival. The present study also demonstrated that *PCDH10* expression is a useful independent prognostic biomarker for the prediction of survival in HCC. Large-scale prospective studies are needed to confirm these preliminary findings.

Author contributions

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Methodology: Maolin Tian, Zhaolai Ma, Liang Wang.
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