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. However, because of the high rate of recurrence and metastasis, the 5-year overall survival rate is still low.[6] 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.
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^-ΔΔCt 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.

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 were 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),
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 \((P = .000; \text{hazard ratio } \text{[HR]} = 4.784; 95\% \text{ confidence interval } \text{[CI]}: 2.550–8.977)\) was an independent prognostic indicator for HCC, as well as smoking status \((P = .014; \text{HR} = 2.691; 95\% \text{ CI: 1.220–5.931})\) and drinking status \((P = .036; \text{HR} = 2.290; 95\% \text{ CI: 1.056–4.967})\) (Table 2).

### Table 2

| Factors          | Univariate analysis | Multivariate analysis |
|------------------|---------------------|-----------------------|
|                  | 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 (0.594–2.133) | .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.

4. **Discussion**

HCC, as a highly malignant cancer, is the most prevalent primary malignant tumor of the liver in the world today.\(^\text{[17]}\) There were about 782,000 new HCC patients around the world during 2012, and more than one-half of the patients were in China.\(^\text{[1]}\) Thus, because of the high incidence and mortality of HCC, HCC has already become the current social health burden of our country.\(^\text{[18]}\) In addition, tumor cells are also likely to invade intrahepatic blood vessels resulting in intrahepatic and extrahe-
The term PCDH10 was associated with HCC patient’s prognosis. The expression of 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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**References**

[1] Torre LA, Bray F, Siegel RL, et al. Global cancer statistics, 2012. CA Cancer J Clin 2015;65:87–108.  
[2] Forner A. Hepatocellular carcinoma surveillance with miRNAs. Lancet Oncol 2015;16:743–5.  
[3] Marrero JA, Fontana RJ, Fa S, et al. Alcohol, tobacco and obesity are synergistic risk factors for hepatocellular carcinoma. J Hepatol 2005;42:218–24.  
[4] Yang JD, Harmsen WS, Slettedahl SW, et al. Factors that affect risk for hepatocellular carcinoma and effects of surveillance. Clin Gastroenterol Hepatol 2011;9:617–23.  
[5] Kew MC. Hepatocellular carcinoma: epidemiology and risk factors. J Hepatol Carcinoma 2014;1:115–25.  
[6] Akin O, Brennan SB, Dershaw DD, et al. Advances in oncologic imaging: update on 5 common cancers. CA Cancer J Clin 2012;62:364–93.  
[7] Liu J, Zhao Q, Deng W, et al. Radiation-related lymphopenia is associated with spleen irradiation dose during radiotherapy in patients with hepatocellular carcinoma. Radiat Oncol 2017;12:90.  
[8] Choi HJ, Kim DG, Na GH, et al. Clinical outcome in patients with hepatocellular carcinoma after living-donor liver transplantation. World J Gastroenterol 2013;19:4737–44.  
[9] Yu B, Yang H, Zhang C, et al. High-resolution melting analysis of PCDH10 methylation levels in gastric, colorectal and pancreatic cancers. Neoplasma 2010;57:247–52.  
[10] Kim SY, Yasuda S, Tanaka H, et al. Non-clustered protocadherin. Cell Adh Migr 2011;5:97–105.  
[11] Deng QK, Lei YG, Lin YL, et al. Prognostic value of Proteocadherin10 (PCDH10) methylation in serum of prostate cancer patients. Med Sci Monit 2016;22:516–21.  
[12] Li M, Yan DG, Liu JL. Methylation status of PCDH10 and RASSF1A gene promoters in colorectal cancer. Zhonghua Yi Xue Za Zhi 2016;96:456–9.  
[13] Hou YC, Deng JY, Zhang RP, et al. Evaluating the clinical feasibility: the direct bisulfitge genomic sequencing for examination of methylated status of proteocadherin10 (PCDH10) promoter to predict the prognosis of gastric cancer. Cancer Biomark 2015;13:567–73.  
[14] Zhong X, Zou Y, Mao J, et al. Frequent epigenetic silencing of PCDH10 by methylation in human colorectal cancer. J Cancer Res Clin Oncol 2015;139:485–90.  
[15] Ye M, Li J, Gong J. PCDH10 gene inhibits cell proliferation and induces cell apoptosis by inhibiting the PI3K/Akt signaling pathway in hepatocellular carcinoma cells. Oncof Rep 2017;37:3167–74.  
[16] Fang S, Huang SF, Cao J, et al. Silencing of PCDH10 in hepatocellular carcinoma via de novo DNA methylation independent of HBV infection or HBX expression. Clin Exp Med 2013;13:127–34.  
[17] Guthle M, Dollinger MM. Epidemiology and risk factors of hepatocellular carcinoma. Der Radiol 2014;54:64–9.
[18] Bruix J, Reig M, Sherman M. Evidence-based diagnosis, staging, and treatment of patients with hepatocellular carcinoma. Gastroenterology 2016;150:835–53.
[19] Maluccio M, Covey A. Recent progress in understanding, diagnosing, and treating hepatocellular carcinoma. CA Cancer J Clin 2012;62:394–9.
[20] Dhir M, Melin AA, Douaiher J, et al. A review and update of treatment options and controversies in the management of hepatocellular carcinoma. Ann Surg 2016;263:1112–25.
[21] Zhang H, Liu H, Bi H. MicroRNA-345 inhibits hepatocellular carcinoma metastasis by inhibiting YAP1. Oncol Rep 2017;38:843–9.
[22] Luo J, Chen P, Xie W, et al. MicroRNA-138 inhibits cell proliferation in hepatocellular carcinoma by targeting Sirt1. Oncol Rep 2017;38:1067–74.
[23] Jiang T, Guan LY, Ye YS, et al. MiR-874 inhibits metastasis and epithelial-mesenchymal transition in hepatocellular carcinoma by targeting SOX12. Am J Cancer Res 2017;7:1310–21.
[24] Waha A, Gunther S, Huang TH, et al. Epigenetic silencing of the protocadherin family member PCDH-gamma-A11 in astrocytomas. Neoplasia 2005;7:193–9.
[25] Pimson C, Ekalaksananan T, Pientong C, et al. Aberrant methylation of PCDH10 and RASSF1A genes in blood samples for non-invasive diagnosis and prognostic assessment of gastric cancer. PeerJ 2016;4:e2112.
[26] Zhou LN, Hua X, Deng WQ, et al. PCDH10 interacts with hTERT and negatively regulates telomerase activity. Medicine 2015;94:e2230.
[27] Harada H, Miyamoto K, Yamashita Y, et al. Prognostic signature of protocadherin 10 methylation in curatively resected pathological stage I non-small-cell lung cancer. Cancer Med 2015;4:1536–46.
[28] Gao Y, Zhang SG, Wang ZH, et al. Down-regulation of miR-342-3p in hepatocellular carcinoma tissues and its prognostic significance. Eur Rev Med Pharmacol Sci 2017;21:2098–102.
[29] Huang W, Xue X, Shan L, et al. Clinical significance of PCDH10 promoter methylation in diffuse large B-cell lymphoma. BMC Cancer 2017;17:815.