RESEARCH ARTICLE

Serum Peroxiredoxin3 is a Useful Biomarker for Early Diagnosis and Assessemnt of Prognosis of Hepatocellular Carcinoma in Chinese Patients

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Abstract

Background: Recently, peroxiredoxin3 (PRDX3) was identified as a novel molecular marker for the progression of hepatocellular carcinoma (HCC). However, its potential clinical application as a serum marker for the early diagnosis and prognosis of HCC has not been investigated. Methods: PRDX3, alpha-fetaprotein (AFP), and other biochemical parameters were measured in serum samples from 297 Chinese patients, including 96 with HCC, 98 with liver cirrhosis (LC), and 103 healthy controls (HCs). Correlations between serum PRDX3 expression and clinicopathological variables and the relationship between serum PRDX3 expression and prognosis were analyzed. Results: Serum PRDX3 was significantly higher in HCC patients than in the LC and HC groups. The sensitivity and specificity of serum PRDX3 for the diagnosis of HCC were 85.9% and 75.3%, respectively, at a cutoff of 153.26 ng/mL, and the area under the curve was 0.865. Moreover, serum PRDX3 expression was strongly associated with AFP level, tumor diameter, TNM stage, and portal vein invasion. Kaplan-Meier curve analysis revealed that HCC patients with high serum PRDX3 expression had a shorter median survival time than those with low PRDX3 expression. Moreover, serum PRDX3 expression was an independent risk factor for overall survival. The inverse correlation between serum PRDX3 and patient survival remained significant in patients with early-stage HCC and in those with normal serum AFP levels. Conclusions: Serum PRDX3 can be used as a noninvasive biomarker for the diagnosis and/or prognosis of HCC.

Keywords: Alpha-fetoprotein - peroxiredoxin3 - hepatocellular carcinoma - biomarker

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Introduction

Hepatocellular carcinoma (HCC) is the sixth most common malignant tumor, and its incidence has been increasing worldwide (Jemal et al., 2011). HCC, like other cancers, involves aberrant changes in multiple molecular pathways and genetic as well as epigenetic factors, which consequently lead to the malignant transformation and progression of HCC (Breuhahn et al., 2011; Marquardt et al., 2012). Despite advances in chemotherapy, surgical management, and the clinical implementation of numerous therapeutic strategies, the mortality rates of patients with HCC remain very high (up to 94%), making HCC the third most common cause of cancer-related death. The incidence and mortality rates for HCC are nearly identical, resulting in a low overall 5-year survival rate for patients with this type of cancer. Therefore, it is critical to identify and characterize sensitive and specific diagnostic and prognostic biomarkers for screening of early HCC in high-risk populations (Altekruse et al., 2009; Shariff et al., 2009; Huang et al., 2010; Mao et al., 2010).

Serum alpha-fetoprotein (AFP) levels and abdominal ultrasound are the most widely used methods for detection of HCC. However, the sensitivity and specificity of both AFP levels and ultrasound for HCC surveillance have some shortcomings, particularly in the early stages of the disease (Yamamoto et al., 2010; Kudo et al., 2011). Therefore, investigators are attempting to identify more effective HCC serum biomarkers.

The majority of studies have demonstrated that the progression of HCC is closely correlated to oxidative stress (Jo et al., 2011). Cancer cells usually exhibit changes in key mitochondrial regulators of cell death or mitochondrial structure and function, which are different from those of normal cells. This enables them to use antioxidant systems to maintain the redox balance under

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high levels of oxidative stress (Trachootham et al., 2009).

Classical antioxidant genes related to cancer include superoxide dismutase (SOD), catalase, GST, glutathione peroxidase, peroxiredoxins (PRDXs), and thioredoxin (Trx) (Basu et al., 2011; Song et al., 2011; Marra et al., 2011). PRDX3 was identified as a member of the peroxiredoxin protein family (Prx), which acts as a cellular defense system against oxidative stress and whose catalytic activity and protein sequences are different from those of other antioxidants (Dietz et al., 2002). Overexpression of PRDX3 has been reported in mesothelioma, HCC, breast cancer, and ovarian cancer, suggesting that it may be involved in tumorigenesis and cancer progression (Huh et al., 2012). Recently, Qiao et al. identified PRDX3 using differential tissue proteome analysis as a novel molecular marker for HCC progression (Qiao et al., 2012). However, because it is difficult to obtain liver tissue, the use of tissue levels of PRDX3 as a diagnostic biomarker is not feasible. Additionally, no studies have yet reported whether serum PRDX3 may be used as a biomarker for HCC. Therefore, in the current study, we sought to determine whether PRDX3 could serve as a novel and potentially noninvasive serum marker for the early diagnosis and prognosis of HCC patients.

Materials and Methods

Study design

Serum samples were obtained from patients attending the First Affiliated Hospital of Wenzhou Medical University, China, between April 2008 and December 2011. In total, 297 patients were enrolled in this study, including 103 health controls (HCs; with normal liver biochemistry, no history of liver disease or alcohol abuse, and no viral hepatitis), 96 patients with liver cirrhosis (LC), and 98 patients with HCC. Demographic and clinical information was obtained, and a blood sample was collected from each patient. No patients underwent surgery or received chemotherapy or radiotherapy before blood sampling. Inclusion criteria were histologically confirmed HCC or pathognomonic results in dynamic imaging and an underlying chronic liver disease. Patients were excluded if they were younger than 18 years of age, had a history of another malignant disease within the last 5 years, or had a history of organ transplantation. Child-Pugh-score and TNM stage were assessed by results of clinical examination, imaging (dynamic computer tomography [CT], magnetic resonance imaging [MRI], or abdominal ultrasound examination), and laboratory parameters. Blood samples from all patients were centrifuged at 3500×g for 5 min at room temperature. Supernatants were transferred into Eppendorf tubes and centrifuged at 12000×g for 10 min to remove cellular fractions. Supernatants were then stored at -80°C and thawed at the time of assay. The Institutional Ethics Review Board of the First Affiliated Hospital of Wenzhou Medical University approved this study, and all patients provided written informed consent to participate in this study.

Biochemical analyses

Serum AFP levels were detected using an Abbott Architect I2000 luminescence analyzer (Abbott Laboratories, Abbott Park, IL, USA) with a direct chemiluminescence method. Aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), glucose (Glu), total proteins (TP), albumin (ALB), and total bilirubin (TB) levels were determined using a Hitachi 7600 chemistry analyzer (Hitachi, Tokyo, Japan) with the kinetic method. Serum concentrations of PRDX3 were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (BioVision Corporation, Milpitas, CA, USA).

Patient follow up

The follow-up period was defined as the time from the date of surgery to the date of patient death or the last follow-up point. Follow up was completed on July 1, 2013. All HCC patients were monitored after surgery; diagnosis of recurrence was confirmed by ultrasound, enhanced CT scan, MRI, and AFP levels.

Statistical analysis

Statistical analyses were performed with SPSS ver. 17.0 (SPSS, Chicago, IL, USA). Analyses were performed using GraphPad Prism 5 software. Median, range, mean, standard deviation, and standard error were used for descriptive statistics as appropriate. Receiver operating characteristics (ROC) analysis was used to validate the diagnostic value of serum PRDX3 compared to serum AFP to identify the optimal cutoff values. Sensitivity, specificity, and positive and negative predictive values of PRDX3 and AFP were profiled by curves. Categorical variables were tested with Fisher's exact test or the χ^2 test. Comparisons of serum PRDX3 levels and clinical characteristics among the different groups were carried out using the Kruskal-Wallis test (a nonparametric analysis of variance [ANOVA] test) and the Mann-Whitney U test (a nonparametric Student's t-test). All tests were two-tailed, and differences were considered statistically significant when p-values were less than 0.05. The χ^2 test was used to analyze the relationship between PRDX3 expression and clinicopathological parameters in patients with HCC. Overall survival rates were calculated by the Kaplan-Meier method and analyzed by the log-rank test. The Cox proportional hazards regression model was used to assess the correlation between survival time and multiple clinicopathological variables.

Results

Patient characteristics

Table 1 shows the sociodemographic and clinical characteristics of all study groups. Epidemiology, body mass index (BMI), and location of residence did not differ significantly between groups. No significant differences were found in terms of comorbidities, etiology of liver disease, ascitis, or Child-Pugh stage between patients with LC and those with HCC. However, significant differences were observed among all groups with regard to measured liver function tests and tumor markers.

Table 1. Demographic, Clinical, and Biochemical Characteristics of the Patients in this Study

Parameters	HCC1	LC2	HC3	p^a	p^b
Epidemiology					
Patients	96	98	103		
Gender, m/f	67/29	63/35	65/38	0.781	0.653
Age, years	52.3 (52.3±11.6)	49.9 (49.9±13.2)	50.5 (50.5±16.1)	0.923	0.814
BMI (kg/m^2)					
Male	35.30±0.75	33.35±0.52	36.40±0.43	0.636	0.775
Female	24.80±0.47	27.80±0.26	22.50±0.91	0.514	0.454
Residence (%)				0.715	0.398
Urban	72 (75.0%)	75 (76.6%)	77 (74.8%)		
Rural	24 (25.0%)	23 (23.4%)	26 (25.2%)		
Comorbidity				0.812	
Diabetes	5 (0.05%)	7 (0.07%)			
Hypertension	9 (0.09%)	8 (0.08%)			
Heart disease	4 (0.04%)	5 (0.04%)			
Etiology				0.675	
Alcohol abuse	21 (21.9%)	25 (25.5%)			
Hepatitis C	13 (13.5%)	10 (10.2%)			
Hepatitis B	47 (49.0%)	42 (42.9%)			
Cryptogenic	10 (10.4%)	12 (12.2%)			
NASH4	4 (4.2%)	6 (6.1%)			
Haemochromatosis	1 (2.1%)	1 (1.0%)			
Ascitis				0.235	
Present	16 (16.7%)	15 (15.3%)	0 (0%)		
Absent	80 (83.3%)	83 (84.7%)	103 (100%)		
Child-Pugh stage				0.717	
A	46 (47.9%)	43 (43.9%)			
B	35 (36.5%)	38 (38.7%)			
C	15 (15.6%)	17 (17.4%)			
Laboratory results					
ALT (U/L)	293.25 (44.12-469.25)	148.87 (26.91-234.15)	37.51 (9.22-47.87)	0	0.033
AST (U/L)	199.45 (32.14-326.85)	74.76 (20.91-123.02)	24.60 (15.24-40.93)	0	0.012
GGT (U/L)	262.56 (57.13-412.06)	117.98 (36.99-185.09)	31.65 (12.55-60.36)	0.003	0.023
ALP (U/L)	564.87 (265.25-890.23)	239.34 (123.45-430.33)	60.15 (45.76-125.36)	0.03	0.04
TP (g/L)	31.21 (24.19-54.32)	42.21 (31.19-74.32)	71.93 (65.02-85.88)	0.043	0.03
ALB (g/L)	19.14 (11.91-33.14)	31.72 (23.22-45.87)	43.07 (40.12-55.79)	0.027	0.04
TB (μmol/L)	358.22 (63.89-498.01)	226.78 (40.64-297.13)	11.90 (1.11-20.12)	0.004	0
AFP (ng/mL)	2690.23 (13.11-4013.55)	132.00 (5.12-234.98)	13.06 (3.12-20.65)	0	0
PRDX3 (ng/mL)	238.61 (26.81-608.16)	101.37 (11.87-276.68)	65.07 (5.72-88.56)	0	0

p^a: p value between LC and HCC groups, p^b: p value among all study groups; $p<0.05$ is considered significant. 1: Hepatocellular carcinoma, 2: liver cirrhosis, 3: healthy control, 4: nonalcoholic steatohepatitis

Serum PRDX3 level as a potential diagnosis marker for HCC

To evaluate whether serum PRDX3 levels could be used as potential diagnostic markers for HCC, ROC curve analyses were performed in pooled sets. We found that serum PRDX3 levels discriminated HCC patients from non-HCC patients, with a higher area under the curve (AUC) of the ROC curve (0.865; 95% confidence interval [CI], 0.809-0.953) compared to that of AFP (0.67; 95% CI, 0.509-0.753; Figure 1A). At the cut-off value of 153.26 ng/mL, the sensitivity was 85.9%, and the specificity was 75.3%.

In order to determine whether serum PRDX3 expression was HCC specific, we examined serum samples in patients with LC. Our data indicated that PRDX3 expression was significantly higher in HCC patients (238.61 [26.81-608.16]) than in patients with LC (101.37 [11.87-276.68], $p<0.001$). ROC curve analyses suggested that serum PRDX3 expression was a useful marker for discriminating patients with HCC from patients with LC. The AUC of the ROC curve was 0.717 (95%CI, 0.641-0.734; Figure 1B). At the cut-off value of 95.13 ng/mL, the sensitivity and specificity were 73.2% and 69.0%, respectively.

Our data also showed that expression level of PRDX3 in the serum was significantly higher in patients with LC (101.37 [11.87-276.68], $p<0.001$) than in HCs (65.07 [5.72-88.56], $p<0.001$). However, the AUC of serum PRDX3 for discriminating LC patients from HCs was only 0.577 (95% CI, 0.417-0.661; Figure 1C). At the cut-off value of 66.82 ng/mL, the sensitivity and specificity were 59.7% and 51.9%, respectively.

Association between serum PRDX3 expression and clinicopathological features in HCC

Next, we divided HCC patients into low and high expression groups based on the median expression of PRDX3 (238.61 ng/mL) and evaluated differences in clinicopathological data between the two groups to identify associations between PRDX3 expression and clinical features of HCC (Table 2). High serum PRDX3 was significantly associated with tumor diameter, TNM

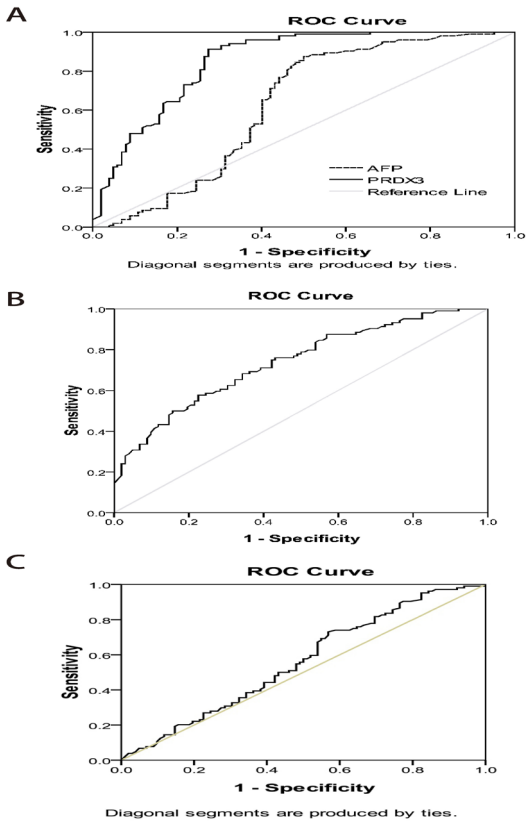

Figure 1. (A) The Diagnostic Sensitivities of PRDX3 and AFP for Discriminating HCC from Non-HCC were 85.9% and73.1%, Respectively, The Specificities of the Two Markers were 75.3% and 63.1%, respectively, At the cutoff 153.26 ng/mL, serum PRDX3 yields a higher area under the curve (0.865) than that of AFP (0.67). (B) At the cutoff 95.13 ng/mL, the AUC was 0.717, with 73.2% sensitivity and 69.0% specificity for discriminating HCC from LC. (C) At the cutoff 66.82 ng/mL, the AUC was 0.577, with 59.7% sensitivity and 51.9% specificity for discriminating LC from HCs

stage, AFP serum levels, and portal vein invasion ($p<0.01$). However, there were no significant associations between PRDX3 expression and other clinicopathological parameters, including age, gender, viral infection, differentiation status, Child-Pugh stage, and intrahepatic metastasis ($p>0.05$, Table 2).

Correlation between the expression of serum PRDX3 and the prognosis of HCC patients

For a better understanding of the potential application of serum PRDX3 as a prognostic indicator for patients with HCC, we explored the relationship between serum PRDX3 levels and overall survival (OS) using the Kaplan-Meier method. The results indicated that HCC patients with high expression of serum PRDX3 had a lower survival rate than those with low expression (median OS, 270 *vs* 641 days; $p<0.001$; Figure 2)

To identify variables with potential prognostic significance in HCC patients, univariate analysis for each variable was performed in relation to survival time. In

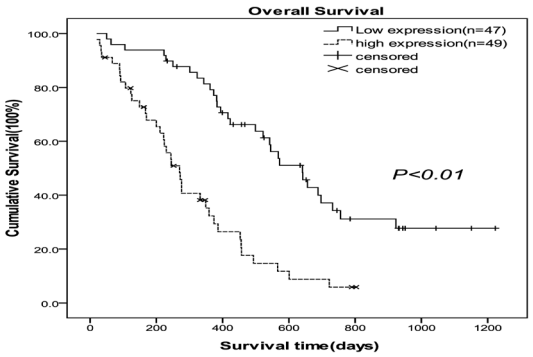

Figure 2. Kaplan-Meier Overall Survival Curves for Patients with High and Low Serum Levels of PRDX3. HCC patients with high expression of serum PRDX3 had significantly lower survival rates than those with low expression

Table 2. Correlation between the Clinicopathological Variables and Serum PRDX3 Expression in HCC.

Variable		Serum PRDX3				
		No. cases	Low expression	High expression	χ^2	p^a
Age (years)	≥ 50	57	28 (49.1%)	29 (50.9%)	1.167	0.268
	≤ 50	39	19 (48.7%)	20 (51.3%)		
Gender	Male	67	33 (49.3%)	34 (50.7%)	0.023	0.866
	Female	29	14 (48.3%)	15 (51.7%)		
Nonviral or viral	Nonviral	36	17 (47.2%)	19 (52.8%)	0.117	0.722
	HCV	13	6 (46.2%)	7 (53.8%)		
	HBV	47	24 (51.1%)	23 (48.9%)		
AFP (ng/mL)	< 20	40	26 (65%)	14 (35%)	12.573	< 0.001
	≥ 20	56	21 (37.5%)	35 (62.5%)		
Tumor diameter	< 3 cm	31	22 (71.0%)	9 (29.0%)	6.1	0.009
	≥ 3 cm	65	25 (38.5%)	40 (61.5%)		
Differentiation	Well-moderate	50	26 (52%)	24 (48%)	0.813	0.319
	Poor-undifferentiated	46	21 (45.7%)	25 (54.3%)		
TNM stage	I+II	30	22 (73.3%)	8 (26.7%)	5.021	0.007
	III+ IV	66	25 (37.8%)	41 (62.2%)		
Child-Pugh stage	A	15	8 (53.3%)	7 (46.7%)	0.384	0.387
	B	35	17 (48.6%)	18 (51.4%)		
	C	46	22 (47.8%)	24 (52.2%)		
Portal vein invasion (Vp)	Vp-	43	27 (62.8%)	16 (37.2%)	14.682	< 0.001
	Vp+	53	20 (37.7%)	33 (62.3%)		
Intrahepatic metastasis(im)	im-	72	35 (46.6%)	37 (53.4%)	0.764	0.265
	im+	24	12 (50%)	12 (50%)		

[a]:Chi-squared test

our univariate analysis, stepwise inclusion of variables in the model showed that elevated levels of serum PRDX3 were significantly correlated with overall survival (hazard ratio [HR]: 5.132; $p<0.001$; Table 3). Moreover, there were significant correlations between OS and clinical features, including serum AFP level, tumor diameter, TNM stage, and portal vein invasion (HR: 1.744, $p=0.041$; HR: 1.906, $p=0.025$; HR: 2.252, $p=0.003$; HR: 2.765, $p<0.001$, respectively; Table 3).

Multivariate Cox regression analysis demonstrated that the expression of serum PRDX3 was an independent prognostic factor for OS (HR: 2.192; $p=0.023$; Table 3).

Prognostic values of serum PRDX3 in different HCC subgroups

To further demonstrate the value of serum PRDX3 expression in predicting survival of HCC patients, multiple analysis methods were performed in this study. A validation cohort was employed to evaluate the prognostic value of PRDX3 for specific subgroups of patients. We used cut-off levels of AFP (20 ng/mL) to subgroup the 96 HCC patients and evaluated the prognostic significance of serum PRDX3 in the patient subgroups. As shown in Figure 3A, HCC patients with different OS times could not be distinguished by conventional AFP tests. Our data suggested that AFP=20 ng/mL was not a prognostic cut-off. However, serum PRDX3 expression was more sensitive toward predicting the prognosis of HCC patients than AFP in the above HCC patient subgroups ($p<0.01$). In another group of HCC patients whose survival is known to be difficult to predict in the clinic, namely those with tumor sizes smaller than 3 cm in diameter, the survival

Table 3. Univariate and Multivariate Analyses of Various Prognostic Parameters in Patients with Liver Cancer by Cox-regression Analysis

	Univariate analysis			Multivariate analysisa		
	HR[b]	(95% CI)[c]	p value	HR[b]	(95% CI)[c]	p value
Tumor diameter	1.906	(1.601-2.482)	0.025	1.255	(0.348-4.532)	0.729
TNM stage	2.252	(1.319-3.879)	0.003	1.232	(0.292-5.199)	0.094
Portal vein invasion	2.765	(1.718-4.133)	0	0.89	(0.068-11.561)	0.165
AFP	1.744	(0.974-2.142)	0.041	1.679	(0.444-6.349)	0.06
PRDX3	5.132	(3.923-7.978)	0	2.192	(1.024-4.691)	0.023

[a]: Backward Wald test was used for variables screened, p=0.05 was chosen as a criterion for significance; [b]:HR, hazard ratio; [c]:95% CI confidence interval

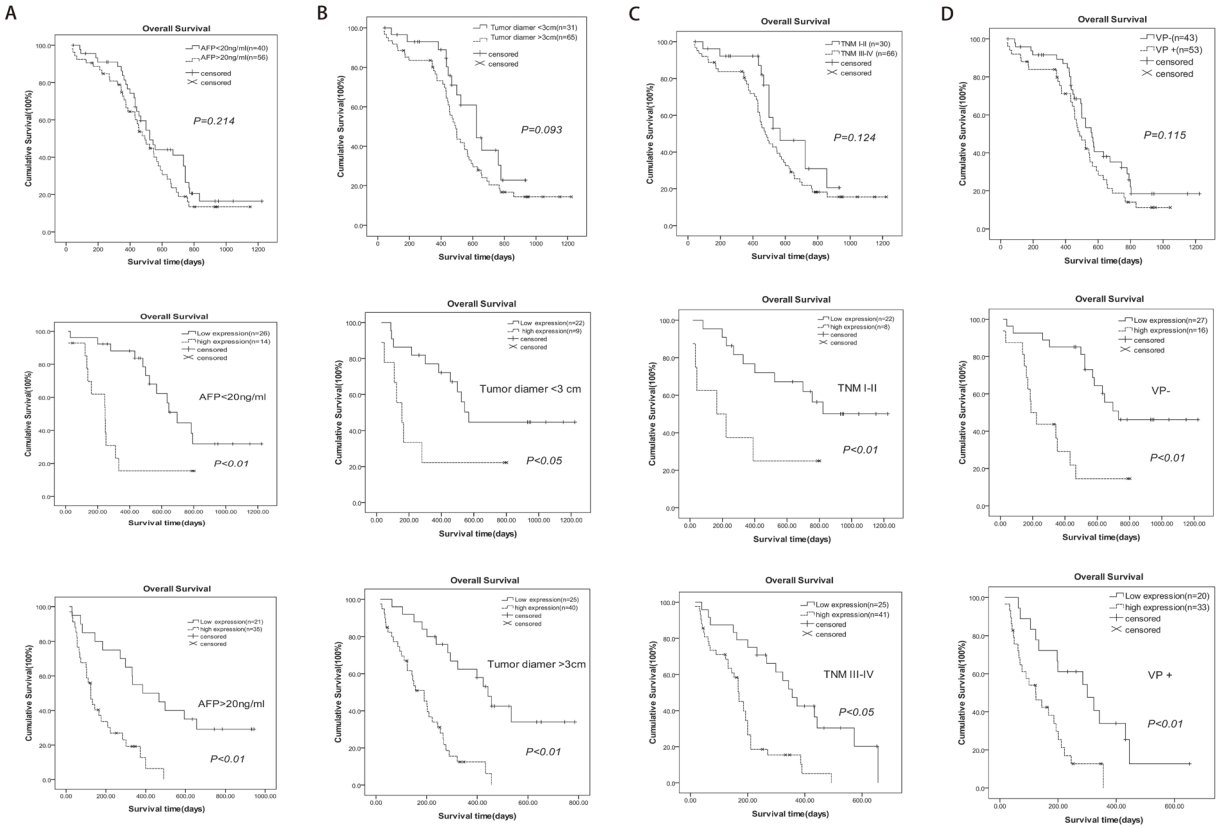

Figure 3. Kaplan-Meier Analysis of OS in 96 Patients Based on Serum PRDX3 Expression in HCC Subgroups According to Clinicopathological Variables. (A) Using AFP level (20 ng/mL) as the cut-off could not separate patients with different OS rates in the study cohort. In contrast, serum PRDX3 levels predicted different OS rates in the subgroups of patients with AFP levels<20 ng/mL and AFP levels>20 ng/mL. (B, C, D) Three other clinicopathological variables, e.g., tumor diameter, TNM stage, and portal vein invasion, could not separate patients with different OS rates in the study cohort. In contrast, serum PRDX3 levels could predict different OS rates in the subgroups of patients with tumor diameters<3 cm or>3 cm, TNM stages of I-II or III-IV, and the presence or absent of portal vein invasion

rate was 44.7% in the low PRDX3 group and only 22.2% for patients exhibiting high PRDX3 expression ($p<0.001$, Figure 3B). In early-stage HCC patients (TNM stages I-II), patients in the low PRDX3 expression group had a survival rate of 50.1%, while those in the high PRDX3 expression group had a 25.3% survival rate ($p=0.016$, Figure 3C). In the clinical subgroup with no portal vein invasion, survival rates were 46.3% and 14.6%, respectively, for patients with low or high PRDX3 expression ($p<0.001$, Figure 3D). Taken together, our data suggested that PRDX3 may have prognostic value for HCC patients in various clinical subgroups for which survival prediction may otherwise be challenging.

Discussion

Both pathologically and clinically, HCC is a heterogeneous cancer with poor prognosis and a very high mortality. The search for valuable biomarkers in the diagnosis of HCC and in predictive prognosis has attracted increasing interest. Hepatic tumor biomarkers should ideally possess both high specificity and sensitivity, especially in the context of distinguishing HCC from LC. Moreover, an ideal biomarker should be easily accessible and quantifiable, and procedures for measuring a biomarker should be minimally invasive, inexpensive, accurate, and acceptable to both the patient and the physician. While a variety of proteins and other characteristics associated with a type of cancer including DNA methylation, circulating tumor cells, and histone modifications, have attracted the attention of researchers for early diagnosis and prognosis of HCC, the availability of clinically applicable biomarkers remains limited (Liu et al., 2012; Shen et al., 2012; Fu et al., 2013; Ji et al., 2014).

PRDX3 is a c-Myc target gene that is required for mitochondrial homeostasis and neoplastic transformation, and predominantly localizes in the mitochondria (Wonsey et al., 2002). Moreover, PRDX3 might play important roles in scavenging reactive oxygen species (ROS) by serving as a first-line of host defense against H_2O_2 produced during respiration, and in protecting cells from H_2O_2 -induced apoptosis (Rabilloud et al., 2002).

Several studies have indicated that PRDX3 is over-expressed in prostate cancer, cervical cancer, colorectal cancer, and ovarian serous cyst adenocarinoma. Aggressively growing cancer cells produce high levels of ROS that disturb the redox balance. PRDX3 is an active responder to oxidative stress, and its expression of concordantly augmented to remove cellular ROS and inhibit apoptosis, which is beneficial to cancer growth and invasion. Furthermore, short hairpin RNA (shRNA)-mediated loss in functional PRDX3 expression led to significant suppression of migration and transendothelial invasion in MCF-7 breast cancer cells in vitro and significant inhibition of breast cancer metastasis *in vivo* (Kim et al., 2009; Li et al., 2009; Liu et al., 2010; Wu et al., 2010). Recently, abundant evidence has also confirmed that tissue-specific expression of PRDX3 plays an important role in hepatocarcinoma progression (Dai et al., 2010). Qiao et al has analyzed the comparative proteomic profiles between HCC tumors and adjacent non-tumor tissues by 2-DE and MALDI-TOF MS, and identified differentially expressed molecules which may help to develop effective therapeutic strategies against HCC. PRDX3 was found and confirmed to predict the poor differentiation of HCC based on proteomics screening and molecular biology confirmation. The results of their study suggest that PRDX3 has substantial clinical impact. However, no further investigations of the relevance of serum PRDX3 in HCC have been carried out to date. In the present study, we focused on the power of serum PRDX3 for early diagnosis and prognosis of HCC patients.

We found that serum levels of PRDX3 were significantly elevated in patients with HCC as compared to patients with LC or HCs. Moreover, serum PRDX3 yielded a higher AUC of 0.865 compared to that of AFP (0.67), with a sensitivity of 85.9% and a specificity of 75.3%. Since LC is a major risk factor for HCC, it may be that elevated serum PRDX3, as observed in patients with HCC, is a feature of liver injury. To determine whether PRDX3 elevation was specific to HCC, we examined serum levels of PRDX3 in patients with LC. Our study showed that PRDX3 could discriminate HCC from LC and yielded an AUC of 0.717 with 73.2% sensitivity and 69.0% specificity. To our knowledge, this is the first study to demonstrate the diagnostic potential of serum PRDX3 for screening HCC.

Further analyses revealed the clinical significance of serum PRDX3. We found that the levels of serum PRDX3 were significantly associated with AFP levels, tumor diameter, TNM stage, and portal vein invasion. HCC patients with tumor diameters of 3 cm or greater, TNM stages III or IV, high AFP serum levels, and evidence of portal vein invasion had higher PRDX3 expression levels than did other patients. This suggested an important role for serum PRDX3 in the development of HCC. However, these results were inconsistent with a previous study measuring the expression of PRDX3 in HCC tissue. Thus, our results showed that the level of PRDX3 in HCC tissues might incompletely represent PRDX3 expression profiles of corresponding sera samples.

Early diagnosis and treatment is key to achieving improved clinical outcomes in HCC patients. Using biomarkers to identify patients presenting with a higher risk of progressing to poor prognoses may thus reduce mortality and medical costs. Notably, in our test cohort, patients with high levels of serum PRDX3 had lower survival rates. Moreover, univariate and multivariate analyses using Cox regression models revealed that serum PRDX3 might serve as an independent risk factor in predictive prognosis of HCC.

Serologic biomarkers, including AFP, are currently used in the clinic to screen mainly for HCC and as an important predictor of patient survival after tumor resection (Nagasue et al., 1998). The diagnostic sensitivity and specificity of AFP are not satisfactory. Therefore, moderately raised levels of AFP are also found in some patients with uncomplicated chronic liver disease. When used alone, a large number of HCC patients that do not display elevated AFP are missed and subsequently progress to late-stage HCC before advancing to a clinically symptomatic stage with detectable AFP. Due to its low

sensitivity in identifying new HCC cases that have not been detected by imaging technology, measuring AFP levels is only marginally effective in specific patient populations (Pateron et al.,1994). Indeed, in our study, only 58.3% of HCC patients were AFP-positive. By contrast, in the patient group in which AFP levels were not prognostically predictive, PRDX3 appeared to indicate the duration of survival. Thus, our study revealed the potential value of PRDX3 in predicting patient survival in subgroups with normal AFP levels or in patients with early-stage HCC, in whom it would otherwise be difficult to predict prognosis using currently available surrogate biomarkers.

In our test cohort, patients with early-stage HCC (TNM stages I-II) displayed significantly higher levels of serum PRDX3 than HCs. Additionally, as the disease progressed to later stages, serum PRDX3 levels increased further. In our validation cohort, early-stage HCC patients (TNM stages I-II, tumor size less than 3 cm, AFP<20 ng/mL, no portal vein invasion) with high levels of serum PRDX3 also displayed a relatively low overall survival as compared to those patients diagnosed with late-stage HCC who had low levels of serum PRDX3. Thus, further efforts to explore the potential usefulness of serum PRDX3 as a biomarker after surgical resection in the identification of patients in early stages of disease with poor prognosis are warranted. Inclusion of metastatic cases in future studies will help address whether high-levels of serum PRDX3 in early-stage HCC patients may contribute to poor survival.

TNM staging is one of the most widely used systems to classify HCC and predict patient prognosis. Although the TNM system successfully predicts patient prognosis based on clinical and pathological variables (e.g., tumor size, tumor number, lymph node metastasis, and distant metastasis), its use remains limited in predicting patient prognosis, which is critical to the optimization of personalized treatment and accurate patient stratification. For example, identification of patients with poor prognosis in early-stage HCC remains highly challenging. Thus, our current findings support the notion that elevated levels of serum PRDX3 in HCC may be important in detecting an aggressive phenotype or a phenotype that can predict poor prognosis. We believe that the use of serum PRDX3 as a diagnostic biomarker of HCC could improve early detection of HCC. Moreover, improved detection rates would have important prognostic implications for patients with HCC.

It is noteworthy that our current study was retrospective in nature and that the number of early-stage HCC patients was small. Clearly, further prospective studies are needed that are designed to include a larger number of early-stage HCC patients and ultimately the diagnostic and prognostic implications of serum PRDX3 in HCC are validated in other large multicenter cohort studies.

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