

# Prostate Tumor Overexpressed 1 Is a Novel Prognostic Marker for Hepatocellular Carcinoma Progression and Overall Patient Survival

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**Abstract:** The gene prostate tumor overexpressed 1 (*PTOVI*) was first found to be upregulated in prostate cancer. This upregulation increased tumor cell proliferation, retinoic acid resistance, and migration. This study investigated the expression and prognostic significance of *PTOVI* in hepatocellular carcinoma (HCC).

Real-time Polymerase Chain Reaction and western blot analysis were performed to examine *PTOVI* expression in 11 HCC cell lines and 2 normal hepatic cell lines. *PTOVI* expression levels were also determined in 8 pairs of tissue samples taken from primary HCC tumors and the matched adjacent noncancerous liver tissue from the same patient. Immunohistochemistry assays assessed *PTOVI* protein expression in paraffin-embedded clinical samples taken from 215 HCC patients. The correlation of *PTOVI* expression with the clinicopathological parameters was evaluated along with the prognostic impact of *PTOVI* expression in these HCC patients.

*PTOVI* mRNA and protein were overexpressed in HCC cell lines compared with normal liver cell lines and were overexpressed in primary HCC samples compared with the matched noncancerous liver tissue samples. In the paraffin-embedded tissue samples from 215 HCC patients, *PTOVI* protein expression was significantly correlated with T classification, N classification, clinical stage, and serum  $\alpha$ -fetoprotein. HCC patients with higher *PTOVI* expression had shorter survival times than patients with lower *PTOVI* expression.

Our study demonstrated that *PTOVI* overexpression is correlated with increased aggressiveness of HCC and could be a prognostic biomarker for patients with HCC.

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**Abbreviations:** AFP =  $\alpha$ -fetoprotein, AR = androgen receptor, HBV = hepatitis B virus, HCC = hepatocellular carcinoma, HCV = hepatitis C virus, IHC = immunohistochemistry, MOD = median optical density, OS = overall survival.

## INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer and, globally, is the third leading cancer-related cause of mortality.<sup>1,2</sup> HCC is most prevalent in South East and East Asia, with an incident rate of 18.3–35.5 per 10,000 people.<sup>3</sup> HCC is a carcinoma with a poor prognosis, largely because of most diagnoses being made at an advanced stage and the lack of a universal HCC prognostic staging system to predict clinical outcomes for patients.<sup>4</sup> Several factors are associated with an increased risk of developing HCC: hepatitis B virus (HBV) or hepatitis C virus (HCV) infection; aflatoxin B exposure; smoking; cirrhosis risk factors including genetic diseases such as hemochromatosis; and genetic diseases including glycogen storage disease type 1 and alpha-1-antitrypsin deficiency.<sup>3,5</sup> However, the molecular mechanisms of HCC development and progression remain largely unknown. Thus, it is of great importance to identify risk factors and biomarkers for early diagnosis and prognostic prediction in patients with HCC.

The gene prostate tumor overexpressed 1 (*PTOVI*) was first identified during screening for genes overexpressed in prostate cancer.<sup>6</sup> *PTOVI* is located at chromosome 19q13.33, a region which is reported to be amplified in HCC.<sup>7</sup> Recently, deregulation of *PTOVI* has been found in prostate cancer, endometrium, bladder, and ovarian cancer and is associated with increased aggressiveness of human carcinomas.<sup>8,9</sup> Ectopic expression of *PTOVI* increased the proliferation of prostate cancer cells and promoted entry at S phase to the cell division cycle.<sup>10,11</sup> These findings suggest that *PTOVI* is important in the development and progression of human malignancies. However, the *PTOVI* expression pattern and its clinical importance in cancer remain to be elucidated.

In this study, we investigated the expression of *PTOVI* in HCC cell lines and 8 pairs of HCC tissue samples and evaluated the clinicopathological significance and prognostic value of *PTOVI* in 215 archived paraffin-embedded HCC clinical samples.

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S-PC, L-SZ, and NJ conceived and designed the experiments. SPC and X-CZ performed the experiments. B-SF analyzed the data. LSZ and H-MY contributed reagents/materials/analysis tools. NJ wrote the article. All the authors read and approved the final manuscript.

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## MATERIALS AND METHODS

### Cell Lines

HCC cell lines (Huh7, QGY7703, HCCC-9810, PLC, QGY7721, Hep3B, HepG2, QGY7701, Bel7404, HCCLM3, and MHCC97H) were purchased from the ATCC Cell Biology Collection and were grown in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS, HyClone, Logan, UT) and 1% penicillin–streptomycin (Invitrogen, Grand Island, NY) at 37°C with 5% CO<sub>2</sub>. Two normal hepatic cell lines were established according to a previous report.<sup>12</sup>

### Tissue Specimens and Patient Information

The study used paraffin-embedded HCC samples taken from 215 HCC patients. The samples had been clinically and histologically diagnosed at the Sun Yat-sen University Cancer Center (Guangzhou, China) between 2007 and 2009. For the use of clinical materials for research purposes, prior patient consents and approval were obtained from the Sun Yat-sen University Cancer Center Institutional Board. The samples were obtained from patients with HCC: 179 (83.3%) men and 36 (16.7%) women. The median age of the cohort was 53 years (range 30–75 years). The follow-up time of the cohort ranged from 1 month to 73 months, with a median follow-up time of 20 months. The clinicopathological information is summarized in Table 1. Eight pairs of HCC tissue samples from Sun Yat-sen University Cancer Center were frozen and stored in liquid nitrogen for future use. Tumor stages were defined according to the 2002 American Joint Committee on Cancer (AJCC) TNM staging system.

### RNA Extraction and Real-Time PCR

Total RNA from cell lines and 8 paired fresh tissue samples were extracted using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instruction. The extracted RNA was pretreated with RNase-free DNase, and 2 µg RNA from each sample was used for cDNA synthesis primed with random hexamers. For PCR-mediated amplification of *PTOVI* cDNA, an initial amplification using *PTOVI*-specific primers was carried out with a denaturation step at 95°C for 10 min followed by 30 cycles of denaturation at 95°C for 60 seconds, primer annealing at 55°C for 30 seconds, and primer extension at 72°C for 30 seconds. On completion of the cycling steps, a final extension at 72°C for 5 minutes was carried out before the reaction was stopped and the products stored at 4°C. Real-time PCR was then employed to determine the fold increases of *PTOVI* mRNA in HCC cell lines relative to normal liver cell lines, and levels of *PTOVI* in each of the primary HCC tumors were compared with the matched noncancerous liver tissue sample from the same patient. Reverse transcription-PCR and real-time PCR primers were designed using the Primer Express v 2.0 software (Applied Biosystems). The sequences of the real-time PCR primers were: *PTOVI* Forward: CGAG TACAGGAGCATGAGCA and Reverse: CTTCACCAACA GAGACTGCG; GAPDH Forward: GACTCATGACCACGTC CATGC and Reverse: AGAGGCAGGGATGATGTTCTG. Expression data were normalized to the geometric mean of the expression of GAPDH and calculated as  $2^{-[(Ct\ of\ PTOVI) - (Ct\ of\ GAPDH)]}$ , where Ct represents the threshold cycle for each transcript. All experiments were performed in triplicate.<sup>13</sup>

### Western Blots

Cells at 70% to 80% confluence were washed twice with ice-cold phosphate-buffered saline, lysed on ice in

**TABLE 1.** Clinicopathological Characteristics of Patient Samples and Expression of *PTOVI* in Hepatocellular Cancer

	Number of Cases	(Percent)
Sex		
Female	36	16.7
Male	179	83.3
Age, y		
<53	101	47.0
≥53	114	53.0
T classification		
1	29	13.5
2	41	19.1
3	71	33.0
4	74	34.4
N classification		
0	207	96.3
1	8	3.7
M classification		
0	209	97.2
1	6	2.8
Stage		
1	25	11.6
2	39	18.1
3	143	66.5
4	8	3.7
AFP		
<200 µg/mL	116	54.0
≥200 µg/mL	99	46.0
Cirrhosis		
0	107	49.8
1	108	50.2
Differentiation		
1	3	1.4
2	177	82.3
3	35	16.3
Tumor size		
T < 5 cm	46	21.4
T ≥ 5 cm	169	78.6
Vascular invasion		
0	131	60.9
1	84	39.1
Vital status (at follow-up)		
0	60	27.9
1	155	72.1
Tumor multiplicity		
0	124	57.7
1	91	42.3
<i>PTOVI</i> expression		
0	113	52.6
1	102	47.4
HBsAg		
0	24	11.2
1	191	88.8
HCV		
0	213	99.1
1	2	0.9

AFP = α-fetoprotein, HBsAg = hepatitis B surface antigen, HCV = hepatitis C virus.

radioimmune-precipitation assay buffer (RIPA, Cell Signaling Technology, Danvers, MA) containing a complete protease inhibitor cocktail (Roche Applied Sciences, Mannheim, Germany), and then heated for 5 minutes at 100°C. Fresh tissue

samples were ground to powder in liquid nitrogen and lysed with sodium dodecyl sulfate polyacrylamide gel electrophoresis sample buffer. Briefly, equal amounts of protein (30 μg) were separated by electrophoresis on a 10.5% sodium dodecyl sulfate polyacrylamide gel and transferred to polyvinylidene fluoride membranes (Immobilon P, Millipore, Bedford, MA). After blocking with 5% milk solution in Tris-buffered saline with Tween-20 (TBST) for 1 hour, the membranes were incubated with primary antibody using anti-PTOV1 antibody (1:100, Sigma, HPA051812) overnight at 4°C. α-Tubulin mouse monoclonal antibody (1:1000, Santa Cruz Biotechnology) was used as an internal loading control. After 3 washes with TBST, the membranes were incubated with secondary antibodies against rabbit immunoglobulin G or mouse immunoglobulin G. *PTOV1* expression was then examined using the enhanced chemiluminescence detection system (Amersham Biosciences Europe, Freiberg, Germany) according to the manufacturer's instructions.

### Immunohistochemistry (IHC) Analysis

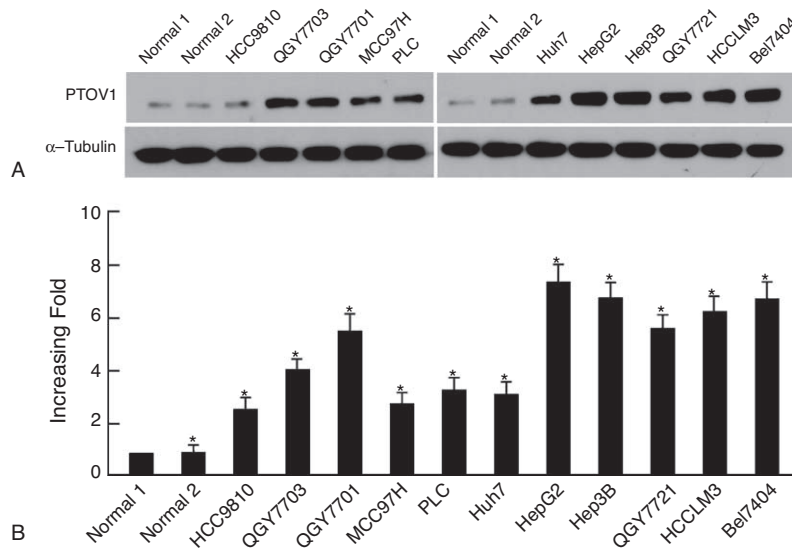
IHC analysis was performed to examine *PTOV1* expression in HCC patient tissues. Briefly, paraffin-embedded specimens were cut into 4-μm sections and baked at 60°C for 2 hours, and then deparaffinized with xylene and rehydrated. Antigenic retrieval was done by submerging the sections into EDTA antigenic retrieval buffer and microwaving. Then, the sections were treated with 3% hydrogen peroxide in methanol to quench the endogenous peroxidase activity, followed by incubation with 1% bovine serum albumin to block the nonspecific binding. Tissue sections were incubated with anti-PTOV1 antibody (1:50, Sigma, HPA 051812) overnight at 4°C. For negative controls, the anti-PTOV1 antibody was replaced with normal goat serum. After washing, the tissue sections were treated with biotinylated anti-rabbit secondary antibody (Abcam), followed by a further incubation with streptavidin-horseradish peroxidase complex (Abcam). The tissue sections were immersed in 3-amino-9-ethyl carbazole,

counterstained with 10% Mayer's hematoxylin, and then dehydrated and mounted in Crystal Mount.

The tissue sections were reviewed and scored independently by 2 investigators based on both the proportion of positively stained tumor cells and the intensity of staining. The proportion of tumor cells was scored as follows: 0 (no positive tumor cells), 1 (<10% positive tumor cells), 2 (10–50% positive tumor cells), 3 (50–75% positive tumor cells), and 4 (>75% positive tumor cells). The intensity of staining was graded as follows: 0 (no staining), 1 (weak staining, light yellow), 2 (moderate staining, yellow brown), and 3 (strong staining, brown). The staining index (SI) was calculated as the product of the staining intensity score and the proportion of positive tumor cells score. Cut-off values for *PTOV1* protein expression were chosen on the basis of a measure of heterogeneity with the log-rank test statistical analysis with respect to overall survival. The strength of staining signals as measured per positive pixel with the mean optical density value (MOD) method. The MOD data were analyzed using t-test to compare the average MOD difference between different groups of tissues. An optimal cut-off value was identified: an SI score of more than or equal to 6 was used to define tumors as having high *PTOV1* expression and a score less than 6 indicating low expression of *PTOV1*.

### Statistical Analysis

All statistical analyses were carried out using the SPSS 16.0 statistical software package (SPSS Inc, Chicago, IL). Spearman correlation test was applied to analyze the correlation between *PTOV1* expression and the clinicopathological characteristics. The  $\chi^2$  test was used to analyze the relationship between *PTOV1* expression and clinicopathological features. Bivariate correlations between study variables were calculated using Spearman rank correlation coefficients. Survival curves were plotted using the Kaplan–Meier method and compared using the log-rank test. The significance of various variables for



**FIGURE 1.** Expression analysis of *PTOV1* in HCC cell lines and 2 normal hepatic cell lines. (A) *PTOV1* protein expression in cultured HCC cell lines (HCC9810, QGY7703, QGY7701, MCC97H, PLC, Huh7, HepG2, Hep3B, QGY7721, HCCLM3, and Bel7404) and 2 normal hepatic cell lines examined by Western blotting. (B) Expression of *PTOV1* mRNA in 2 normal hepatic cell lines and cultured HCC cell lines by real-time reverse transcription-PCR. Expression levels were normalized for α-Tubulin and GAPDH, respectively. Columns and bars represent mean from 3 parallel experiments and SD, respectively. \**P* < 0.05.

survival was evaluated using univariate and multivariate Cox regression analyses. All reported *P* values are two sided. *P* < 0.05 was considered statistically significant in all the cases.

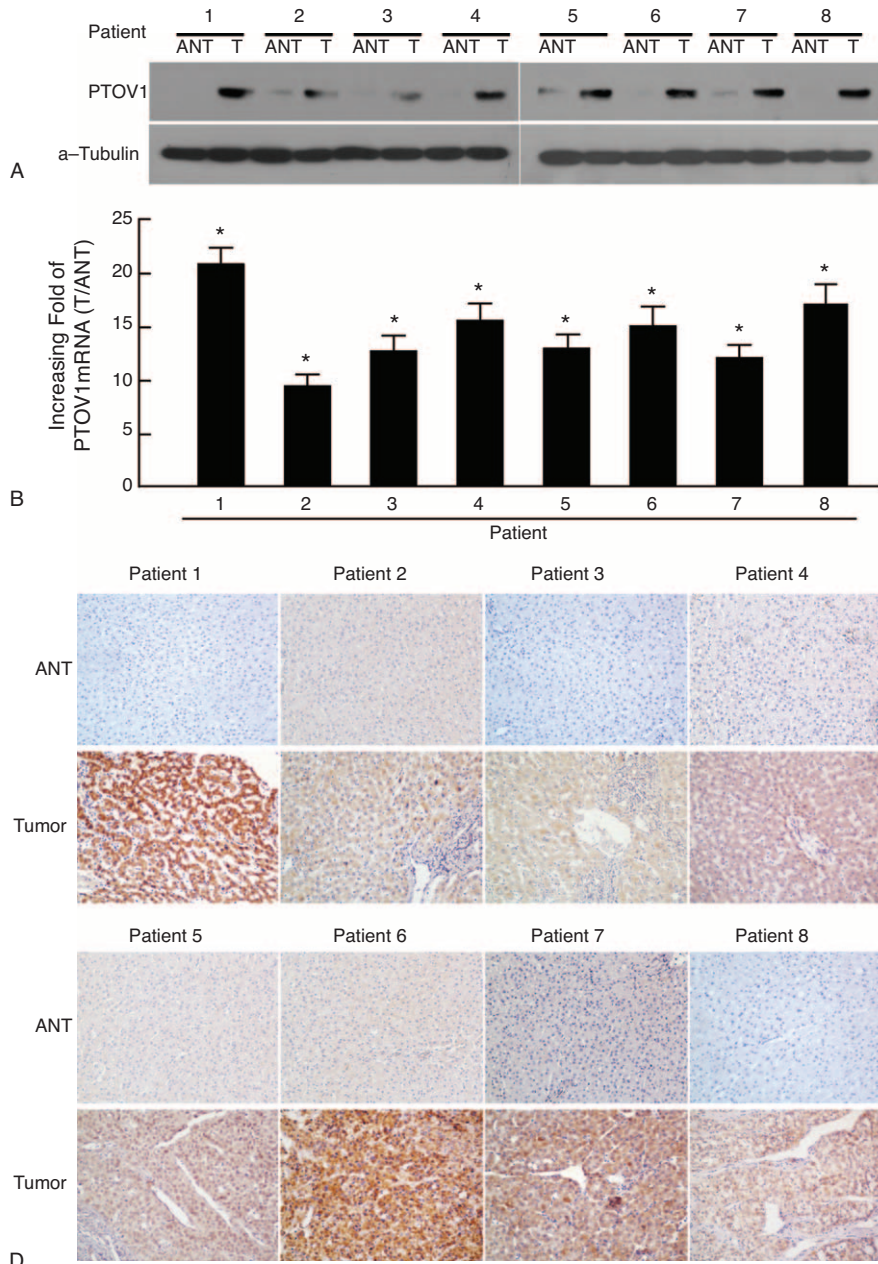
**RESULTS**

**PTOV1 Is Upregulated in HCC**

Western blot analysis showed a higher level of *PTOV1* expression in all HCC cell lines than in the normal liver cell

lines, Normal 1 and Normal 2, which showed marginal *PTOV1* expression (Figure 1A). To explore whether *PTOV1* is also upregulated at the transcriptional level, real-time PCR was performed. The mRNA levels of *PTOV1* in all HCC cell lines were significantly higher than in the 2 normal liver cell lines (Figure 1B). These results demonstrated that *PTOV1* is over-expressed at both the mRNA and protein levels in HCC cell lines compared with normal liver cell lines.

To examine whether the elevation of *PTOV1* expression in HCC cell lines is also correlated with clinical HCC samples, 8



**FIGURE 2.** Overexpression of PTOV1 in HCC tissues. Expression of PTOV1 protein and mRNA in 8 pairs of breast tissues (T) and matched adjacent noncancerous tissues from the same patient were determined by western blotting (A) and real-time PCR (B), respectively. (C) PTOV1 protein expression in each of the primary HCC tissues and matched adjacent noncancerous tissues examined by immunohistochemistry. \**P* < 0.05.



pairs of a primary HCC sample and a noncancerous tissue sample from the same patient were subjected to western blot and real-time PCR. Western blots showed that *PTOVI* expression levels were upregulated in the 8 primary HCC samples when compared with their noncancerous counterparts (Figure 2A). Real-time PCR revealed that expression of the tumor tissue (T)/adjacent noncancerous tissue ratio of the *PTOVI* mRNA level was at least 9.7-fold in these samples, and the highest ratio was approximately 21.3-fold (Figure 2B). The IHC analyses demonstrate that *PTOVI* expression was much higher in all cancer tissues compared with the matched noncancerous tissues, in which *PTOVI* expression was either marginally detectable or undetectable (Figure 2C). Furthermore, our IHC revealed that increasing *PTOVI* staining was positively correlated with advancing clinical stage (Figure 3A). In addition, the *PTOVI* staining median optical density (MOD) values were significantly increased with the progression of tumor stages from I to IV (Figure 3B). Thus, *PTOVI* appears to be upregulated in HCC cell lines and tissues.

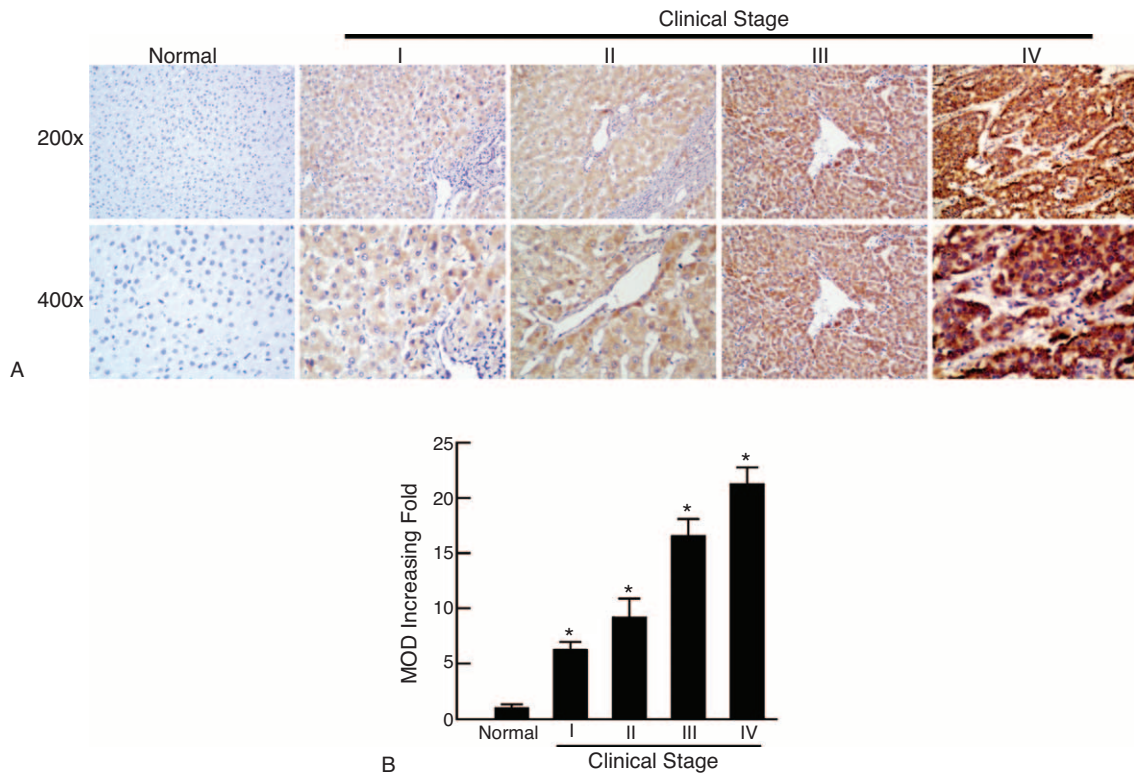
**PTOVI Is Associated With HCC Progression**

To determine the clinical significance of *PTOVI* in HCC, *PTOVI* expression was investigated using IHC analysis in 215 paraffin-embedded, archived HCC tissue samples, including samples from 25 patients with stage I tumors, 39 patients with stage II tumors, 143 patients with stage III tumors, and 8 patients with stage IV tumors. As summarized in Table 1, high expression of *PTOVI* was detected in 102 (47.44%) samples and

low *PTOVI* expression was detected in 113 samples (52.56%). Further investigations were done to explore the correlation between *PTOVI* expression and clinicopathological characteristics. *PTOVI* expression was positively associated with the clinical stage of HCC ( $P < 0.001$ ), T classification ( $P = 0.004$ ), N classification ( $P = 0.002$ ), serum  $\alpha$ -fetoprotein (AFP) ( $P < 0.001$ ), tumor multiplicity ( $P = 0.003$ ), and vascular invasion ( $P < 0.001$ , Table 2). Spearman correlation analysis showed that the high *PTOVI* expression level was closely correlated with clinical stage ( $P < 0.001$ ), T classification ( $P = 0.001$ ), N classification ( $P = 0.002$ ), M classification ( $P = 0.009$ ), tumor multiplicity ( $P = 0.003$ ), vascular invasion ( $P < 0.001$ ), and serum AFP ( $P < 0.001$ , Table 3). However, our results did not show significant correlations between *PTOVI* expression and sex, age, or tumor size (Table 3).

**High PTOVI Expression Is Correlated With Poor Prognosis of Patients With HCC**

Kaplan–Meier analysis and the log-rank test were used to calculate the effects of *PTOVI* expression on survival. HCC patients with higher *PTOVI* expression levels had shorter survival times, whereas HCC patients with lower *PTOVI* expression levels had substantially longer survival times (Figure 4A,  $P < 0.001$ ). Univariate Cox regression analysis demonstrated that *PTOVI* expression, T classification, N classification, clinical stage, vascular invasion, and serum AFP levels were significant worse prognostic factors. Moreover, multivariate Cox regression analysis revealed that *PTOVI* expression, clinical stage, tumor



**FIGURE 3.** *PTOVI* protein overexpression in archived paraffin-embedded HCC tissue sections by immunohistochemistry. (A) Representative images from immunohistochemistry analyses of *PTOVI* expression in normal liver tissues and different clinical stages of HCC tissues. (B) Statistical analyses of the average MOD of *PTOVI* staining between normal liver tissues and HCC specimens of different clinical stages. \* $P < 0.05$ .

**TABLE 2.** Clinicopathological Characteristics of Patient Samples and Expression of PTOV1 in Hepatocellular Cancer and Correlation Between PTOV1 Expression and Clinicopathological Characteristics of Hepatocellular Cancer Patients

Characteristics		PTOV1			$\chi^2$ Test P-Value	Fisher Exact Test P-Value
		Total	Low Expression	High Expression		
Sex	Female	36	22 (66.1%)	14 (38.9%)	0.26	0.278
	Male	179	91 (50.8%)	88 (49.2%)		
Age	0	101	55 (54.5%)	46 (45.5%)	0.600	0.682
	1	114	58 (50.9%)	56 (49.1%)		
T	1	29	23 (79.3%)	6 (20.7%)	0.004	0.004
	2	41	24 (58.5%)	17 (41.5%)		
	3	71	36 (50.7%)	35 (49.3%)		
	4	74	30 (40.5%)	44 (59.5%)		
N	0	207	113 (54.6%)	94 (45.4%)	0.002	0.002
	1	8	0 (0%)	8 (100%)		
M	0	209	113 (54.1%)	96 (45.9%)	0.009	0.011
	1	6	0 (0%)	6 (100%)		
Stage	1	25	23 (92.0%)	2 (8.0%)	<0.001	<0.001
	2	39	24 (61.5%)	15 (38.5%)		
	3	143	66 (46.2%)	77 (53.8%)		
	4	8	0 (0%)	8 (100%)		
HBsAg	0	24	10 (41.7%)	14 (58.3%)	0.257	0.284
	1	191	103 (53.9%)	88 (46.1%)		
HCV	0	213	111 (52.1%)	102 (47.9%)	0.177	0.499
	1	2	2 (100%)	0 (0%)		
AFP	<200 $\mu\text{g/mL}$	116	74 (63.8%)	42 (36.2%)	<0.001	<0.001
	$\geq 200 \mu\text{g/mL}$	99	39 (39.4%)	60 (60.6%)		
Cirrhosis	0	107	87 (81.3%)	20 (18.7%)	<0.001	<0.001
	1	108	26 (24.1%)	82 (75.9%)		
Tumor size	$T < 5 \text{ cm}$	46	30 (65.2%)	16 (34.8%)	0.052	0.067
	$T \geq 5 \text{ cm}$	169	83 (49.1%)	86 (50.9%)		
Tumor multiplicity	0	124	76 (61.3%)	48 (38.7%)	0.003	0.004
	1	91	37 (40.7%)	54 (59.3%)		
Vascular invasion	0	131	82 (62.6%)	49 (37.4%)	<0.001	<0.001
	1	84	31 (36.9%)	53 (63.1%)		
Vital status	0	60	42 (70.0%)	18 (30.0%)	0.001	0.001
	1	155	71 (45.8%)	84 (54.2%)		

AFP =  $\alpha$ -fetoprotein, HBsAg = hepatitis B surface antigen, HCV = hepatitis C virus.

**TABLE 3.** Spearman Correlation Analysis Between PTOV1 and Clinical Pathologic Factors

Variables	PTOV1 Expression	
	Spearman Correlation	P Value
Sex	0.077	0.262
Age	0.036	0.602
T classification	0.234	0.001
N classification	0.207	0.002
M classification	0.178	0.009
Clinical staging	0.332	<0.001
AFP (200 $\mu\text{g/mL}$ )	0.244	<0.001
Cirrhosis	0.573	<0.001
Tumor size (5 cm)	0.132	0.053
Tumor multiplicity	0.204	0.003
Vascular invasion	0.251	<0.001
Vital status	0.217	0.001

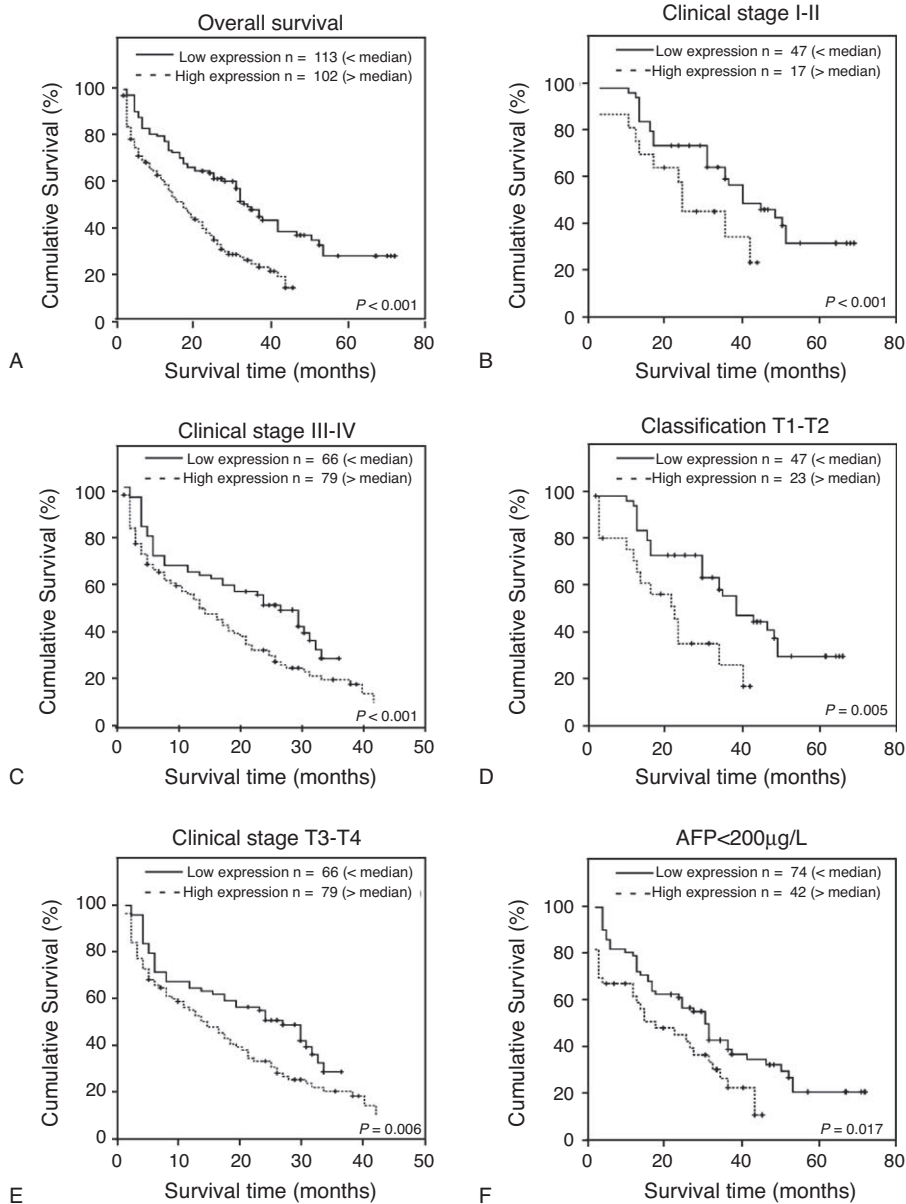
AFP =  $\alpha$ -fetoprotein.

size, and sex were independent prognostic factors for patients with HCC (Table 4).

In addition, the prognostic value of *PTOV1* expression was analyzed in specific subgroups of HCC patients stratified according to clinical stage and T classification. As shown in Figure 4, the clinical outcomes between patients with high and low *PTOV1* expression levels were compared between a subgroup of patients with stage I–II tumors (Figure 4B,  $P < 0.001$ ) and a subgroup of patients with stage III–IV tumors (Figure 4C,  $P < 0.001$ ). Similar results were observed in the subgroups of patients with T classifications T1–T2 (Figure 4D,  $P = 0.005$ ) and T3–T4 (Figure 4E,  $P = 0.006$ ), and serum AFP  $< 200 \mu\text{g/L}$  (Figure 4F,  $P = 0.017$ ). These results indicated that *PTOV1* might be a useful prognostic biomarker for HCC patients.

## DISCUSSION

In this study, we report that aberrant *PTOV1* expression is present in HCC cell lines compared with normal immortal cell lines. Aberrant *PTOV1* expression is also present in the cancer tissue of patients when compared with the matched noncancerous tissue from the same patient at both transcriptional and translational levels. IHC analysis of a cohort of 215 patients revealed that



**FIGURE 4.** Kaplan–Meier analysis of overall survival in HCC patients based on PTOV1 expression. (A) Overall survival (OS) of all patients with high PTOV1 expression versus low PTOV1 expression. OS rate for patients with high PTOV1 expression versus patients with low PTOV1 expression in patients with T1–T2 grade tumors (B) and T3–T4 grade tumors (C). The overall survival of patients of high PTOV1 expression and low PTOV1 expression compared between patients with early stage disease (stage I–II) (D) and late stage disease (stage III–IV) (E) and between patients with serum AFP level < 200 µg/L (F). *P* values were calculated by log-rank tests.

upregulation of *PTOV1* is correlated with clinicopathological characteristics and the aggressiveness of the HCC. Our data imply that aberrant *PTOV1* expression is frequently observed in HCC cell lines and clinical samples and might be an independent prognostic marker for patients with HCC.

*PTOV1* was first identified as a novel gene that was upregulated in prostate cancer, and its overexpression has been implicated in other neoplasm, such as skin, ovary, and bladder cancers.<sup>12–14</sup> *PTOV1* is located on chromosome region 19q13 and possesses 2 repeated homology PTOV blocks, each containing a potential nuclear localization signal.<sup>9</sup> It is thought that

*PTOV1* plays an essential role in tumor progression in prostate cancer.<sup>8–11</sup> *PTOV1* protein has been reported to shuttle between the cytoplasm and the nucleus and to promote entry into the S phase of the cell cycle, thus increasing the proliferation of prostate cancer cells.<sup>15–17</sup> In addition, it is reported that *PTOV1* enables the nuclear translocation and enhances the mitogenic activity of Flotillin-1.<sup>10</sup> *PTOV1* is closely related to the proliferation marker Ki67, both in vivo and in vitro.<sup>8,10</sup> Overexpression of *PTOV1* in prostate cancer cells promotes the translation of c-Jun, thus inducing cell invasion and migration, whereas *PTOV1* silencing significantly reduced the levels of

**TABLE 4.** Univariate and Multivariate Cox-Regression Analyses of Various Prognostic Parameters in Patients With Hepatocellular Cancer

Variables	No. patients	Univariate Analysis		Multivariate Analysis			
		Regression (SE)	<i>P</i>	<i>P</i>	Relative Risk	95% Confidence Interval	
<i>PTOVI</i>		0.809 (0.167)	<0.001	0.002	1.692	1.204	2.739
Low expression	113						
High expression	102						
T classification		0.604 (0.093)	<0.001				
1	29						
2	41						
3	71						
4	74						
N classification		1.276 (0.423)	0.003				
0	207						
1	8						
Stage		0.964 (0.146)	<0.001	<0.001	2.209	1.630	2.995
1	25						
2	39						
3	143						
4	8						
AFP		0.423 (0.165)	0.01				
<200 µg/L	116						
≥200 µg/L	99						
Cirrhosis		0.622 (0.165)	0.01				
0	107						
1	108						
Tumor size		0.927 (0.235)	<0.001	0.038	1.64	1.027	2.618
<i>T</i> < 5 cm	46						
<i>T</i> ≥ 5 cm	169						
Sex		0.493 (0.207)	0.018	0.007	0.568	0.376	0.858
Female	36						
Male	179						
Vascular invasion		0.453 (0.168)	0.007				
0	131						
1	84						

AFP = α-fetoprotein.

phosphorylated c-Jun and the proportion of cells in which the protein was translocated to the nucleus.<sup>11</sup> These reports suggested that *PTOVI* may serve as a positive regulator of tumor cell proliferation and progression in HCC. However, further investigations are required to clarify the molecular mechanisms of the involvement of *PTOVI* in HCC carcinogenesis. In the present study, *PTOVI* was shown to be significantly associated with aggressive features of HCC and unfavorable clinicopathological characteristics. Patients with high *PTOVI* expression had a substantially poorer overall survival than patients with low *PTOVI* expression, and Cox regression analysis revealed that *PTOVI* might be an independent factor for patients with HCC. Our study demonstrates that *PTOVI* is correlated with aggressive characteristics of HCC and might serve as a useful prognostic marker for patients with HCC.

Cancer development and progression is a multistep process and gene mutations, and alterations in gene transcription and translation, can potentially serve as specific biomarkers for disease.<sup>18</sup> A better knowledge of the molecular mechanisms involved in the pathogenesis of HCC, especially the genetic alterations implicated in HCC, will provide new insights into diagnoses and therapy options for HCC patients, thus improving clinical outcomes. Serum AFP is currently the most widely used tumor marker for the diagnosis and the prediction of the

prognosis for patients with HCC, with a sensitivity of 41–65% and specificity of 80–94% at the cut-off value of 200 µg/L.<sup>19</sup> However, serum AFP levels rise during pregnancy and in other diseases, such as hepatitis, cirrhosis, teratoma, and germ cell tumors.<sup>20</sup> HCC is predominantly found in men, with the male:female incidence ratio ranging from 2.1 to 4.0.<sup>21</sup> This discrepancy is attributed to the elevated expression and enhanced activity of the androgen receptor (AR) in men, which could also be a potential therapeutic target for HCC.<sup>22,23</sup> The male predominance in HBV-related HCC is significantly high, with a ratio of 5–7:1.<sup>24</sup> HBV infection accounts for 50% of the total cases of HCC in Asia and sub-Saharan Africa.<sup>25</sup> Moreover, it is reported that AR contributes to HBV-induced hepatocarcinogenesis and HBV enhances AR-responsive gene expression, indicating that HBV and AR cooperate with each other to promote HCC carcinogenesis.<sup>26,27</sup> *PTOVI* has been mapped to 19q13, which is a region that has been demonstrated to harbor a large number of genes modulated by androgens.<sup>9</sup> In addition, *PTOVI* itself can be induced by exposure to androgens.<sup>9,14</sup> Consistent with these findings, our study revealed that sex is an independent prognostic factor for HCC patients and males are more likely to have unfavorable outcomes. These reports imply that *PTOVI* may promote HCC development and progression via the AR signaling pathway.



## CONCLUSION

We found that in both HCC cell lines and clinical samples *PTOV1* was upregulated at both the mRNA and protein level when compared with normal liver cell lines and matched noncancerous tissues. *PTOV1* was closely associated with unfavorable pathologic features of HCC, including clinical stage, T classification, and N classification. Patients with high *PTOV1* expression exhibited worse survival rates than those with low *PTOV1* expression. *PTOV1* could serve as a novel prognostic marker for HCC patients.

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