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 (PTOV1) 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 PTOV1 in hepatocellular carcinoma (HCC).

Real-time Polymerase Chain Reaction and western blot analysis were performed to examine PTOV1 expression in 11 HCC cell lines and 2 normal hepatic cell lines. PTOV1 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 PTOV1 protein expression in paraffin-embedded clinical samples taken from 215 HCC patients. The correlation of PTOV1 expression with the clinicopathological parameters was evaluated along with the prognostic impact of PTOV1 expression in these HCC patients.

PTOV1 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, PTOV1 protein expression was significantly correlated with T classification, N classification, clinical stage, and serum α-fetoprotein. HCC patients with higher PTOV1 expression had shorter survival times than patients with lower PTOV1 expression.

Our study demonstrated that PTOV1 overexpression is correlated with increased aggressiveness of HCC and could be a prognostic biomarker for patients with HCC.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer and, globally, is the third leading cancer-related cause of mortality.1,2 HCC is most prevalent in South East and East Asia, with an incident rate of 18.3–35.5 per 10,000 people.3 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.3 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.3,5 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 (PTOV1) was first identified during screening for genes overexpressed in prostate cancer.6 PTOV1 is located at chromosome 19q13.33, a region which is reported to be amplified in HCC.7 Recently, deregulation of PTOV1 has been found in prostate cancer, endometrium, bladder, and ovarian cancer and is associated with increased aggressiveness of human carcinomas.8,9 Ectopic expression of PTOV1 increased the proliferation of prostate cancer cells and promoted entry at S phase to the cell division cycle.10,11 These findings suggest that PTOV1 is important in the development and progression of human malignancies. However, the PTOV1 expression pattern and its clinical importance in cancer remain to be elucidated.

In this study, we investigated the expression of PTOV1 in HCC cell lines and 8 pairs of HCC tissue samples and evaluated the clinicopathological significance and prognostic value of PTOV1 in 215 archived paraffin-embedded HCC clinical samples.
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% CO₂. Two normal hepatic cell lines were established according to a previous report.12

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 PTOV1 cDNA, an initial amplification using PTOV1-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 PTOV1 mRNA in HCC cell lines relative to normal liver cell lines, and levels of PTOV1 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: PTOV1 Forward: CGAG TACAGGAGCATGAGCA and Reverse: CTTCAACCAACA GAGACTGCG; GAPDH Forward: GACTCATGACCACGT CATGC and Reverse: AGAGGCAAGGATGATGTTCTG. Expression data were normalized to the geometric mean of the expression of GAPDH and calculated as 2^([-Ct of PTOV1] - [Ct of GAPDH]), where Ct represents the threshold cycle for each transcript. All experiments were performed in triplicate.13

Western Blots

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

| Number of Cases (Percent) |
|---------------------------|
| **Sex**                   |
| Female                    | 36 | 16.7 |
| Male                      | 179| 83.3 |
| **Age, y**                |
| <53                       | 101| 47.0 |
| 53–100                    | 114| 53.0 |
| **Stage**                 |
| 1                        | 29 | 13.5 |
| 2                        | 41 | 19.1 |
| 3                        | 71 | 33.0 |
| 4                        | 74 | 34.4 |
| **AFP**                   |
| <200 μg/mL                | 116| 54.0 |
| ≥200 μg/mL                | 99 | 46.0 |
| **Cirrhosis**             |
| 0                         | 107| 49.8 |
| 1                         | 108| 50.2 |
| **Differentiation**       |
| 0                         | 3  | 1.4  |
| 1                         | 177| 82.3 |
| 2                         | 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 |
| **PTOV1 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.
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. Antigen 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 χ² 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
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 overexpressed 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

![Graph showing overexpression of PTOV1 in HCC tissues.](image)

**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 PTOV1 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 PTOV1 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 PTOV1 expression was much higher in all cancer tissues compared with the matched noncancerous tissues, in which PTOV1 expression was either marginally detectable or undetectable (Figure 2C). Furthermore, our IHC revealed that increasing PTOV1 staining was positively correlated with advancing clinical stage (Figure 3A). In addition, the PTOV1 staining median optical density (MOD) values were significantly increased with the progression of tumor stages from I to IV (Figure 3B). Thus, PTOV1 appears to be upregulated in HCC cell lines and tissues.

PTOV1 Is Associated With HCC Progression

To determine the clinical significance of PTOV1 in HCC, PTOV1 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 PTOV1 was detected in 102 (47.44%) samples and low PTOV1 expression was detected in 113 samples (52.56%). Further investigations were done to explore the correlation between PTOV1 expression and clinicopathological characteristics. PTOV1 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 PTOV1 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 PTOV1 expression and sex, age, or tumor size (Table 3).

High PTOV1 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 PTOV1 expression on survival. HCC patients with higher PTOV1 expression levels had shorter survival times, whereas HCC patients with lower PTOV1 expression levels had substantially longer survival times (Figure 4A, $P < 0.001$). Univariate Cox regression analysis demonstrated that PTOV1 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 PTOV1 expression, clinical stage, tumor

FIGURE 3. PTOV1 protein overexpression in archived paraffin-embedded HCC tissue sections by immunohistochemistry. (A) Representative images from immunohistochemistry analyses of PTOV1 expression in normal liver tissues and different clinical stages of HCC tissues. (B) Statistical analyses of the average MOD of PTOV1 staining between normal liver tissues and HCC specimens of different clinical stages. *$P < 0.05$. 

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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 µg/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

| 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 | Total | Low Expression | High Expression | χ² Test P-Value | Fisher Exact Test P-Value |
|-----------------|-------|----------------|----------------|----------------|-------------------------|
| 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 µg/mL  |       |                |                |                |                         |
| ≥200 µg/mL      | 116   | 74 (63.8%)     | 42 (36.2%)     | <0.001         | <0.001                  |
| 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 cm         | 46    | 30 (65.2%)     | 16 (34.8%)     | 0.052          | 0.067                   |
| T ≥5 cm         | 169   | 83 (49.1%)     | 86 (50.9%)     |                |                         |
| Tumor multiplicity |   | 124   | 76 (61.3%)     | 48 (38.7%)     | 0.003          | 0.004                   |
| 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 = α-fetoprotein, HBsAg = hepatitis B surface antigen, HCV = hepatitis C virus.

| TABLE 3. Spearman Correlation Analysis Between PTOV1 and Clinical Pathologic Factors |
|-----------------------------------------------|
| 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 µg/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 = α-fetoprotein.
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. PTOV1 is located on chromosome region 19q13 and possesses 2 repeated homology PTOV blocks, each containing a potential nuclear localization signal. It is thought that

*PTOV1* plays an essential role in tumor progression in prostate cancer. *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. In addition, it is reported that *PTOV1* enables the nuclear translocation and enhances the mitogenic activity of Flotillin-1. Over-expression 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

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**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.
phosphorylated c-Jun and the proportion of cells in which the protein was translocated to the nucleus. These reports suggested that PTOV1 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 PTOV1 in HCC carcinogenesis. In the present study, PTOV1 was shown to be significantly associated with aggressive features of HCC and unfavorable clinicopathological characteristics. Patients with high PTOV1 expression had a substantially poorer overall survival than patients with low PTOV1 expression, and Cox regression analysis revealed that PTOV1 might be an independent factor for patients with HCC. Our study demonstrates that PTOV1 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. 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. However, serum AFP levels rise during pregnancy and in other diseases, such as hepatitis, cirrhosis, teratoma, and germ cell tumors. HCC is predominantly found in men, with the male:female incidence ratio ranging from 2.1 to 4.0. 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. The male predominance in HBV-related HCC is significantly high, with a ratio of 5–7:1. HBV infection accounts for 50% of the total cases of HCC in Asia and sub-Saharan Africa. 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. PTOV1 has been mapped to 19q13, which is a region that has been demonstrated to harbor a large number of genes modulated by androgens. In addition, PTOV1 itself can be induced by exposure to androgens. 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 PTOV1 may promote HCC development and progression via the AR signaling pathway.

### 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)     | P         | Relative Risk | 95% Confidence Interval |
|                    |              | 0.809 (0.167)       | <0.001   | 1.692        | 1.204                    | 2.739                     |
| PTOV1              | Low expression | 113                |          |              |                          |
|                    | High expression | 102               | 0.604 (0.093)       | <0.001   | 2.209        | 1.630                    | 2.995                     |
|                    | T classification | 29                | 0.964 (0.146)       | <0.001   | 1.276        | 0.003                     |
|                    | 1            | 25                 |          |              |                          |
|                    | 2            | 41                 |          |              |                          |
|                    | 3            | 71                 |          |              |                          |
|                    | 4            | 74                 |          |              |                          |
| N classification   | 0            | 207                | 1.726 (0.423)       | 0.003    | 0.423        | 0.165                     | 0.01                       |
|                    | 1            | 8                  |          |              |                          |
| Stage              | 1            | 25                 | 0.038 (0.235)       | 0.038    | 0.493        | 0.207                     | 0.007                      |
|                    | 2            | 39                 |          |              |                          |
|                    | 3            | 143                |          |              |                          |
|                    | 4            | 8                  |          |              |                          |
| AFP                | <200 μg/L    | 116                | 0.927 (0.235)       | <0.001   | 1.64         | 1.027                    | 2.618                      |
|                    | ≥200 μg/L    | 99                 |          |              |                          |
| Cirrhosis          | 0            | 107                | 0.622 (0.165)       | 0.01     | 0.453        | 0.168                     | 0.007                      |
|                    | 1            | 108                |          |              |                          |
| Tumor size         | T<5 cm       | 46                 | 0.493 (0.207)       | 0.018    | 0.568        | 0.376                     | 0.858                      |
|                    | T≥5 cm       | 169                |          |              |                          |
| Sex                | Female       | 36                 | 0.493 (0.207)       | 0.018    | 0.568        | 0.376                     | 0.858                      |
|                    | Male         | 179                |          |              |                          |
| Vascular invasion  | 0            | 131                | 0.493 (0.207)       | 0.018    | 0.568        | 0.376                     | 0.858                      |
|                    | 1            | 84                 |          |              |                          |

AFP = α-fetoprotein.
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.

ACKNOWLEDGMENTS
This study was supported by the National Natural Science Foundation of China (No. 81000959), Guangdong Natural Science Foundation (No. 10451130001004472, No.S2013010 016023), Science and Technology Programme of Guangzhou Municipal Government (No.2013J4100081), and the Supported Foundation of Science and Technology Innovation of Zengcheng (No. ZC201004), Science and Technology Planning Project of Guangdong Province, China (No. 2009B030801007), The Fundamental Research Funds for the Central Universities (No. 12ykpy47), and National 12th Five-Year Science and Technology Plan Major Projects of China (No.2012ZX10002017-005).

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